

RAMIRAN managing organic resources in a changing environment 2023

Edited by

Rachel E. Thorman, David Robert Chadwick, Tom Misselbrook,
Fiona Nicholson and Harald Menzi

Published in

Frontiers in Sustainable Food Systems



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ISSN 1664-8714
ISBN 978-2-8325-6532-2
DOI 10.3389/978-2-8325-6532-2

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RAMIRAN 2023: managing organic resources in a changing environment

Topic editors

Rachel E. Thorman — Agricultural Development Advisory Service, United Kingdom

David Robert Chadwick — Bangor University, United Kingdom

Tom Misselbrook — Department of Sustainable Agriculture Sciences, Rothamsted Research, United Kingdom

Fiona Nicholson — Agricultural Development Advisory Service, United Kingdom

Harald Menzi — Independent researcher, Bern, Switzerland

Citation

Thorman, R. E., Chadwick, D. R., Misselbrook, T., Nicholson, F., Menzi, H., eds. (2025). *RAMIRAN 2023: managing organic resources in a changing environment*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-6532-2

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OPEN ACCESS

EDITED BY

Rachel E. Thorman,
Agricultural Development Advisory Service
(United Kingdom), United Kingdom

REVIEWED BY

James Steve Robinson,
University of Reading, United Kingdom
Ruben Sakrabani,
Cranfield University, United Kingdom

*CORRESPONDENCE

Tomas Javier Sitzmann
✉ tomas.sitzmann@unito.it

RECEIVED 31 October 2023

ACCEPTED 10 January 2024

PUBLISHED 23 January 2024

CITATION

Sitzmann TJ, Sica P, Grignani C and
Magid J (2024) Testing biowaste materials as
peat replacement in organo-mineral fertilizers.
Front. Sustain. Food Syst. 8:1330843.
doi: 10.3389/fsufs.2024.1330843

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Testing biowaste materials as peat replacement in organo-mineral fertilizers

Tomas Javier Sitzmann^{1*}, Pietro Sica², Carlo Grignani¹ and
Jakob Magid²

¹Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Turin, Italy,

²Department of Plant and Environmental Science, University of Copenhagen, Copenhagen, Denmark

Organo-mineral fertilizers (OMFs) can have higher efficiency than mineral fertilizers. In Europe, peat is commonly used as an organic matrix in OMFs, as it is a highly stable organic material. However, peat extraction releases long-term stored organic C. Stabilized biowaste materials could replace peat in OMFs. Thus, this study aimed to understand how the variety of chemical properties in biowastes can influence OMF-soil interactions and nutrient availability to plants. Peat, green compost (GC), municipal solid waste compost (MSWC), and vermicompost (VC) were used as the organic matrix of OMFs with a C-N-P₂O₅ content of 7.5, 10, and 5%, respectively. OMFs were tested first in a ten-day plant-free incubation to measure Hedley P fractionation, nitrate, ammonium, total N in the soil, and CO₂ and NH₃ emissions. Further, a 30-day greenhouse trial measured maize yield and N and P use efficiencies. Controls included no fertilization (N₀P₀) and mineral N and P fertilization (MF_{NP}). No differences were found in the incubation experiment for mineral N fractions in the soil. Fertilization significantly increased CO₂ emissions, which were slightly higher in OMFs over MF_{NP}, whereas OMFs significantly increased ammonia volatilization compared to MF_{NP}. Available P had consistent results in the incubation and the greenhouse trials. Peat and MF_{NP} had the highest water- and bicarbonate-P pools in the first experiment and higher yield and P use efficiency by maize. Therefore, OMFs from biowaste materials exhibited limited suitability for short growth cycles due to lower P use efficiency and higher ammonia volatilization.

KEYWORDS

organo-mineral fertilizers, biosolid, bio-waste, peat replacement, nutrient immobilization, pruning compost, municipal solid waste, vermicompost

1 Introduction

Organo-mineral fertilizers (OMFs) combine organic and mineral components designed to provide a balanced supply of nutrients to plants, harnessing the benefits of both organic materials and inorganic minerals (Smith et al., 2020). The degree of influence of the organic material on the mineral fertilizer depends on the proportion between organic and mineral fractions (Pare et al., 2010). Therefore, comparisons across different OMFs are rather complex when OMFs are made with different mineral fertilizers, mixtures of organic and mineral nutrients, and organic materials proportions. Low organic C (C_{org}) OMFs primarily utilize the organic material as a carrying matrix for mineral nutrients rather than a primary source of nutrients. In Europe, the standard establishes a minimum of 7.5% of C_{org} in solid OMFs (EC, 2019). Thus, low C_{org} OMFs have almost negligible amounts of macronutrients provided by the organic material, while they

can be a source of some microelements such as Fe, Cu, and Zn (Sitzmann et al., 2023). However, the organic material ranges ideally between 30 to 50% of the total OMF volume (Pare et al., 2010). Consequently, organic materials with a medium C_{org} content are considered desirable as they allow sufficient addition of mineral fertilizers while providing an optimal organic matrix for efficient nutrient delivery.

In low C_{org} OMFs, the combination of an organic matrix with mineral nitrogen (N) and phosphorus (P) has proven effective in reducing N losses and enhancing N use efficiency (Richards et al., 1993; Antille et al., 2014; Florio et al., 2016). Additionally, OMF application increases plant P uptake by increasing the availability of mineral P (Antille et al., 2013b). The increase of nutrient use efficiency can be attributed to a variety of underlying processes: (i) the reduction of mineral sorption of P to soil minerals (Parent et al., 2003), thereby facilitating absorption by plant roots while simultaneously inhibiting the transformation of available P into forms that are inaccessible to plants (Khari and Parent, 2005); (ii) an increase in microbial immobilization of both N and P, leading to a gradual nutrient release (Mandal et al., 2007) and a reduced N leaching (Richards et al., 1993); (iii) a nutrient electrostatic attraction onto the charged surface of the organic material, thereby reducing nutrient mobility in soil (Gwenzi et al., 2018; Luo et al., 2021); (iv) chemical interactions between the inorganic nutrients and the organic fraction that lead to the formation of new compounds with lower solubility (Mazeika et al., 2016; Carneiro et al., 2021; Luo et al., 2021); (v) the OMF acts as a physical barrier to soil humidity, thereby decreasing fertilizer solubility (Limwikran et al., 2018).

Traditionally, OMFs with humic C content have been considered high quality fertilizers (Alianiello et al., 1999; Florio et al., 2016). Humic-related substances have been reported to influence microbial and physicochemical properties of soil increasing crop growth (Li et al., 2019), having a biostimulant effect on plant metabolism (Asli and Neumann, 2010; Vujanović et al., 2020), and ameliorated soil contaminants thanks to a high sorption capacity (Piccolo, 2002; Conte et al., 2005). Therefore, in low C_{org} OMFs, Peat has been appointed as a high-quality organic material for OMF (Alianiello et al., 1999; Florio et al., 2016) as it is a material typically rich in humic C substances and with a high humification degree (Cavani et al., 2003). Among other geogenic materials, peat in OMFs is allowed in Europe (EC, 2019). However, there is a rising interest in replacing peat with affordable local alternatives that do not require long-distance transportation (Taparia et al., 2021) to protect peatlands to avoid the release of long-term stored C as CO_2 and the emissions of other greenhouse gasses (Saarikoski et al., 2019; Humpenöder et al., 2020). Among possible alternatives to peat, biowaste materials have been proposed to replace peat substrates for horticultural production (Taparia et al., 2021) and mushroom cultivation (Schmilewski, 2008).

Biowaste are easily accessible and local resources, thereby offering the benefit of reducing transportation expenses and costs related to peat extraction. Indeed, the incorporation of biowaste materials into OMFs promotes a circular value proposition, as it establishes a closed-loop system that repurposes and recycles waste materials to create agricultural benefits (Barros et al., 2020; Chojnacka et al., 2020; Zeller et al., 2020). For that, biowaste materials should be degraded through digestion, composting, or vermicomposting to achieve the biochemical stability that will allow the production of a homogeneous OMF with stable interactions between the mineral and organic fractions over time (Sakurada et al., 2016; Bouhia et al., 2022). Therefore, using

biowaste materials as an organic matrix seems promising for OMF manufacturing (Chojnacka et al., 2020, 2022) and, thus, as peat replacement.

Although multiple interactions between organic and mineral fractions have been described, there is still limited knowledge about the main influences of a biowaste material as the organic matrix on the mineral fertilizer in a blended OMF.

Based on this background, we hypothesized that the close contact of an organic material and mineral fertilizers in a blended OMF will cause:

- (H1) slower nutrient release patterns related to the granule integrity in soil due to a higher recalcitrant C_{org} content.
- (H2) an increase of the organic immobilization of nutrients related to a more labile C_{org} fraction boosting the microbial activity.
- (H3) changes in N losses through ammonia volatilization either by reducing it because microbial immobilization into organic N (N_{org}) or increasing it because the interaction between the organic matrix pH and the mineral fertilizer in the OMF.

These hypotheses were tested by analyzing changes in soil respiration, ammonia emissions, and N and P forms in incubated soils fertilized with granular OMFs made with organic materials from different origins. We also tested if potential changes in nutrient availability in soil affect the initial growth and nutrient use efficiency by maize.

This study aimed to contribute to a deeper understanding of the interactions between organic and mineral fractions in low C_{org} OMFs while also validating alternatives to peat for more sustainable agricultural practices.

2 Materials and methods

This study conducted two independent experiments: (i) a soil incubation trial and (ii) a plant growth trial with maize as the selected crop. For both trials four organo-mineral fertilizers (OMFs) with different organic matrices were used: peat, green compost (GC), municipal solid waste compost (MSWC), and vermicompost (VC). Two control treatments were also included: (1) no fertilization (N_0P_0); (2) mineral fertilization (MF_{NP}), consisting of a blend of ammonium sulfate, urea, and triple superphosphate (TSP) at an N:P ratio of 10–2.1. In the pot trial, two additional control treatments were used: (3) fertilization with only mineral N (MF_N), using ammonium sulfate and urea, and (4) fertilization with only mineral P (MF_P), utilizing TSP.

2.1 Organo-mineral fertilizers production

Four different organo-mineral fertilizers (OMFs) were produced using organic materials collected from industries across Italy. The chosen organic materials were green compost based on pruning residues (GC), municipal solid waste compost (MSWC), peat (Peat), and manure-based vermicompost (VC). The organic materials organic C, heavy metals, degrees of stability, and other additional chemical

parameters are presented in Table 1. For more details about the characterization of the organic materials, see Sitzmann et al. (2023).

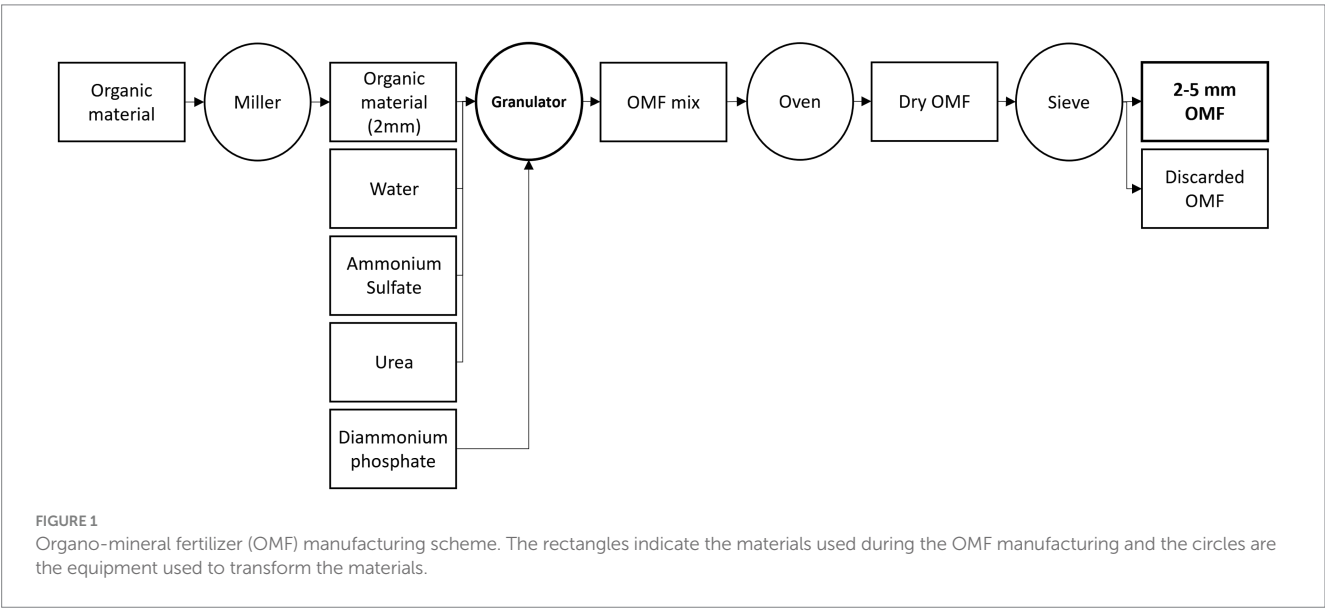
The OMFs were provided by SCAM S.p.a. (Modena, Italy). The OMFs were obtained by a granulation process (Figure 1). The organic material was mixed with mineral N – ammonium sulfate + urea - and water into a granulator. The water was added to facilitate a partial dissolution and impregnation of mineral N into the organic material. The dissolved urea acted as a binder between the organic material and the undissolved mineral N. Diammonium phosphate (DAP) granules is soon thereafter added to the granulator. DAP granules serve as the core for the OMF granules. No additional binders were added. After the granulation, the granules were dried in an oven during 24 h at 60°C to reduce their moisture content to approximately 4%. The OMFs were sieved to give a product of 2 to 5 mm in diameter. The organic material and mineral fertilizers mixture produced OMFs with a C_{org} -N-P₂O₅ of 7.5–10.5 (2.1% of total P).

2.2 Soil

The soil used for this study was collected from the unfertilized treatment, serving as the negative control, within the CRUCIAL long-term fertilization trial that was initiated in 2003 at the University of Copenhagen's experimental farm in Taastrup, Denmark (55°40'N, 12°16'E). This trial predominantly involved the cultivation of spring cereals. The soil is a low-P (Olsen-P: 5 mg kg⁻¹) sandy loam, characterized as a Luvisol according to the FAO classification, comprising 12.6% clay, 14.3% silt, and 69.8% sand. The soil pH was

TABLE 1 Characterization or organic materials used for OMF production.

Parameter	Unit	Peat	GC	MSWC	VC
C _{org}	mg kg ⁻¹ DM	166.9	193.8	212.7	198.1
Total Humic C	mg kg ⁻¹ DM	79.5	60.3	92.6	97.6
Humification degree	%	79.0	53.0	68.6	72.1
C/N		16.2	17.7	10.1	14.7
Alkyl C/O-Alkyl C		0.9	0.6	0.8	0.6
N _{total}	mg kg ⁻¹ DM	10.0	11.0	23.1	14.8
N _{org}	mg kg ⁻¹ DM	9.3	10.7	21.4	14.1
P	mg kg ⁻¹ DM	2.4	4.4	8.4	8.7
K	mg kg ⁻¹ DM	10.3	13.9	10.0	13.4
Mg	mg kg ⁻¹ DM	14.0	11.7	11.5	12.0
Ca	mg kg ⁻¹ DM	40.7	52.9	46.5	40.5
Fe	mg kg ⁻¹ DM	30.2	17.5	16.3	15.0
CEC	meq 100 g ⁻¹	57.5	64.6	49.4	61.8
EC	mS cm ⁻¹	1.5	1.1	2.2	1.5
pH		8.0	9.5	9.3	9.9



6.8 (measured at a 1:5 ratio with water), with a water holding capacity (WHC) of 31 g per 100 g of dry soil and a water-extractable P content of 1.78 mg kg⁻¹ in terms of dry soil weight. Further comprehensive insights into the CRUCIAL long-term trial and the specific soil employed in our investigation can be found in López-Rayó et al. (2016) and Lemming et al. (2019). After collection, the soil was air-dried and sieved to a size of 4 mm.

The soil was pre-fertilized with all the essential nutrients (except N and P), independently of its corresponding treatment with nutrient solutions. These were the nutrient application rates: 150 mg K, 50 mg Ca, 40 mg Mg, 1.2 mg Zn, 0.1 mg Mo, 3 mg Fe, 0.3 mg B, 2 mg Mn, 1.5 mg Cu, and 0.1 mg Co per kg⁻¹ soil to avoid any possible complementary nutrient deficiency. The application rates for macro and micronutrients were determined based on preliminary studies conducted with the soil used in this study, and based on Sica et al. (2023). Nitrogen and phosphorus were only provided by the fertilization treatments used in this study.

For both experiments in this study the soil was mixed with quartz sand (0.4 to 0.8 mm) in a proportion of 3 to 1. The sand was added to increase the water infiltration rate and facilitate root growth throughout the experiment. The soil and sand mixture are hereafter called 'soil.' Then, the soil was humidified to 40% of water holding capacity and pre-incubated for 7 days at room temperature aiming to reactivate the microbial community, as described by Oehl et al. (2004).

2.3 Incubation setup

For the incubation, 120 mL plastic containers, with a surface area of 18 cm², were filled with the equivalent of 100 g of dry soil packed to a bulk density of 1.00 g cm⁻³. Before being added to the plastic containers, each experimental unit was fertilized homogeneously with 600 mg N kg⁻¹ soil and 131 mg P kg⁻¹ soil and humidified to 50% of its water-holding capacity. The following treatments were used in this experiment: (1) no P, no N; (2) N and P mineral fertilizers (MF_{NP}); (3) peat OMF; (4) GC OMF; (5) MSWC OMF; (6) VC OMF.

Thereafter, containers were placed into a 750 mL glass jar with an airtight lid. Also, in the same jar two open traps were placed consisting of (1) 5 mL of 0.2 M sulfuric acid (H₂SO₄) in an open plastic container (26 mL airtight plastic container, Frisenette ApS, Knebel, Denmark) to trap ammonia (NH₃) that could potentially volatilize (Ndegwa et al., 2009); (2) 10 mL of 1 M sodium hydroxide (NaOH) to trap the CO₂ released by the soil. In addition, 10 mL of deionized water was placed into a plastic shot glass to maintain a high humidity inside the jar preventing desiccation of the fertilized soils. The jars were incubated in a dark room set at approximately 15°C for 10 days. Thereafter, fresh soil samples for inorganic analysis were collected avoiding OMF granules that may not have dissolved completely.

2.4 Plant trial setup

The pot trial was carried out with the same soil, bulk density, and pre-fertilization as the incubation. Soils were homogeneously fertilized with OMFs and controls to achieve 300 mg N kg⁻¹ soil and 65.5 mg P kg⁻¹ soil and added to 1-liter pots with the equivalent of 1 kg of dry soil packed to a bulk density of 1.00 g cm⁻³. The following treatments were used in this study: (1) no P, no N (N₀P₀); (2) no P, mineral N

(MF_N); (3) no N, mineral P (MF_P); (4) mineral N and P (MF_{NP}); (5) peat OMF; (6) GC OMF; (7) MSWC OMF; (8) VC OMF.

One day after fertilization, one pre-germinated seed of maize (*Zea mays*) cv. "Ambition" (Limagrain SE) was transplanted to each pot, ensuring that all seedlings were similar height. The pots were rotated randomly four times a week to mitigate the potential impact of temperature or light gradients within the greenhouse. Additionally, watering was conducted based on weight measurements to sustain the soil moisture content at 60% of the soil water holding capacity (WHC). Throughout the experiment, the daytime (14 h) temperatures fluctuated between a minimum of 13°C and a maximum of 28°C, while the nighttime (10 h) temperatures ranged from 6°C to 18°C.

2.5 Analyses

2.5.1 Incubation

For the incubation, all treatments were done in triplicates. After 10 days, the fresh soil was homogenized to analyze N-NH₄⁺, N-NO₃⁻, and Hedley sequential extraction fractionation.

For the ammonium and nitrate, the fresh soil was extracted with KCl at a ratio of 1:5, by shaking in an end-over-end shaker for 1 h. The samples were filtered using Whatman filter papers and analyzed for N-NH₄⁺ and N-NO₃⁻ by a flow-injection analyzer (FIAstar 500, FOSS, Denmark).

A simplified Hedley P fractionation scheme (Hedley et al., 1982) was used to evaluate the impact of OMFs on distinct soil inorganic P pools. This adapted fractionation technique consisted of four sequential extractions from an equivalent of 0.5 g of dry soil. The first extraction was with 30 mL of deionized water (at a 1:60 ratio) in the soil, followed by 16 h of extraction using an end-over-end shaker and subsequent centrifugation (15 min, 5,000 rpm). The supernatant was filtered, and the residual solids were employed for the subsequent extraction stage, with 30 mL of 0.5 M NaHCO₃. The same procedure — 30 mL of extractant, 16 h of shaking, 15 min of centrifugation at 5,000 rpm, and supernatant filtration — was repeated for the subsequent extractions employing 0.1 M NaOH and 1 M HCl. The ortho-P content in all extracted solutions was determined using the molybdenum blue method on a flow injection analyzer (FIAstar 5,000, Foss Analytical, Denmark).

N_{org} was calculated as the difference between total N and N-NH₄⁺ and N-NO₃⁻. Residual P was measured as the differences between the soil total P and the P extracted by water, bicarbonate, NaOH, and HCl fractions.

The microbial respiration was determined by adding a saturated barium chloride solution to the 1 M NaOH trap solutions and back titrating it with 1 M HCl, adapted from Alef (1995). The ammonia emissions were determined by measuring the NH₄⁺ contents in the H₂SO₄ traps.

2.5.2 Plant analyses

For the plant trial, all treatments were performed with four replicates. Plant shoots were harvested 33 days after transplanting and dried at 60°C for 48 h to determine the dry matter (DM). After that, shoots were placed in a container with three zirconia balls and milled by shaking for 5 min. A subsample was collected to determine the total N content in the shoots using an Elemental Analyzer (Vario macro cube, Elementar Analysensysteme GmbH, Germany).

The total P was determined as follows: around 100 ± 10 mg of the sample powder was precisely weighed into crucibles. The crucibles with the samples underwent incineration at 550°C for 1 h. Following incineration, the resulting ash was extracted using 50 mL of 0.5 M sulfuric acid, utilizing an end-over-end shaker overnight. The subsequent step involved filtering the extract through Whatman No. 5 ashless filter papers to eliminate any residual solid particles. Ortho-P content in the extract was quantified using ammonium molybdate by a flow-injection analyzer (FIAstar 500, FOSS, Denmark).

2.6 Calculations

The N and P uptake, in mg kg^{-1} soil, were calculated by multiplying the shoots dry matter, in g DM kg^{-1} soil, by the N and P content, respectively, in mg g^{-1} DM.

The nutrient index is calculated according to the Eq. (1):

$$\text{Nutrient index} = 100 \times \frac{\text{Nutrient content}}{\text{Nutrient critical content}} \quad (1)$$

These values indicate the plant's nutrition level, with 100 indicating an ideal situation, whereas a higher value indicates a luxurious consumption by the crop, and a value below 100 indicates a deficiency (Duru and Ducrocq, 1996).

For N, the critical content was calculated according to Eq. (2), a model obtained by Ziadi et al. (2008) for maize:

$$N \text{ content } (\%) = 3.4 \times DM^{-0.37} \quad (2)$$

Where N critical content is the N content at which below the plant will be under deficiency of N (in %) and DM is the plant dry matter (in g DM kg^{-1} soil).

The P critical content was calculated according to Eq. (3), based on the model developed by Gagnon et al. (2020) for maize:

$$P \text{ critical content } (\text{mg g}^{-1} \text{DM}) = 0.82 + N_{\text{content}} \times 0.097 \quad (3)$$

Where the P critical content is the P content at which below the plant will be under P deficiency and the N content is the N measured concentration in the maize shoots in mg g^{-1} .

The N or P use efficiency (NUE, PUE respectively) were calculated according to Eq. (4):

$$\begin{aligned} N \text{ or } P \text{ use efficiency } (NUE \text{ or } PUE, \%) \\ = \frac{N \text{ or } P \text{ uptake} - N \text{ or } P \text{ average uptake } N_0P_0}{N \text{ or } P \text{ fertilization rate}} \times 100 \quad (4) \end{aligned}$$

2.7 Statistical analyses

The pot trial was set up in a randomized complete block design, while the incubation had a complete randomized design. A General linear model (GLM) procedure was used to analyze variables considering

the treatment as a categorical variable. A *post hoc* Tukey's HSD test (<0.05) was performed when differences were found between treatments. Analyses were performed using the statistical software R, version 4.3.1.

3 Results and discussion

3.1 Organic matter stability effect on OMF coating (hypothesis I)

We hypothesized that the stability of the organic material used to coat the mineral fertilizer within the OMF would increase the granule's resistance to degradation in soil, thereby prolonging nutrient release. After 10 days of incubation, N-NH_4^+ remained higher than N-NO_3^- in all treatments. The N-NH_4^+ in fertilized treatments was 3 times higher than in the control N_0P_0 , while the N-NO_3^- content was only 1.5 to 2 times higher than in N_0P_0 . Fertilization increased the total mineral N content in soil by an average of 28 to 42%. No significant differences in the N_{org} content were found between treatments (Table 2).

Using the Alkyl C/O Alkyl C ratio (Baldock et al., 1997; Pizzanelli et al., 2023) and the humification degree (Zaccone et al., 2018) as indicators of C_{org} stability, it is shown that Peat would be more difficult to degrade in soil than GC, MSWC and VC. However, with no differences in N-NH_4^+ and N-NO_3^- concentrations it was not possible to confirm the hypothesis that the granule integrity will slow down the N release. Additionally, in the pot trial, among the OMFs and the control MF_{NP} , NNI ($>100\%$) demonstrated that the N dosage used was sufficient to sustain plant needs through the growth trial, not limiting growth (Table 3).

Adding P increased the different P fractions in the soil compared to the control N_0P_0 . Treatments MSWC and VC had on average half of the water-extractable P (Water P) than the control MF_{NP} , GC, and Peat (Table 2). Among the fertilizers, the bicarbonate-P pool was significantly higher for peat OMFs, compared to MSWC and VC. The NaOH P pool was significantly higher in VC than in GC and MSWC. Compared to the control N_0P_0 , the HCl P pool was higher only in Peat (31%), with intermediate values in MF_{NP} (29%), GC (12%), MSWC (17%), and VC (21%). The residual P was significantly lower in the mineral control MF_{NP} (-10%) than in MSWC, with intermediate values for the rest of the treatments.

Despite differences in P availability, the initial hypothesis can also be dismissed for P. Peat had the highest C_{org} stability among all samples (Table 1); however, water and bicarbonate P concentrations in soil – considered indicators of plant-available P (Hedley et al., 1982; Teng et al., 2020) – were higher for peat OMF compared to the other OMFs (Table 2). This higher plant-available P was consistent in both experiments, as peat OMF also had the highest PUE (Table 3) among the fertilizers in the plant trial experiment.

It has been reported that physical forms that promote compacted proximity between the organic and mineral components in OMFs, such as pellets and grains, exhibit a more gradual release of nutrients compared to mineral controls or powdered mixtures of these constituents (Rodrigues et al., 2021; Fachini et al., 2022). However, this observation was not corroborated by the present study. Despite the granulated peat-based OMF's physical form, there were no discernible differences in the availability of N and P in the soil and their uptake by maize compared to the mineral control MF_{NP} . Consequently, the differences in nutrient availability observed in the cases of GC, MSWC,

TABLE 2 N forms in soil, fractionated P extractions.

Treatment	N content in soil				Fractionated P in soil				
	N-NH4	N-NO3	Norg	N-Total	Water-P	Bicarbonate-P	NaOH-P	HCl-P	Residual-P
	mg N kg ⁻¹ soil				mg P kg ⁻¹ soil				
N ₀ P ₀	181.9 ± 16.4b	17.8 ± 1.8b	1000.3 ± 17.5	1200.0 ± 0.0	7.0 ± 0.4c	18.4 ± 0.6b	35.2 ± 1.4c	130.6 ± 7.1b	166.8 ± 9.1ab
MF _{NP}	606.7 ± 23.4a	34.6 ± 1.2a	925.4 ± 130.8	1566.7 ± 124.7	67.6 ± 4.6a	45.8 ± 5.6ab	96.1 ± 1.1ab	168.5 ± 4.1ab	111.1 ± 4.1b
Peat	568.9 ± 73.7ab	32.0 ± 2.4a	1099.1 ± 188.6	1700.0 ± 141.4	57.0 ± 9.0a	60.2 ± 15.4a	86.3 ± 10.7ab	170.7 ± 20.2a	114.9 ± 39.7ab
GC	610.3 ± 57.0a	27.1 ± 5.6ab	896.0 ± 163.8	1533.3 ± 124.7	59.3 ± 1.0a	36.0 ± 10.4ab	80.7 ± 8.0b	145.6 ± 13.2ab	167.4 ± 30.1ab
MSWC	607.8 ± 25.8a	33.1 ± 1.9a	892.4 ± 183.6	1533.3 ± 170.0	34.4 ± 6.0b	30.2 ± 3.0b	83.0 ± 0.3b	153.3 ± 1.4ab	188.2 ± 8.7a
VC	567.7 ± 32.1ab	32.0 ± 4.1a	1005.9 ± 133.0	1600.0 ± 100.0	30.6 ± 0.7b	28.0 ± 3.9b	103.3 ± 6.1a	158.0 ± 13.0ab	169.2 ± 19.3ab
Treatment P(F)	*	**	ns	ns	***	***	***	*	*

Letters on the right indicate differences between treatments.

and VC OMFs are more closely linked to interactions between the soil and the OMF rather than the organic matrix stability in the OMF.

3.2 Organic matter effect on nutrient organic immobilization (hypothesis II)

We also hypothesized that adding a less recalcitrant C_{org} source through the OMF in the soil might enhance nutrient immobilization by stimulating microbial activity. Soil CO₂ emission was used as a microbial activity indicator (Barnard et al., 2020). The fertilization was the primary driver for higher respiration compared to the control N₀P₀ (Figure 2), as it increased it by 34 to 43 times, indicating nitrogen deficiency in the turnover of native soil carbon. A modest yet consistent increase (11 to 23%) in CO₂ emissions was observed in OMFs compared to the mineral control MF_{NP}. This increase may have been caused by adding C_{org} to C-limited microorganisms in the soil (Traoré et al., 2016; Zheng et al., 2020). Although some authors point out that the fertilizer-soil interface can immobilize N (Moritsuka et al., 2004) and P (Sica et al., 2023) in organic forms, this has not been observed in this study.

On the other hand, in the case of P, it was observed that the GC, MSWC, and VC OMFs had higher residual P fractions in soil compared to the mineral control and the Peat OMF (Table 2). In the simplified Hedley extraction method, the residual P also accounts for organic P fractions in the soil (Alvarenga et al., 2017; Sica et al., 2023). Differences between the OMFs on the increase of residual P can be linked to the stability of the organic material and its resistance to microbial breakdown (Bernal et al., 1998; Grigatti et al., 2020). However, no statistical differences exist for the residual P between the control N₀P₀ and the biowaste OMFs, suggesting that adding low C_{org} quantities does not immobilize P into the organic fraction.

Nevertheless, it is possible to estimate that the addition of C_{org} alone will cause an almost neglectable nutrient immobilization compared to the other nutrients added. An indicative microbial nutrient immobilization can be calculated assuming that 20% of the respired C-CO₂ from the OMFs treatments was incorporated into the microbial biomass C (Holland and Coleman, 1987; Sistla et al., 2012) and a stoichiometric relation for soil microbial biomass C:N:P of 60:7:1 (Cleveland and Liptzin, 2007). During the incubation, the soil respiration was between 4 to 8 mg C-CO₂ kg⁻¹ soil higher in the OMFs treatments than in the mineral control MF_{NP} (Figure 2). Therefore, adding C_{org} through the OMFs would immobilize only 0.3 to 0.6 mg N kg⁻¹ soil and 0.02 to 0.04 mg P kg⁻¹ soil into the microbial biomass. The differences in water- and bicarbonate-extractable P between GC, MSWC, and VC and the mineral control MF_{NP} ranged between 8 to 37 and 10 to 19 mg P kg⁻¹ soil, respectively. This indicates that differences in available P between low C_{org} biowaste OMFs and MF_{NP} and Peat OMF are related to chemical interactions between the organic material and the mineral fertilizer rather than microbial immobilization.

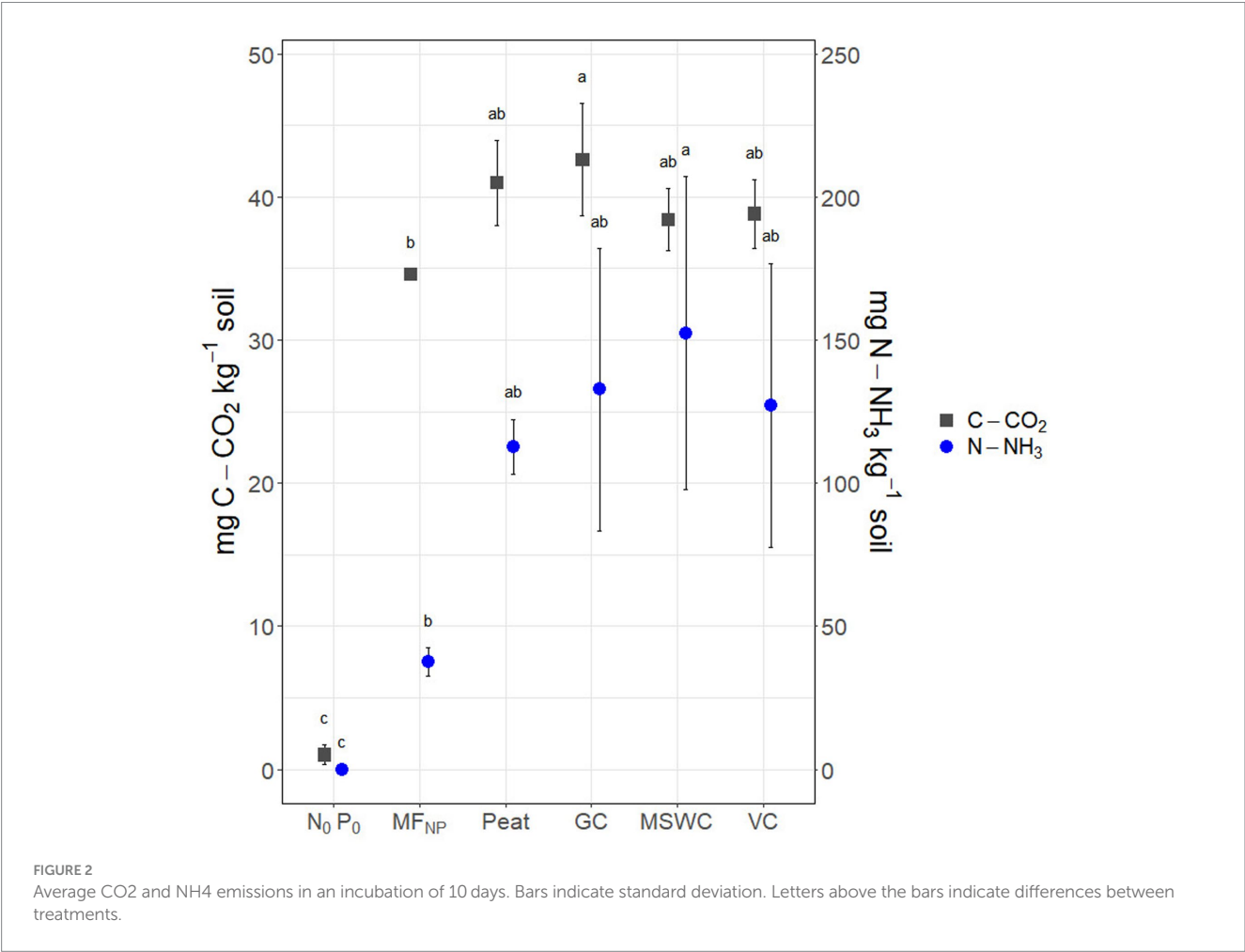
3.3 Organic matter effect on nutrient chemical immobilization

As the organic matrix stability does not significantly influence the granule integrity nor the microbial immobilization of nutrients, it is necessary to examine further chemical properties that may influence

TABLE 3 N and P nutrient index (NNI and PNI, respectively), and N and P use efficiency (NUE and PUE, respectively) average and standard deviation.

Treatment	NNI ^a		PNI ^b		NUE		PUE	
	%							
N ₀ P ₀	42.1 ± 2.4	c	59.1 ± 4.8	b				
MF _N	145.1 ± 3.3	b	44.7 ± 1.0	b	20.7 ± 1.7	c		
MF _P	34.4 ± 2.6	c	279.2 ± 46.5	a			10.3 ± 2.3	c
MF _{NP}	177.7 ± 10.6	ab	67.1 ± 4.4	b	61.5 ± 2.3	ab	23.2 ± 1.6	ab
Peat	200.7 ± 28.5	a	63.0 ± 5.1	b	73.5 ± 11.2	a	25.5 ± 5.0	a
GC	197.1 ± 6.6	a	54.7 ± 4.0	b	55.8 ± 2.9	b	15.8 ± 1.6	bc
MSWC	208.5 ± 21.5	a	67.3 ± 7.1	b	58.6 ± 7.7	ab	21.0 ± 4.7	ab
VC	192.4 ± 15.4	a	59.9 ± 4.5	b	54.1 ± 5.5	b	17.2 ± 2.5	abc
Treatment P(F)	<0.001		<0.001		<0.001		<0.001	

Letters on the right indicate differences between treatments.
*NNI calculated as N concentration/N critical *100. Where N critical is calculated as 3.4*Dry matter production −0.37.
^bPNI, calculated as P concentration/P critical *100. Where P critical is calculated as 0.82 ± N concentration *0.097.



nutrient availability. Unexpectedly no link was found between the organic matrix properties and N availability in soil. A slower N release by OMFs was reported in studies using urea combined with potash to coat a biosolid granule used as a P source (Antille et al., 2013a, 2014) and ammonium nitrate pelletized with peat (Richards et al., 1993). However, comparing those studies with our results is rather complex due to differences in organic materials, elaboration process, and

formulation. In a study by Florio et al. (2016), a peat OMF similar to the one used in this study, with 7.5% C_{org} produced with 20% N (12% ammoniacal N, 8% ureic N, and 1% N_{org}), reported a reduction of N release into the soil after measuring at different days less nitrate content in leachates but similar N-NH₄⁺ emissions and N plant uptake than a mineral control. Differences between that study and ours can be related to time differences (10 vs. 36 days of incubation).

A potential explanation for the lack of differences in soil N fractions was that the soil used in the incubation showed a low nitrification capacity for the duration of the experiment as most of the N was found as N-NH_4^+ . The low nitrification capacity could be caused by the high N fertilization used during the incubation. High N inputs have been associated with a lower soil quality that decreases the soil microbial functional diversity, inhibiting the N transformation in soil (Shen et al., 2010). Differences between fertilizers' release patterns can be observed in soils with a high nitrification capacity (Sahrawat, 2008). Shortly after fertilization, fertilizers with an earlier release of N will have a lower N-NH_4^+ concentration and a higher N-NO_3^- concentration than a fertilizer with a slow N release (Sahrawat, 2008).

On the other hand, a plausible explanation for the differences in P availability can be related to the higher alkalinity of the biowaste materials compared to Peat. Biowaste materials surpassed pH 9 (Table 1), which may reduce P availability to plants (Hartemink and Barrow, 2023). Additionally, a potential chemical interaction between OMF and soil that reduces plant available P is the formation of bonds between Ca and phosphate (Lindsay and Stephenson, 1959; Penn and Camberato, 2019). Peat and VC had a similar Ca content, while GC and MSWC have a slightly higher Ca content (Table 1); however, the higher pH in GC, MSWC, and VC may have stimulated the formation of Ca-phosphates bonds compared to Peat (Devau et al., 2011). Although no differences in N soil fractions were found, the acid–base properties of the organic material can limit the plant available P fractions in soil.

3.4 Organic material acid–base properties influence N losses (hypothesis III)

We hypothesized that the organic matrix of an OMF can influence ammonia volatilization, reducing or increasing it. The average ammonia volatilization in the soil after 10 days was 3 to 4 times higher in the OMF treatments than in the mineral control MF_{NP} (Figure 2). It is worth noting that for NH_3 soil emissions, the standard deviation in the OMFs was considerably higher (2 to 10 times higher) than in the MF_{NP} .

Our findings of OMFs increasing the ammonia volatilization are similar to the results from Florio et al. (2016), who found that OMFs with low C_{org} quantity made with peat as an organic matrix tend to increase the ammonia volatilization compared to a mineral control. Florio et al. (2016) hypothesized that the increase in ammonia volatilization was caused by a rising pH in the soil surrounding the OMF granules caused by the hydrolyzation of labile N_{org} by soil urease enzymes. The N_{org} values of the organic materials were correlated to the ammonia emissions since the highest ammonia values were found in MSWC, which was the organic material with the higher N_{org} content. At the same time, Peat had the lowest N_{org} content and the lower ammonia volatilization among OMFs. It is unclear if OMFs with less than 0.5% of total N_{org} will cause a discernible pH increase in the fertilizer hotspot.

In addition, the pH of the organic material itself could cause higher ammonia volatilization. It is well known that a high pH in the soil area where the fertilizer is present can increase the N losses through ammonia volatilization (Fenn and Hossner, 1985; Fan and Mackenzie, 1993); shifting the ammonium–ammonia equilibria toward the gaseous form (Körner et al., 2001). All organic materials used in this experiment were alkaline, but peat had one to two points less pH than other organic materials (Table 1). The organic material alkalinity explains a trend of lower volatilization in Peat than in the rest of the

OMFs. Further, the organic materials used in this experiment would provide more than 1% of CaO to 7.5% C_{org} OMFs, which may cause a localized liming effect (Maguire et al., 2006; de Campos et al., 2022), increasing the NH_3 volatilization. On this base, an acidification process of the organic matrix can be suggested (Mari et al., 2005; Turan, 2008; Fangueiro et al., 2015) as adding small quantities of organic materials to the mineral fertilizer promotes ammonia volatilization.

3.5 OMF effect on maize nutrient use efficiency

The sum of the hypothesized interactions between the organic matrix and the mineral fertilizers in the OMF and soil can cause an apparent slow release of nutrients by the fertilizers. Therefore, we tested if nutrients differences will cause an effect on early growth of maize plants. Differences in yield and nutrient uptake were linked to differences in plant available P in the incubation experiment. Maize dry matter ranged between 1.2 and 7.9 g (Figure 3A). Fertilization

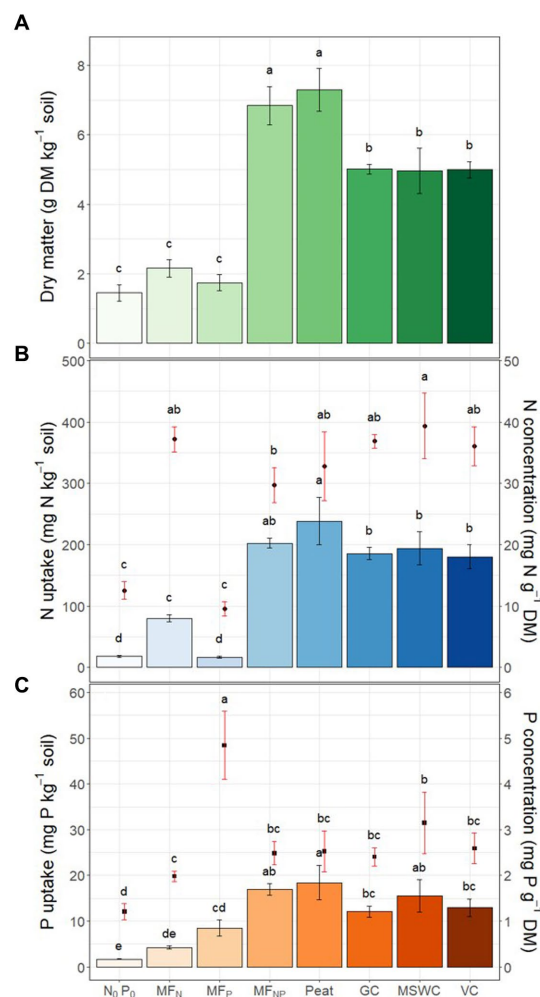


FIGURE 3
OMF influence in crop: (A) Above ground dry matter production; (B) N concentration in tissues (as points) and N uptake (as bars) by maize shoots; (C) P concentration in tissues (as points) and P uptake (as bars) by maize shoots. Letters above the standard deviation bars indicate differences between treatments.

with only N (MF_N) or P (MF_P) did not increase the yield compared to the control N_0P_0 , suggesting a co-limitation of nutrients in the soil used in this experiment. The lower maize yield in N_0P_0 , MF_N , and MF_P was expected due to previous reports of low soil N and P availability (Gómez-Muñoz et al., 2018; Lemming et al., 2019). For the treatments receiving combined mineral N and P, the yield increased 3 to 5 times compared to N_0P_0 , with Peat showing a similar yield to the mineral control MF_{NP} . However, all three biowaste materials OMFs had significantly lower DM compared to peat OMF and MF_{NP} .

The addition of N and P also increased the N and P contents in the shoots (Figure 3B). The addition of only mineral P did not influence the plant N concentration. However, when mineral P was added in MF_P , MF_{NP} , Peat, GC, MSWC, and VC, the P concentration was 2 to 4 times higher than in control N_0P_0 , being higher in MF_P and with no differences between treatments with combined mineral N and P (Figure 3C). Using the concentrations to calculate the N and P nutrient indexes, it was observed that after one month, the limiting nutrient for plant growth was P, except for MF_P , which was strongly limited by N, whereas the N reserves in shoots surpassed 1.5 to 2 times the minimum content required for its plant growth, except for the control N_0P_0 and MF_P (Table 3).

The N uptake by shoots followed a similar trend as biomass production. Adding combined N and P (MF_{NP} and OMFs) increased N uptake 10 to 13 times compared to the control N_0P_0 (Figure 3B). Compared to other OMFs, Peat significantly increased N uptake by 23 to 32%. The P uptake was significantly higher in Peat than in GC and VC by 53 and 42%, respectively. The nutrient uptake reflected the efficiency of the different fertilizers, with higher NUE and PUE found for Peat and the lowest NUE and PUE efficiency in GC and VC (Table 3).

Treatments with both mineral N and P (MF_{NP} , Peat, GC, MSWC, and VC) had the same content of mineral nutrients; however, the organic matrix could provide additional N and P to the OMFs (Rady, 2012; Antille et al., 2013a,b; Anetor and Omuetti, 2014). However, with a content of 7.5% C_{org} in OMFs, the N_{org} in the organic matrix would represent less than 0.5% of the total N in fertilizer while the P addition is practically neglectable and does not influence P uptake. Thus, as N was not limiting plant growth in this study, the additional N_{org} may not have affected the plant growth.

In soils receiving N and P fertilization, the nutrient index values revealed that P (PNI < 70%) rather than N (NNI > 170%) acted as a growth-limiting factor. Consequently, plant growth and N uptake differences could be attributed to variations in P release or immobilization (Hertzberger et al., 2020). Also, although OMF had higher N losses through ammonia volatilization, it did not impact the N uptake in 30-day-old maize plants. The biowaste organic matrix effect on P availability in 10 days caused plant differences after 30 days, indicating that crops require longer periods of time to recover this immobilized P.

4 Conclusion

The results obtained in this study show that even small quantities of organic material with high pH should not be used in OMFs containing mineral N. It is necessary to reduce N losses in biowaste OMFs before using them as effective peat OMF replacements. In addition, biowaste-based OMFs used in this experiment are not recommendable for short-term growing crops

because of a lower initial P availability that reduces the P use efficiency. These biowaste OMFs require to be tested in long-term trials to determine if the unavailable P can be taken up by plants over extended periods.

The main differences between organo-mineral fertilizer (OMF) treatments are related to whether the organic material in OMFs originated from geogenic sources or from biowastes that underwent a composting process. However, our hypotheses of the organic carbon stability influencing the granule integrity and the microbial immobilization were discarded. The main factors influencing the P availability in soil are related to the Ca and pH of the organic matrixes. Our third hypothesis was confirmed as the pH of the organic matrix from the OMFs increased the ammonia volatilization. The organic matrix influence on the mineral fertilizers remained after 30 days, as the initial P immobilization correlated to a lower P use efficiency by early maize plants. However, N losses may not have affected the fertilizer use efficiency, as for all OMFs, plants were not limited by N uptake as indicated by the Nitrogen Nutrient Index.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

TS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. PS: Conceptualization, Investigation, Methodology, Writing – review & editing. CG: Funding acquisition, Writing – review & editing. JM: Conceptualization, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement no. 860127.

Acknowledgments

The authors appreciate the collaboration of Stefano Tagliavini from SCAM S.p.A. for manufacturing the organo-mineral fertilizers used in this experiment. The authors thank Laura Zavattaro for her help in correcting the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

EDITED BY

Tom Misselbrook,
Rothamsted Research, United Kingdom

REVIEWED BY

Mesfin Tsegaye Gebremikael,
Ghent University, Belgium
Ana I. M. Natalio,
Harper Adams University, United Kingdom

*CORRESPONDENCE

Christina van Midden
✉ christina.van-midden@cranfield.ac.uk

RECEIVED 15 December 2023

ACCEPTED 04 March 2024

PUBLISHED 22 March 2024

CITATION

van Midden C, Harris J, Shaw L, Sizmur T,
Morgan H and Pawlett M (2024)
Immobilisation of anaerobic digestate
supplied nitrogen into soil microbial biomass
is dependent on lability of high organic
carbon materials additives.
Front. Sustain. Food Syst. 8:1356469.
doi: 10.3389/fsufs.2024.1356469

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Immobilisation of anaerobic digestate supplied nitrogen into soil microbial biomass is dependent on lability of high organic carbon materials additives

Christina van Midden^{1*}, Jim Harris¹, Liz Shaw², Tom Sizmur²,
Hayden Morgan³ and Mark Pawlett¹

¹School of Water, Energy, and Environment, Cranfield University, Bedford, United Kingdom,

²Department of Geography and Environmental Science, University of Reading, Reading, United Kingdom, ³Bioprocess Department, Future Biogas Ltd., Guildford, United Kingdom

Anaerobic digestate is a nutrient rich slurry by-product derived from biogas production, often used as a fertiliser due to its high nitrogen content. However, nitrogen losses from its application can lead to environmental pollution. In a laboratory experiment, the addition of high organic carbon materials to digestate-amended soil as a potential means to stimulate microbial immobilisation of digestate supplied nitrogen was investigated. Soil was incubated in pots for 5 months with digestate (equivalent to 250 kgN ha⁻¹). The impact of adding carbon into the digestate (equivalent to 540 kgC ha⁻¹) as either glycerol, straw, woodchip, or biochar on soil microbial and chemical parameters was quantified. Glycerol amended soils had significantly higher microbial biomass compared to digestate alone during the first month and at 30 days after application had a 4x higher on average microbial N. The digestate + straw treatment resulted in a 2.5x significantly greater nitrogen immobilisation compared to digestate alone after 3 months of incubation. The digestate + woodchip had a 2x higher mean microbial N after 5 months, whilst the biochar amendment did not stimulate significant nitrogen immobilisation at any time. These results suggest that mixing a labile to moderately labile organic carbon amendment, such as straw, with digestate has the greatest potential to reduce nitrogen losses following digestate application through microbial immobilisation.

KEYWORDS

biogas residue, nitrogen immobilisation, microbial community, glycerol, straw, woodchip, biochar

1 Introduction

Anaerobic digestate is a nutrient-dense slurry generated from biogas production. The process to create biogas requires the breakdown of a biodegradable feedstock such as animal waste, purpose grown energy crops, sewage waste, and the organic fraction of household, municipal and industrial wastes, in the absence of oxygen. During anaerobic digestion between 20% and 95% of the carbon in the feedstock is converted into methane and carbon dioxide (Möller, 2015) and collected as biogas. As the organic matter is digested, between 15% and

82% of the organic nitrogen bound within is mineralised into ammonium (Bareha et al., 2018). As this form of nitrogen is readily available for plants to utilise, digestate can be applied to land as a fertiliser. However, due to its high liquid content it is expensive for the biogas industry to store and transport the digestate (Al-Seadi and Lukehurst, 2012). A solution to reduce costs is to separate the digestate into its fibrous (solid) and liquid fractions, which reduces the volume of the liquid by 20%–30% (Lyons et al., 2021).

The liquid fraction of the separated digestate has been extensively studied for its use as a fertiliser and been found to produce yields comparable to synthetic nitrogen (Šimon et al., 2015; Riva et al., 2016; Walsh et al., 2018; Barzee et al., 2019). However, sustainable use of digestate as a fertiliser on agricultural land is dependent on good management practises. On average, over 80% of the nitrogen content of the liquid fraction is in the form of ammonium and nitrate (Czekala, 2022), which are the N forms most susceptible to loss processes by volatilisation, denitrification, nitrification and leaching. Therefore, land spreading digestate can result in groundwater and atmospheric pollution (Nkoa, 2014). These nitrogen losses from the soil reduce the nitrogen use efficiency of digestate fertilisers. Therefore, methods need to be developed to keep nitrogen supplied by the liquid digestate in the soil and available for crop uptake, whilst minimising losses and detrimental impacts on the environment.

Ammonia volatilisation losses represent a key nitrogen loss pathway when applying anaerobic digestate with 35%–60% of total nitrogen applied lost (Tiworthy et al., 2015). However, these losses are reduced by 40%–50% using precision slurry spreading techniques such as band spreading or injecting digestate directly into the soil compared to the traditional practise of broadcast spreading (Nicholson et al., 2018). Similarly adding sulphuric acid into digestate to lower its pH can effectively reduce ammonia volatilisation with reductions ranging from 45% (Wagner et al., 2021) to 95% (Sánchez-Rodríguez et al., 2018) when compared to non-acidified digestate. Once in the soil, the ammonium nitrogen from digestate is converted by nitrifying and denitrifying microorganisms, resulting in nitrogen losses before plant uptake as nitrate leaching and N₂O emissions. Research is ongoing on the applicability of using nitrification inhibitors, which are chemical compounds added to a fertiliser to delay the conversion of ammonium into nitrate and therefore reduce these losses. Huf and Olf (2020) and Giacometti et al. (2020) observed that the addition of nitrification inhibitors to digestate reduced N₂O and leaching losses, respectively, within a month of application. Hegewald et al. (2021) observed lower annual N₂O emissions by 36% over a three-year period. However, the cost of using nitrification inhibitors can be significant because factors such as climate, soil type and crop influence their effectiveness (Macleod et al., 2015; Drame et al., 2023). Furthermore, these nitrification inhibitors are agrochemicals which cannot be used in organic farming systems. As such, it is necessary to investigate alternative methods of reducing nitrogen losses from the soil.

Soil microorganisms can immobilise nitrogen into their biomass, as they use nitrogen to build proteins, nucleic acids and other cellular components. This nitrogen forms part of the necromass when the microbes die, which is subsequently remineralised when the necromass is primed by plant root exudates (Meier et al., 2017). This provides plants with a source of nitrogen when they need it as opposed to farmers applying synthetic nitrogen, which can result in a mismatch between time of input and plant demand. Most soil microorganisms

are heterotrophic and require an external source of organic carbon as a precursor to synthesising their own molecules. Digestate has a low total carbon content, typically between 0.43% and 3.4% (Risberg et al., 2017). The nutrient flush following digestate application causes temporary increases in microbial activity and abundance that subside within 24 h of application (Alburquerque et al., 2012; Iocoli et al., 2019) and disappear within a few weeks (Walsh et al., 2012; Johansen et al., 2013; Mortola et al., 2019; Gebremikael et al., 2020; Ren et al., 2020; Różyło and Bohacz, 2020). When digestate is separated into its solid and liquid fractions and applied separately to land, the organic carbon richer solid fraction increases microbial growth and immobilised nitrogen into microbial biomass, which is not seen when liquid digestate is applied (de la Fuente et al., 2013). This observation indicates that to stimulate microbial immobilisation of nitrogen in liquid digestate, more carbon is needed to satisfy microbial stoichiometry.

Organic materials high in carbon but low in nitrogen are known to immobilise nitrogen into soil microorganisms; as the microbes utilise the bioavailable carbon, they simultaneously use the material supplied nitrogen to meet their own, lower, carbon to nitrogen (C:N ratio) requirements (Robertson and Groffman, 2007). However, C:N ratio alone does not determine the rate of microbial growth, as the carbon in material can be of varying accessibility to microbes. For example, carbon that forms a simple monomer can be readily taken up through diffusion or active transport into microbial cells (da Silva et al., 2009; Dobson et al., 2012). More complex carbon structures include plant polymers such as hemicellulose, cellulose and lignin. To break down these materials into assimilable molecules, microbes require a variety of externally excreted enzymes, with residues containing a high lignin content predominately decomposed by enzymes secreted by fungi and bacterial cells that form multicellular assemblages such as *Actinomycetes* (Mekonnen, 2021).

This research aimed to investigate the effects of adding different sources of organic carbon to liquid digestate on soil microbial biomass, microbial nitrogen immobilisation and microbial community dynamics. The objective was to determine which type of organic material, in terms of carbon accessibility, was most effective at inducing nitrogen immobilisation. It was hypothesised that adding organic carbon additives to liquid digestate would stimulate microbial immobilisation of nitrogen, and that the magnitude and the timing of the effect would be influenced by the accessibility of the carbon substrates to soil microorganisms. As the additives vary in structural complexity, they are utilised by different microbial groups as a carbon source for growth and energy, it was predicted that the microbial community would shift to one with a higher fungi-to-bacteria ratio with the addition of complex carbon materials into the digestate. A pot experiment was established to test these hypotheses in the absence of confounding environmental variables, such as temperature.

2 Materials and methods

2.1 Soil, digestate and high organic carbon materials

A sandy loam topsoil (69% sand, 20% silt, 11% clay) bought from Bourne Amenity Ltd., was used for the study. Prior to the experiment the soil was passed through a 2 mm sieve to remove any stones and

large debris and thoroughly mixed to homogenise. Liquid anaerobic digestate was supplied by Future Biogas Ltd. from a biogas plant, managed to BSI PAS110 standards, using a mixed feedstock of 85 tonne maize silage, 7.5 tonne cow manure, and 18 tonne chicken manure. The plant is mesophilic, operating at 43°C with a retention time of 98 days. The liquid fraction was mechanically obtained from the whole digestate after screw press separation. The biochar applied in the experiment (CreChar™, supplied by Carbogenics Ltd) was produced from office waste in a kiln run at 700–800°C for 60 min. Wheat straw was obtained from a farmer (Bedfordshire United Kingdom) and woodchips from the Milton Keynes council parks department. Straw and woodchips were air-dried and chopped into smaller pieces (1–2 cm) to fit into the pots. Glycerol was bought from Sigma Aldrich. Details of material properties are in [Table 1](#).

2.2 Soil incubations

The incubation experiment was carried out in the dark at 20°C ± 4°C for 5 months. 250 g (dry weight basis) soil was added to 330 mL capacity PVC containers (top diameter 8 cm, bottom diameter 5 cm, height 12 cm) and pots were gently tapped on the worktop to ensure soil settled to a bulk density of 1 g cm³. Before the start of the experiment the soils were adjusted to 40% water holding capacity and pre-incubated at 20°C ± 4°C in the dark under aerobic conditions for a week, to allow soil microbial activity to recover after being sieved. Water holding capacity was determined using a saturate and drain method modified from [Harding and Ross \(1964\)](#). 50 g of soil was added to a stoppered funnel and saturated with 100 mL of deionised water for 30 min. The stopper was then removed, and the water drained for 30 min. The volume of water retained in the soil was

combined with the known moisture content of the soil to calculate the water holding capacity.

The experiment consisted of six treatments, arranged in a randomised block design with four replications: (1) liquid digestate control (LD); (2) liquid digestate with glycerol (LD-G); (3) liquid digestate with straw (LD-S); (4) liquid digestate with woodchip (LD-W); (5) liquid digestate with biochar (LD-B); and (6) unfertilised control (CONT). Sufficient sets were set-up to allow for destructive sampling for soil biochemical analysis on four occasions: 3 h after application, then 30, 90 and 150 days after application. This gave a total of 96 experimental units (6 treatments × 4 replicates × 4 sampling times).

Digestate was applied at 23 mL per pot, a rate equivalent to 250 kg-N ha⁻¹, which supplied 0.59 mg-N g⁻¹ dry soil. This rate of nitrogen was selected as it is the maximum amount farmers are allowed to apply in a 12 month period from organic sources in areas designated as Nitrate Vulnerable Zones under UK law ([DEFRA and EA, 2018](#)). Prior to application, the digestate was mixed with additives at a rate of 12 kg-C m³ of digestate (equivalent to 540 kg-C ha⁻¹), resulting in a material with a C:N ratio of approximately 5:1. This equalled an addition to the pots of 0.7 mL glycerol and 0.68, 0.59, or 0.51 g of straw, woodchip, and biochar, respectively. The treatments were then mixed into the soil. A volume of water equal to the volume of digestate was added to the non-amended control pots and similarly mixed. The rationale for this amendment rate is given in the supporting information. The pots were loosely covered with lids to reduce moisture loss and weighed twice a week to check the moisture content and deionised water was added to maintain soils at 40% water holding capacity, which is optimal for microbial development ([Gulledge and Schimel, 1998](#)). At each sampling time, the pots were destructively sampled and the soil passed through a 2 mm sieve to

TABLE 1 The biophysiochemical properties of the materials used in this study.

Properties	Soil	Liquid digestate	Biochar	Woodchips	Straw	Glycerol
Dry matter (%)	90.4	5.2	97.7	86.2	93.1	-
Organic matter (%)	5.3	3.9	-	-	-	-
pH	8.1	8.3	11.3	-	-	-
Total Carbon (g/kg fw)	37.5	20.6	628	546	479	391.9
Nitrogen						
Total (g/kg)	2.85	6.3	5.22	11.2	6.0	-
Ammonium (mg/kg)	-	4,147	-	-	-	-
Nitrate (mg/kg)	-	<10	-	-	-	-
Phosphorous						
Total (mg/kg)		521	1.02	-	-	-
Available (mg/L)	49	-	-	-	-	-
Potassium						
Total (mg/kg)	-	4,275	21.5	-	-	-
Available (mg/L)	755	-	-	-	-	-
C:N ratio	12.95	3.27	120.39	48.97	79.85	-
Microbial biomass Carbon (mg-C/kg)	392	-	-	-	-	-
Microbial Biomass Nitrogen (mg-N/kg)	98	-	-	-	-	-

Data for the liquid digestate, biochar, glycerol and select soil properties (LOI, P, K) were provided by the suppliers.

break down the aggregates that had formed when mixing treatments into the soil, in order to homogenise the sample ready for analysis.

2.3 Microbial analyses

Microbial biomass carbon and nitrogen (microbial C and N respectively) were determined following the fumigation-extraction method (Vance et al., 1987). After extraction, the extracts were analysed on a Shimadzu TOC with a TN module. The microbial C and N were calculated using K_{EC} and K_{EN} values of 0.45 and 0.54, respectively (Brookes et al., 1985; Vance et al., 1987). PLFA profiles were determined using a modified method from Frostegård et al. (1991) by freeze-drying the soil after sieving. Lipids were extracted from 10 g freeze-dried soil using the Bligh and Dyer (1959) solvent ratio 1:2:0.8 v/v/v of chloroform, methanol, and a pH 4 citrate buffer, fractionated, and the phospholipids derivatised by mild alkaline methanolysis. The resultant fatty acid methyl esters were separated by gas chromatography (Agilent Technologies, Santa Clara, CA, United States) using a HP-5 (Agilent Technologies) capillary column (30 m length, 0.32 mm ID, 0.25 μ m film). The GC conditions were reported in Pawlett et al. (2013). Resultant peak areas were integrated using G2070 ChemStation (Agilent Technologies) for gas chromatography and calculated as relative abundance (mol %). Bacteria were identified by the PLFA bioindicators 14:00, 15:0i, 15:0ai, 15:00, 16:0i, 16:1 ω 7c, 16:00, 17:0i, cyc17:0, ai17:0, 17:0br, 17:1 ω 8c, 17:1 ω 8t, 17:1 ω 7, 18:00, 18:0 (10Me), 18:1 ω 13 and 20:00 (Frostegård and Bååth, 1996; White et al., 1996; Zelles, 1997, 1999; Bossio and Scow, 1998; Kourtev et al., 2002). Fungi were identified by the biomarker 18:2 ω 6,9 (Frostegård and Bååth, 1996). The fungi-to-bacteria ratio was calculated by dividing the mol % of the fungal biomarker (18:2 ω 6,9) by the summed mol % of bacterial fatty acids (Frostegård and Bååth, 1996).

2.4 Chemical analyses

Soil total nitrogen was determined by dry combustion according to the British Standard Institution (BS EN 13654-2:2001) and analysed using an elemental analyser (Elementar, Vario EL III). Available nitrogen as the sum of ammonia and total oxides of nitrogen was determined using the potassium chloride (KCl) extraction method (MAFF, 1986). 20 g of soil was eluted with 100 mL of 2 mol KCl solution and filtered, after which the extracts were analysed on an analytical segmented flow multi-chemistry analyser (Seal, AA3).

2.5 Statistics

Data were first tested for normality using Kolmogorov–Smirnov & Lilliefors test and homoscedasticity using Levene's Test, following this the available N, microbial C and N datasets were box-cox transformed. As the independent experimental variable of interest was treatment, a one-way ANOVA was used to determine treatment effects at each sampling time on the soil parameters, whilst time as factor was not included due to unequal variance in data between sampling times. Significant differences between treatments for available and total N, and fungi:bacteria (F:B) ratio were determined by Tukey's *post hoc* test.

The microbial C and N datasets still failed homoscedasticity so a one-way Welch ANOVA was used as it can tolerate unequal variance (Wilcox, 2003) and significant differences between treatments were determined by Games-Howell's *post hoc* test. Principle Component Analysis was run on the PLFA profile data for each timepoint, which was normalised by measuring each biomarker as the relative abundance (%mol) to all the biomarkers. The resultant factor scores for each timepoint were analysed using a one-way ANOVA. All differences were considered statistically significant if $p < 0.05$. All statistical analysis was carried out in Statistica version 14.

3 Results

3.1 Biological properties of the soil

The addition of digestate alone to the soil did not result in higher microbial biomass (C) than the soil only control treatment at any timepoint. At 3 h after application, the addition of glycerol to digestate resulted in a significantly ($p = 0.004$) higher microbial biomass (C) by 344% compared to the digestate only control (Figure 1A). At 90 days the digestate with either straw or woodchip had a higher microbial biomass than soil only, but not the digestate control. At 150 days, the ANOVA recorded a significant effect due to treatment ($p = 0.001$), but the *post hoc* test did not identify any significant differences between individual treatments.

Microbial N was not significantly ($p = 0.95$) affected by treatments at 3 h after application (Figure 1B). At 30 days the LD + biochar had a significantly ($p < 0.05$) lower concentration than soil only control, but not the digestate only control. At 90 days after application digestate applied with straw significantly ($p = 0.009$) immobilised 80 μ g-N g⁻¹ (dry weight soil), equivalent to 75 kg-N ha⁻¹ (see Supplementary material for calculation) compared to the digestate only control, a difference of 309%. After 90 days no further significant treatment effect was observed, however digestate with woodchip had a 2x higher on average microbial N content compared to digestate only treatment.

The first two principal components (PC) on each of the PLFA datasets accounted for $\geq 50\%$ of the total variation (Figure 2), with significant ($p < 0.05$) treatment effects on PC1 axis at every timepoint except 150 days after application. Fatty acid loadings (≥ 0.8 and ≤ 0.8) that contributed the most included 16:00, 16:1 ω 5, 17:0c, 18:2 ω 6,9 and 19:1 ω 6 for PC1 and 17:0br, 17:1 ω 8c and 18:1 ω 7t for PC2, for information on specific PLFAs for each PCA see Supplementary Table 1. At both 3 h and 30 days after application the microbial communities between the digestate and the soil only controls were distinctly separate (Figures 2A,B; $p = 0.01$) with a higher fungi:bacteria (F:B) ratio in the digestate control at day 30 (Table 2). At 30 days the addition of woodchips and biochar resulted in separate cluster with the soil only control, distinct from a group made of the glycerol additive and the digestate control (Figure 2B; $p < 0.001$), with a lower F:B ratio in the former treatments compared to the latter (Table 2), whilst the straw treatment lay non-distinctly between the two groups. At 90 days the woodchip amended grouped separately from the glycerol and straw amended digestate treatments (Figure 2C; $p = 0.02$), with a lower F:B ratio in the woodchip treatment compared to the straw and glycerol treatments (Table 2). By day 150 there were no distinct groupings between the digestate

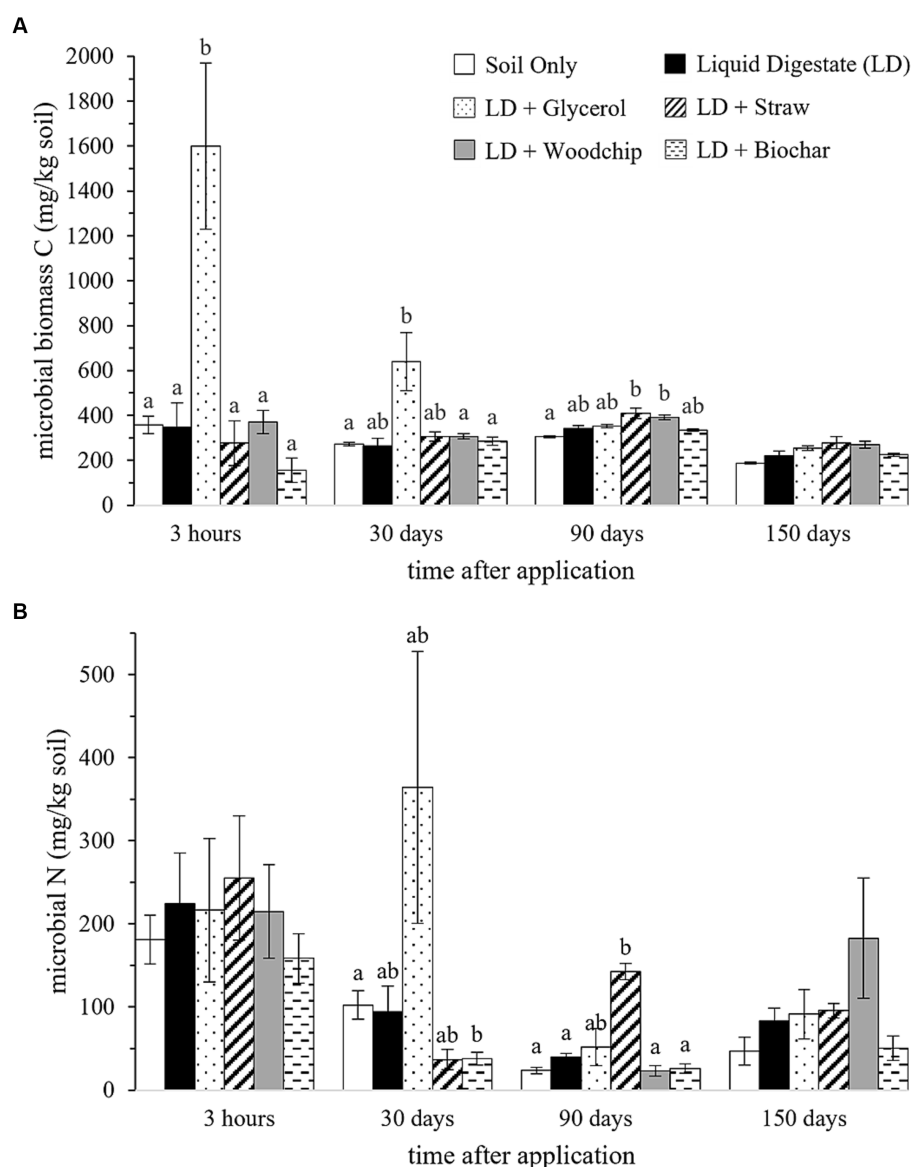


FIGURE 1

Changes in soil microbial biomass carbon (A) and microbial N (B). At each sampling time points denoting different lower-case letter have statistically different treatments effects according to Games-Howell's test at 5% probability on box-cox transformed data. Error bars denote the standard error from the mean, $n = 4$. In graph B, LD + woodchip at 30 days was not included into the analysis, due to only one replicate producing analysable data.

with and without additives (Figure 2D; $p = 0.06$) and no difference in the F:B ratios (Table 2).

3.2 Chemical properties of the soil

3h after treatment applications, the amount of total available nitrogen was significantly greater ($p = 0.02$) in the digestate (LD) control treatment compared to the soil only control and remained so for the experimental duration (Figure 3). The addition of additives into digestate had no effect on total available N compared to digestate control at all timepoints, however at day 90 the digestate with glycerol had a lower content compared to the digestate mixed with either woodchip or biochar.

The liquid digestate resulted in a significantly ($p \leq 0.004$) greater amount of total soil nitrogen compared to the soil only control from 30 days onwards (Table 3). Mixing additives into digestate did not result in any further significant differences to the total soil nitrogen content apart from at 90 days after application where there was a lower total nitrogen in the digestate with straw treatment compared to woodchip and biochar amended digestates.

4 Discussion

The addition of straw to digestate elevated microbial N in the soil by 2.5× compared to digestate alone, by an amount equivalent to 75 kg-N ha⁻¹, demonstrating that straw stimulated the immobilisation

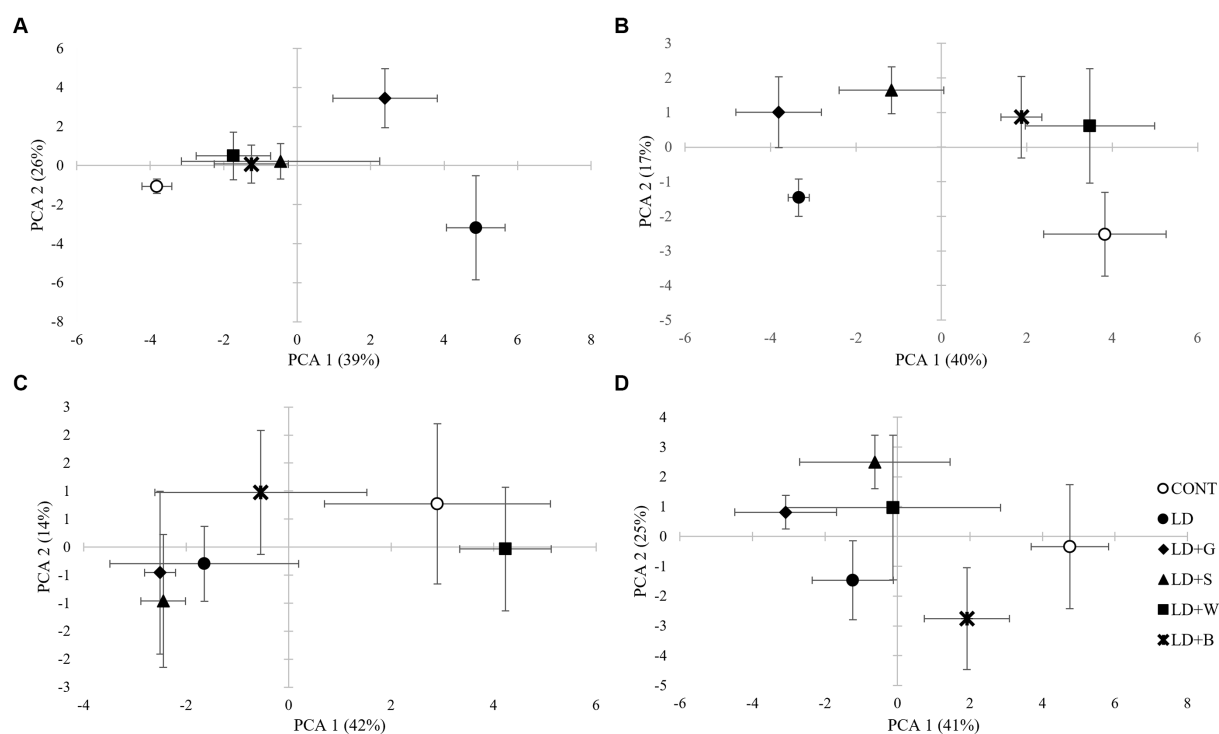


FIGURE 2

Principal component analysis on the PLFA profiles (mean \pm SE, $n = 4$) of the microbial community as affected by the treatments at each sampling time: **A** = 3 hrs, **B** = 30 days, **C** = 90 days and **D** = 150 days, after application. Cont, soil only control; LD, liquid digestate control; LD + G, liquid digestate + glycerol; LD + S, liquid digestate + straw; LD + W, liquid digestate + woodchips; LD + B, liquid digestate + biochar.

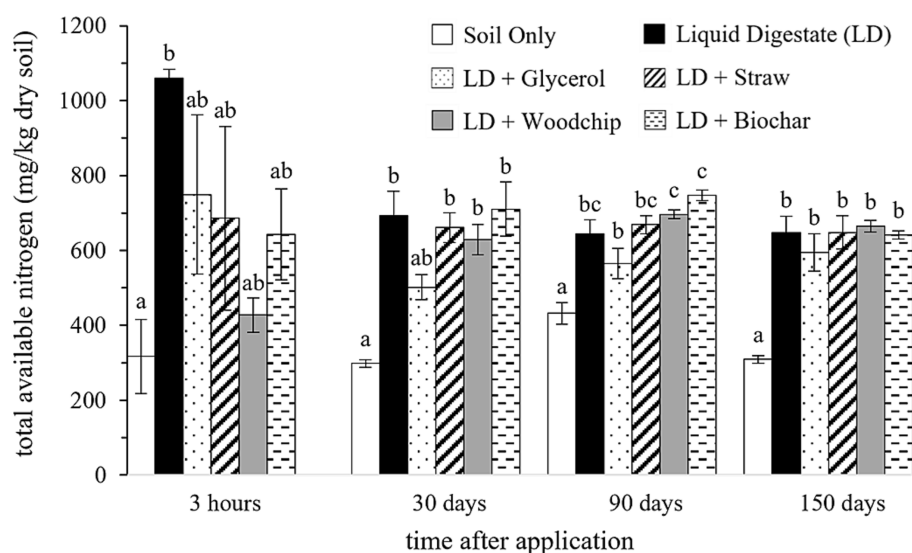


FIGURE 3

Changes in soil available nitrogen. At each sampling time point different lower-case letters between treatments have statistically different effects according to Tukey's test at 5% probability on box-cox transformed data. Error bars denote the standard error from the mean ($n = 4$).

of N into microorganisms. This immobilisation is in agreement with studies by [Cao et al. \(2018\)](#) and [Reichel et al. \(2018\)](#) who applied straw with synthetic nitrogen fertilisers, [Chaves et al. \(2005\)](#) who added straw with nitrogen rich crop residue, and [Wang et al. \(2019\)](#) who applied digestate with rice straw. However, in this study, microbial

growth cannot explain the uptake of N in biomass as the microbial biomass carbon under straw + digestate was not significantly greater than the digestate treatment. Microbes do not only uptake N as they grow and reproduce, but their C:N stoichiometry can change to adapt to nutrient limitations, or alleviation thereof ([Heuck et al., 2015](#); [Chen](#)

TABLE 2 Fungi:bacteria (F:B) ratio (mean \pm SE) in the soil after treatment manipulations.

Parameter	Days after application	Soil only control	Liquid digestate	LD + Glycerol	LD + Straw	LD + Woodchip	LD + Biochar
F:B ratio (%mol)	0	0.118 \pm 0.001	0.125 \pm 0.017	0.173 \pm 0.025	0.153 \pm 0.026	0.139 \pm 0.017	0.146 \pm 0.009
	30	0.084 \pm 0.005 ^a	0.202 \pm 0.007 ^c	0.172 \pm 0.019 ^c	0.154 \pm 0.016 ^{bc}	0.091 \pm 0.004 ^a	0.104 \pm 0.009 ^{ab}
	90	0.161 \pm 0.023 ^{ab}	0.215 \pm 0.020 ^{ab}	0.218 \pm 0.003 ^a	0.219 \pm 0.005 ^a	0.142 \pm 0.007 ^b	0.187 \pm 0.024 ^{ab}
	150	0.124 \pm 0.011	0.186 \pm 0.013	0.208 \pm 0.013	0.183 \pm 0.024	0.174 \pm 0.028	0.148 \pm 0.010

Mean ($n=4$) values between treatments in a row (sampling time) denoted with a different lower-case letter are statistically different according to Tukey's test at the 5% probability level.

TABLE 3 Total nitrogen content of soils (mean \pm SE) following treatment incorporations.

Parameter	Days after application	Soil only control	Liquid digestate	LD + Glycerol	LD + Straw	LD + Woodchip	LD + Biochar
Total N (mgN/kg ⁻¹)	0	2,600 \pm 115	2,966 \pm 33	2,933 \pm 120	2,833 \pm 120	2,933 \pm 120	3,000 \pm 100
	30	2,575 \pm 48 ^a	2,950 \pm 65 ^b	2,725 \pm 25 ^{ab}	2,925 \pm 25 ^b	2,850 \pm 87 ^{ab}	2,975 \pm 111 ^b
	90	2,800 \pm 41 ^a	3,125 \pm 103 ^b	3,050 \pm 96 ^{ab}	3,000 \pm 41 ^a	3,125 \pm 48 ^b	3,300 \pm 58 ^b
	150	2,575 \pm 25 ^a	3,025 \pm 48 ^b	3,050 \pm 29 ^b	2,875 \pm 25 ^b	3,075 \pm 75 ^b	3,050 \pm 29 ^b

Mean ($n=4$) values between treatments in a row (sampling time) denoted with a different lower-case letter are statistically different according to Tukey's test at the 5% probability level.

et al., 2019). In fungi, Khan and Joergensen (2019) suggested that N immobilisation can occur by storage of nitrogen-based compounds in vacuoles. Therefore, it is likely that a combination of both biomass incorporation and storage in fungal vacuoles caused the significant microbial N effect due to straw addition.

Contrary to the hypothesis, the addition of glycerol to digestate did not result in a significant microbial nitrogen immobilisation, despite the significantly greater growth in the first month from application. However, its addition did lead to a 4 \times higher on average microbial N at 30 days after application compared to digestate control. Significant results may be masked by experimental artefacts that can occur when comparing comparatively low amounts of extracted nitrogen between fumigated and non-fumigated soil relative to the large inputs of soluble N (Widmer et al., 1989), as from digestate. Time of sampling could also have been an issue, as de la Fuente et al. (2013) observed that digestates made from a feedstock mix of cattle slurry with 4% and 6% glycerol stimulated a peak nitrogen immobilisation within a week of application. Since we did not sample between 3 h and 30 days after application, it is possible that the peak of N immobilisation was missed.

The addition of woodchips to digestate had no significant effect on microbial growth or microbial N compared to digestate alone, although at 150 days microbial N was 2 \times higher on average than digestate alone. Similarly, Tahboub et al. (2007) observed no effect on nitrogen immobilisation when adding woodchips with synthetic fertiliser. However, Reichel et al. (2018) observed significant nitrogen immobilisation when adding sawdust with synthetic N, as did Chaves et al. (2005) when adding sawdust with nitrogen rich celery leaves. These significant effects could be due to the smaller particle size of sawdust (~1 mm) compared to woodchips (majority between 2.5–5 cm) used by Tahboub et al. (2007) and the 1–2 cm woodchips used in our study. This meant that our samples had less surface area per volume exposed for microbial colonisation which reduces decomposition rates (Idler et al., 2019). Furthermore, woodchips are mainly decomposed by fungi (Noll and Jirjis, 2012), yet the fungi to bacteria ratio in the woodchip amended soil of this study was never greater than digestate alone amended soil. This indicates that the

fungal community in the soil did not include those that could effectively utilise the woodchips as carbon source. This could also have been a result of sampling bias, as the soil was sieved prior to PLFA analysis, removing pieces of woodchip greater than 2 mm and excluding any fungi colonising those woodchips from the analysis.

Mixing biochar into digestate did not have any effect on microbial biomass or nitrogen immobilisation. This corroborates results from Martin et al. (2015) who also observed no effect of adding biochar with digestate on either microbial C or N. In biochar the carbon is formed into complex aromatic structures (Schmidt et al., 2000) that are extremely resistant to decomposition (Wang et al., 2016). Additionally, the highly porous structure of biochar absorbs dissolved organic carbon, reducing the amount available to microbes to utilise (Mukherjee et al., 2016). Yet, Holatko et al. (2021) observed increased microbial biomass C from digestate amended with biochar after 6 weeks, with a correlated decrease in soil nitrogen that could indicate nitrogen immobilisation had occurred. It is possible that the differing effects of biochar addition on nitrogen immobilisation between the current study and Holatko et al. (2021) may be due to differences in the amount and type of biochar used. Holatko et al. (2021) applied twice the concentration of biochar (0.4 kg per litre of digestate compared to 0.2 kg per litre) and used agricultural grain-waste biochar pyrolysed at 600–650°C (cf 700°C–800°C office waste biochar used here). Biochar feedstock and processing temperatures, of which there are numerous options (Lehmann et al., 2011; Wang et al., 2016), determine the size of a small biochar-associated labile carbon pool. It is possible that the biochar used by Holatko et al. (2021) had a greater concentration of labile carbon and therefore stimulated microbial growth.

The immobilisation of nitrogen observed when digestate was mixed with straw demonstrates that microorganisms can be used as a potential mechanism to reduce the nitrogen losses from anaerobic digestate application. However, the quantity of N immobilised should be reflected in a lower concentration of soil available nitrogen. Yet contrary to this, the greater microbial N under straw addition did not result in a lower soil available nitrogen. This could be due to the extent of change in microbial N being too small to make a noticeable

difference in the soil available N pool. There are several studies that supplied greater quantities of carbon than the 0.5 t-C ha^{-1} in this study. For example, 1.3 t-C ha^{-1} was applied by Cao et al. (2018) and 4.5 t-C ha^{-1} was applied by Reichel et al. (2018) as either straw or sawdust with synthetic nitrogen fertilisers, and 2.5 t-C ha^{-1} was applied by Chaves et al. (2005) who added straw and sawdust separately with nitrogen rich crop residue. All these studies recorded significant N immobilisation. de la Fuente et al. (2013) used digestates with a feedstock of 4% and 6% glycerol and saw N immobilisation, whereas our digestate mix contained 3% glycerol. However, for the amount of carbon added in this experiment ($1,400\text{ mg-C kg}^{-1}$), the changes in microbial N and soil available N under the digestate and glycerol did meet expectations. Working on the assumption that microbes use 1 unit of N per 8 units of carbon to satisfy stoichiometry for growth (Sinsabaugh et al., 2016), then the increase of $1,200\text{ mg-C kg}^{-1}$ in microbial biomass due to glycerol addition at 3 h after application would immobilise 155 mg-N kg^{-1} . The measured average reduction in available N due to glycerol addition compared to the digestate alone was higher than the estimate at 195 mg-N kg^{-1} , due to variability in sample measurements between replicates. It appears that the high volume of nitrogen added by the digestate, requires a larger N uptake by microbes than what occurred in this study to significantly reduce the soil available N pool. This indicates that adding more carbon than we did into the digestate is necessary to increase the magnitude of microbial growth.

Whilst our system was carbon limited, we were still able to identify trends in the influence of carbon source on its bioavailability and subsequent impact on the magnitude and timing of soil microbial N uptake. Glycerol resulted in the greatest and quickest growth that occurred in the first month with a microbial N higher on average by $4\times$ than digestate, followed by straw with a microbial N concentration greater by $2.5\times$ than digestate. In an experiment comparing glycerol, straw and grass as N immobilisers, Redmile-Gordon et al. (2014) observed that the addition of glycerol with nitrogen had a quicker and greater magnitude of immobilisation compared to the straw-N mix, attributable to the greater lability, and therefore bioavailability, of carbon in glycerol compared to straw. Soil incubation experiments by Reichel et al. (2018) and Chaves et al. (2005), who used both straw and sawdust as immobilisers of nitrogen-rich sources, observed that nitrogen immobilisation under sawdust was of lower magnitude and occurred later compared to straw. Similarly, our woodchip-digestate had a later and lower on-average microbial N peak compared to the straw addition. This is attributable to the higher lignin content in woody material, which slows decomposition rates and therefore carbon availability to microbes (Melillo et al., 1982; Lehmann et al., 1995; Rahn and Lillywhite, 2002). Biochar was the most recalcitrant material used in our study and its addition to digestate had no positive influence on microbial N, as observed by Manirakiza et al. (2019) when they mixed biochar into biosolids. Alotaibi and Schoenau (2016) mixed biochar with urea to a C:N ratio of 20:1, higher than our digestate + biochar mix, and measured a reduction in microbial biomass after 4 months compared to urea alone. In contrast, the microbial biomass under their glycerol-urea mix was no different to urea control, which corroborates our results, confirming that glycerol has a quick and short-term influence. As such the hypothesis that the lability of carbon in the organic material influences the time and magnitude of microbial N uptake can be accepted.

The phenotypic (PLFA) profile of the soil community changed with the application of digestate, with the soil only treatment resulting in a group distinct from the digestate treated soil. A month after application two distinct groups emerged: digestate alone and with glycerol and straw, and digestate with woodchip and biochar and the unamended soil. A higher ratio of fungi to bacteria was present in the former group. This is contrary to the hypothesis, which expected that the additives containing more labile carbon would increase the relative abundance of bacteria compared to fungi. Approximately a third of the fungi in digestate are yeasts and moulds, which are quick growing (Coelho et al., 2020). The labile carbon supplied by the digestate, glycerol and straw provided a carbon source for these quick growing fungi from both the digestate and the soil. Meanwhile biochar, being able to sorb carbon and nutrients (Mukherjee et al., 2016; Ding et al., 2020), may have reduced the availability of these compounds, which may be a reason that digestate with biochar did not result in a change in the community composition compared to the soil only control. The merging of groups after 3 months is consistent with decreases in abundance of quick growing microorganisms as they exhaust their labile carbon supply (Meidute et al., 2008), shifting the community to a composition similar to the control soil.

There are practical implications for adding materials into digestate. In this study chopped straw was used, which resulted in significant immobilisation, however this is difficult in terms of mixing it into the digestate to ensure a homogeneous distribution followed by difficulties spreading it evenly onto a field, particularly with band or injection spreading equipment and systems where the digestate is transported to the applicator unit by an umbilical system, where straw addition may increase the chances of blockages occurring. An alternative would be to apply digestate and straw separately. Adding digestate to straw on the soil surface is problematic, as this reduces the infiltration rate of the digestate, resulting in increased ammonia gaseous losses (Cao et al., 2018). Whilst there are applicators that inject digestate straight into the soil through a straw layer to reduce ammonia losses, this results in spatial separation of the straw on the soil surface and the digestate below the surface. Therefore, microorganisms in the soil do not have access to the straw and cannot acquire the carbon they need to immobilise nitrogen from the digestate, as evidenced by Aita et al. (2012) who observed increased decomposition rates when slurry and straw were incorporated into the soil as opposed to straw remaining on soil surface. This would mean that the addition of straw to digestate is a mixture that is limited for use on crops that combine fertilisation with seedbed preparation.

This study demonstrated that the use of a high organic carbon material additives can result in microbial growth and uptake of digestate supplied nitrogen. To meet its potential as a mechanism to reduce nitrogen losses from the application of anaerobic digestate, more carbon is required than was applied in this study, however we have demonstrated that materials high in carbon, of which the majority is in an easily degradable form (i.e., low in lignin or char content) have the greatest potential to do this. Under field conditions the nitrogen immobilisation and remineralisation may be slower than found in this study, due to fluctuating temperatures and variable rainfall (Sun et al., 2019). Potentially less nitrogen would be immobilised overall as leaching and volatilisation would remove some nitrogen from the soil alongside immobilisation. Therefore, our findings that high organic carbon materials immobilise digestate

supplied nitrogen need to be verified under field conditions. Should it prove possible to control digestate supplied nitrogen using this approach, then further work will be required to determine agronomic effects.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repository and accession number(s) can be found at: <https://doi.org/10.17862/cranfield.rd.24808605>.

Author contributions

CM: Conceptualization, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing, Formal analysis. JH: Conceptualization, Methodology, Supervision, Writing – review & editing. LS: Conceptualization, Methodology, Supervision, Writing – review & editing. TS: Conceptualization, Methodology, Supervision, Writing – review & editing. HM: Conceptualization, Funding acquisition, Methodology, Resources, Writing – review & editing. MP: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This research was funded by UKRI BBSRC FoodBioSystems Doctoral Training Partnership (DTP), grant number BB/T008776/1 and Future Biogas Ltd.

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Acknowledgments

The authors would like to thank Denise Cysneiros at Future Biogas Ltd. for her input to the project. We are very grateful for the help and support by the EAF technical team, in particular Richard Andrews, Nuannat Simmons, Paul Barton, Maria Biskupska, Tammy Cowley, and Dave Sharpe. We would like to thank Jan Mumme at Carbogenics for providing us with the biochar used in this study.

Conflict of interest

HM was employed by Future Biogas Ltd., from whom this study received funding. The funder had the following involvement with the study: provision of digestate used.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2024.1356469/full#supplementary-material>

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APPROVED BY
Frontiers Editorial Office,
Frontiers Media SA, Switzerland

*CORRESPONDENCE
Christina van Midden
✉ christina.van-midden@cranfield.ac.uk

RECEIVED 15 April 2024
ACCEPTED 16 April 2024
PUBLISHED 02 May 2024

CITATION
van Midden C, Harris J, Shaw L, Sizmur T,
Morgan H and Pawlett M (2024) Corrigendum:
Immobilisation of anaerobic digestate
supplied nitrogen into soil microbial biomass
is dependent on lability of high organic
carbon materials additives.
Front. Sustain. Food Syst. 8:1417696.
doi: 10.3389/fsufs.2024.1417696

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Corrigendum: Immobilisation of anaerobic digestate supplied nitrogen into soil microbial biomass is dependent on lability of high organic carbon materials additives

Christina van Midden^{1*}, Jim Harris¹, Liz Shaw², Tom Sizmur²,
Hayden Morgan³ and Mark Pawlett¹

¹School of Water, Energy, and Environment, Cranfield University, Bedford, United Kingdom,

²Department of Geography and Environmental Science, University of Reading, Reading, United Kingdom, ³Bioprocess Department, Future Biogas Ltd., Guildford, United Kingdom

KEYWORDS

biogas residue, nitrogen immobilisation, microbial community, glycerol, straw, woodchip, biochar

A corrigendum on

Immobilisation of anaerobic digestate supplied nitrogen into soil microbial biomass is dependent on lability of high organic carbon materials additives

by van Midden, C., Harris, J., Shaw, L., Sizmur, T., Morgan, H., and Pawlett, M. (2024). *Front. Sustain. Food Syst.* 8:1356469. doi: 10.3389/fsufs.2024.1356469

In the published article, there was an error in the Data Availability statement. The doi link to the dataset was incorrect. The correct Data Availability statement appears below.

“The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: <https://doi.org/10.17862/cranfield.rd.24808605>”.

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Sven Gjedde Sommer,
Aarhus University, Denmark

*CORRESPONDENCE

Peter Dörsch
✉ peter.doersch@nmbu.no

RECEIVED 14 January 2024

ACCEPTED 13 March 2024

PUBLISHED 26 March 2024

CITATION

Nyvold M and Dörsch P (2024) Complete
elimination of methane formation in stored
livestock manure using plasma technology.
Front. Sustain. Food Syst. 8:1370542.
doi: 10.3389/fsufs.2024.1370542

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Complete elimination of methane formation in stored livestock manure using plasma technology

Magnus Nyvold^{1,2} and Peter Dörsch^{1*}

¹Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, Ås, Norway, ²N2 Applied, Asker, Norway

Plasma-based nitrogen fixation has recently been shown to be applicable in the domain of manure management, as it has the ability to reduce ammoniacal nitrogen losses and increase the nitrogen content of organic wastes, with air and electricity as the only input. In addition, the plasma treatment confers antimicrobial properties, which we hypothesize to be transferable to methanogenic archaea and hence prevent methane formation during manure storage – a major contributor to global anthropogenic greenhouse gas emissions. In this work we compared the methane formation from cow manure to the methane formation in nitrogen enriched cow manure, kept in two outdoor storage tanks for 70–80 summer days over three consecutive years. In all instances, the methane formation was eliminated completely. To investigate the cause of inhibition, a controlled incubation experiment was conducted to show that neither the acidification nor the addition of nitrate or nitrite, alone or in combination, could explain the inhibition of methanogenesis and denitrification that occurred in plasma treated cow manure at moderate pH.

KEYWORDS

nitrogen fixation, plasma, methane emissions, sustainable livestock production, organic fertiliser

1 Introduction

The potent greenhouse gas methane (CH₄) has received recent attention for its short lifespan in the atmosphere (IEA, 2022). Compared to the 120-year half-life of carbon dioxide (CO₂), CH₄ has an atmospheric lifespan of only 12 years (Lynch et al., 2020). The combination of its potency and short lifespan results in a time-dependent global warming potential (GWP) where the GWP over a 100-year period (GWP100) will be 27 times higher than that of CO₂, whereas a 20-year time-horizon (GWP20) will result in a GWP of 81 CO₂ equivalents. Consequently, measures that significantly reduce CH₄ emissions will have an impact on the rate of global warming, particularly in the short term. The Intergovernmental Panel on Climate Change (IPCC, 2018) estimate that a 40–45% reduction of CH₄ emissions can limit the global temperature rise to 1.5°C above pre-industrial level if implemented by 2030. The resultant delay in global warming may thus leave sufficient time for the complex task of decarbonizing power production, transportation, and other fossil fuel-dependent industries, and increase the probability of limiting global warming to 1.5°C throughout the 21st century.

Of global methane emissions, close to one third stems from agriculture, whereof the livestock sector is the main contributor. Methane emissions from livestock are dominated by enteric fermentation in ruminants and are estimated to account for 70% of global

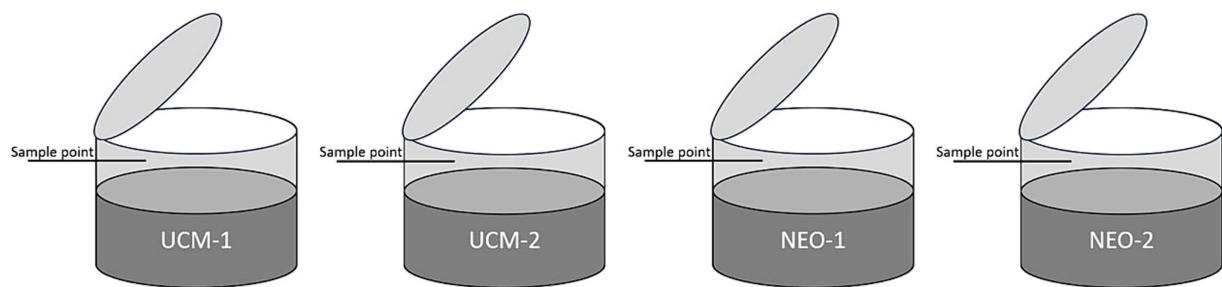


FIGURE 1

The four outdoor tanks had a retractable lid to ensure realistic exposure to the environment, except during the brief period of measurement. Samples were drawn through the sampling point with a syringe, transferred to a gas vial and the CH_4 concentration was measured on a gas chromatograph.

agricultural CH_4 emissions, followed by manure management (7%) (Kuylenstierna et al., 2021). However, there are regional differences in the distribution of sources: in livestock-intense regions, where liquid manure is stored for prolonged periods, such as California, CH_4 emissions from manure management equal those from enteric fermentation (CARB, 2020). Identifying and implementing measures that reduce CH_4 emissions from manure management is therefore central to the 1.5°C target, as these measures must counteract a growing global demand for milk and meat (OECD-FAO, 2022).

Anaerobic digestion of livestock manure (AD) has been presented as a sensible mitigatory measure, as it captures the CH_4 emitted from livestock manure and uses it to replace fossil gas. However, AD will often lead to increased methane production downstream (Balde et al., 2016; Rodhe et al., 2018) and exacerbated ammonia (NH_3) emissions (Holly et al., 2017). An alternate or complimentary approach is acidification of livestock manure, commonly with sulfuric acid (H_2SO_4), to reduce the slurry pH and hence the ammonia volatilization and methanogenic activity (Hou et al., 2017). Retaining the effect during storage and at field application does however lead to a sulphur dose that often exceeds crop needs and increases the risk of soil acidification and sulphate leaching (Lamers et al., 1998).

A novel approach to manure management, that can work alone or in conjunction with the above technologies, is plasma treatment. In this process, a strong electric field ionises air, leading to the formation of a reactive nitrogen gas (NO_x). The reactive gas is absorbed in livestock manure as nitrogen oxyanions (NO_x^-) resulting in a nitrogen enriched organic fertiliser (NEO), containing more plant available nitrogen at a lower pH, thus reducing NH_3 volatilisation (Ingels and Graves, 2015). Since crops require more nitrogen than sulfur, the nutrient balance is improved, which in turn reduces the risk of soil acidification. The treatment is energy intense but electricity-based and is therefore suitable for integration with intermittent renewable energy.

In addition to the electricity-based nitrogen production and reduced losses of NH_3 , the plasma treatment has been shown to have antimicrobial effects (Hiis et al., 2023). This is important, as microbial activity in NEO would lead to denitrification and the subsequent loss of the plasma-generated nitrate (NO_3^-) and nitrite (NO_2^-). In this work we hypothesise that the inhibitory effect is transferable to methanogenic archaea. We measured the impact on CH_4 emissions during the summer months over three

consecutive years, in an outdoor storage facility, to approximate a farm scenario. To elucidate the mechanism behind the inhibition of CH_4 emissions, we also conducted a controlled laboratory experiment investigating the effect of acidification (by H_2SO_4) and NO_2^- alone and in combination to imitate the effect of the plasma treatment.

2 Methodology

2.1 Farm-scenario experiment

Four outdoor monitoring tanks, each holding 2 m³ of slurry were used to estimate the CH_4 emissions from untreated cow manure (UCM), which served as the non-plasma treated control, and nitrogen enriched organic fertiliser (NEO). All tanks were equipped with a lid that remained open, except during the 40-min measurement period when the lid was closed and gas samples were drawn at 10-min intervals to estimate CH_4 emission. Figure 1 shows a schematic of the system. Continuous measurements of the pH and temperature in all tanks were conducted the first year.

The UCM originated from Skollenborg, Norway and was filtered with a screw press before being transferred to two of the tanks. The same filtered UCM was used as input material in the plasma process, which converts UCM to NEO. Figure 2 illustrates the plasma process and Table 1 shows the difference in chemical composition between the UCM and NEO (averaged over the three years). Each year, the tanks were drained before a new batch of UCM and NEO was transferred to the tanks, with approximately the same composition as the year prior.

2.2 Laboratory experiment

In the laboratory experiment, the UCM was compared to UCM acidified with sulfuric acid (H_2SO_4) to pH 6.3 and pH 5.0 ($\text{UCM}_{\text{pH}6.3}$ and $\text{UCM}_{\text{pH}5.0}$) and to NEO at pH 6.3 and pH 5.0 ($\text{NEO}_{\text{pH}6.3}$ and $\text{NEO}_{\text{pH}5.0}$). Each UCM treatment was set up in nine gas tight serum-vials, the NEO treatment in three. Conditions in the vials were made anoxic by washing the bottles (repeated cycles of evacuation and He-filling while stirring the slurries) to accelerate methanogenesis. The vials were incubated at 30°C for 180 h in a water bath connected with a multi-column GC (Model

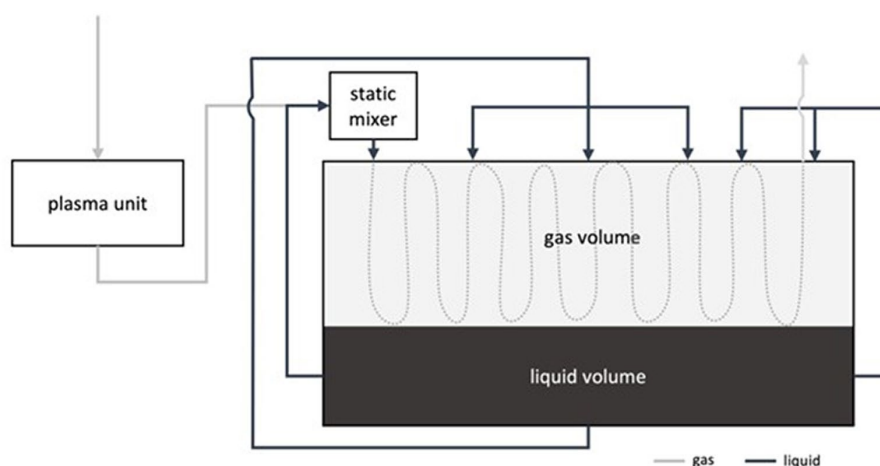


FIGURE 2

A schematic representation of the enrichment process. Air passes through the plasma generator, the resultant reactive nitrogen gas is subsequently absorbed in the organic substrate as NO_3^- and NO_2^- . The process is pH-controlled and produces material at a requested pH.

TABLE 1 The pH and nitrogen composition of the cow slurry before and after nitrogen enrichment.

Treatment	pH	[kgN m ⁻³]		
		NH_4^+	NO_3^-	NO_2^-
UCM	7.1	1.3	0	0
NEO	5.3	1.3	1.2	0.7

7890A, Agilent, Santa Clara, CA, USA) and a chemoluminescence NO analyser (Model 200 A, Advanced Pollution Instrumentation, San Diego, USA) via an autosampler and a peristaltic pump for repeated headspace analyses as described by Molstad et al. (2007). The methane concentrations in the headspace were measured six times a day.

Approximately halfway through the incubation, three vials from the UCM and UCM_{pH6.3} received a potassium nitrate (KNO_3) dose resulting in a concentration of 1,000 mg NO_3^- - N/L, and another three vials received a potassium nitrate (KNO_3) and potassium nitrite (KNO_2) dose resulting in a concentration of 1,000 mg NO_3^- - N/L and 750 mg NO_2^- - N/L, which corresponds to the nitrogen oxyanion concentration in NEO. No CH_4 formation was detected in the UCM_{pH5.0} vials. Therefore, no NO_3^- or NO_2^- was added to the UCM_{pH5.0} treatments. The setup aimed to determine whether pH, nitrate or nitrite, or a certain combination of the three, yielded a complete inhibition of methanogenesis or whether the inhibition is a result of particular properties of the plasma treatment.

3 Results and discussion

3.1 Farm-scenario experiment

Temperature readings suggested differences in microbial activity between the two treatments. As shown in Figure 3, the

temperature at a slurry depth of 30 cm displayed higher peaks in UCM than in NEO, and more pronounced diurnal fluctuations, likely caused by higher microbial activity in the UCM that affected the slurry temperature, whereas air temperature was the main driver in NEO. However, the minima are lower in the UCM – resulting in comparable average temperature over the entire storage period.

The pH during storage also indicated a clear inhibition of microbial activity in NEO (Figure 4). The pH in NEO rose slowly from pH 5.3 to pH 5.9 throughout the 80 days of storage. The slow increase can be attributed to deprotonation of carbonates, combined with the reaction of NO_2^- with organic matter. The onset of microbial denitrification would have been associated with an abrupt pH increase and elevated temperatures as described by Hiis et al. (2023), suggesting an absence of denitrification in the stored NEO.

The apparent inhibition of microbial activity had a clear effect on the CH_4 fluxes. Figure 5 shows the cumulative CH_4 emissions for all tanks in the 2021–2023 measurement season, where the methane formation is completely inhibited by the nitrogen enrichment. A minimal yet consistent CH_4 uptake is found in all NEO treatments.

The low measurement frequency in 2022 did not yield an appropriate resolution of the fluxes, especially since the deviation in emissions between the two tanks in 2021 was large, mainly as a result of crust formation on UCM-1 and no crust formation on UCM-2. CH_4 emissions were substantially higher in 2023 compared to 2022, a finding we currently have no explanation for, other than speculating that the annual temperature variations may have influenced the rate of CH_4 formation. Despite emission-variation in the UCM, the reduction resulting from the plasma-based nitrogen enrichment was reliably above 100% throughout the storage periods. The complete emission reduction distinguishes the plasma-based nitrogen enrichment from acidification alone, which reports CH_4 reductions in the range of 69 to 84% (Habtewold et al., 2018).

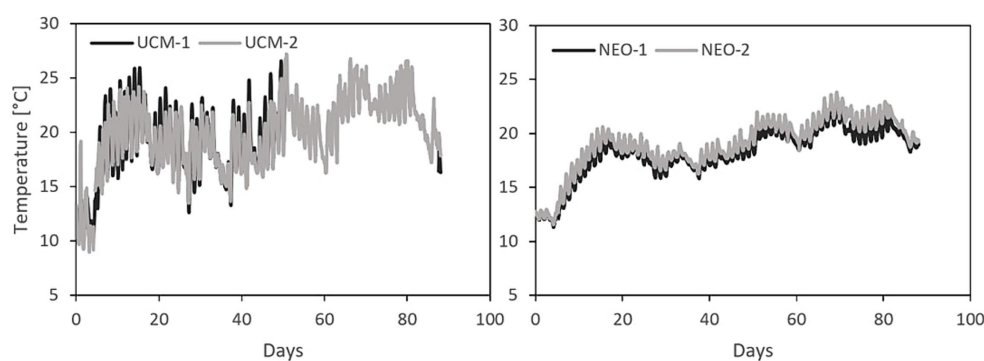


FIGURE 3

The temperature at 30 cm depth in all tanks, year 2 – ambient temperatures were used to determine the impact of temperature the following years.

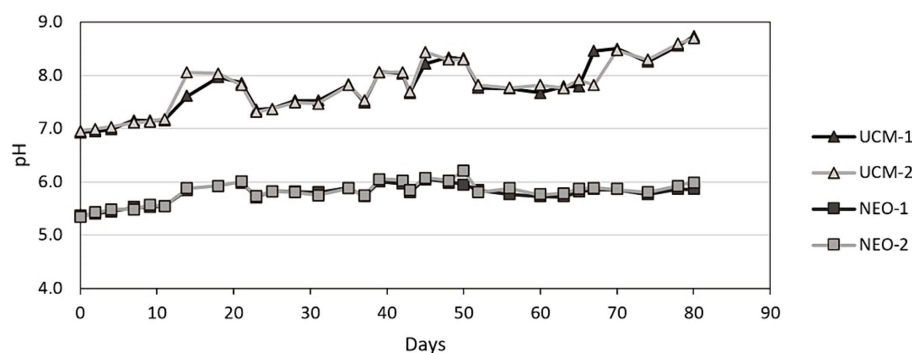


FIGURE 4

The pH development in all tanks, year 2.

3.2 Laboratory experiment

The controlled incubation experiments demonstrated a pronounced effect of acidification and NO_3^- and NO_2^- addition on CH_4 production, although their combinatory effect did not match that of the plasma treatment entirely. The unacidified UCM (pH 7.1) showed a steady methane formation, until either NO_3^- or $\text{NO}_3^- + \text{NO}_2^-$ was added (Figure 6). The addition of $\text{NO}_3^- + \text{NO}_2^-$ resulted in inhibition of methanogenesis throughout the incubation period, whereas the methane formation resumed in the NO_3^- treatment.

Both treatments where the nitrogen oxyanion concentration was artificially elevated resulted in denitrification, as was evident from N_2 formation shown in Figure 7. The pronounced drop in CH_4 concentration was unexpected; the increased formation of N_2 , N_2O , NO and CO_2 and could not entirely explain the reduced concentration. A potential explanation of the effect is denitrifying anaerobic methane oxidation (DAMO), as described by Wei et al. (2022), a thermodynamically favourable denitrification pathway where CH_4 acts as electron donor and is oxidised to CO_2 .

Figure 8 shows the impact of acidifying the UCM with H_2SO_4 to pH 6.3. Acidification alone resulted in a 45% reduction in CH_4 accumulation. The nitrogen oxyanions were added in identical concentrations as in the prior experiment after 86 h. Both treatments

resulted in complete inhibition of methanogenesis. NEO (UCM plasma treated to pH 6.3) showed a complete inhibition of methanogenesis throughout the experiment.

Both $\text{UCM}_{\text{pH}6.3 + \text{NO}_3^-}$ and $\text{UCM}_{\text{pH}6.3 + \text{NO}_3^- + \text{NO}_2^-}$ exhibited clear signs of denitrification. The pH at the end of the experiment was 8.1 and 8.2, which indicates that the alkalization occurred due to consumption of nitrogen oxyanions. In contrast, pH in NEO rose moderately from pH 6.3 to pH 6.5 and did not display the same N_2O peaks as the comparable UCM treatments, as shown in Figure 9.

When acidifying the UCM to pH 5.0, no methane formation occurred over the duration of the experiment. However, the amount of H_2SO_4 required to reach this pH results in a sulphur content that often exceeds agronomic needs ($5.4 \text{ kg } 98\% \text{H}_2\text{SO}_4 \text{ m}^{-3}$). Targeting such low pH levels with sulfuric acidification should therefore be avoided.

The exact inhibitory mechanisms of plasma-based nitrogen enrichment cannot be determined by this set of experiments alone. However, some important observations can be made: the addition of nitrogen oxyanions suppresses CH_4 formation, likely because of a shift to NO_3^- and NO_2^- as the favoured electron acceptors. Consequently, denitrifiers consume the nitrogen oxyanions whereafter methanogenesis is likely to resume (as in $\text{UCM}_{\text{NO}_3^-}$). Our results also indicate the occurrence of the DAMO

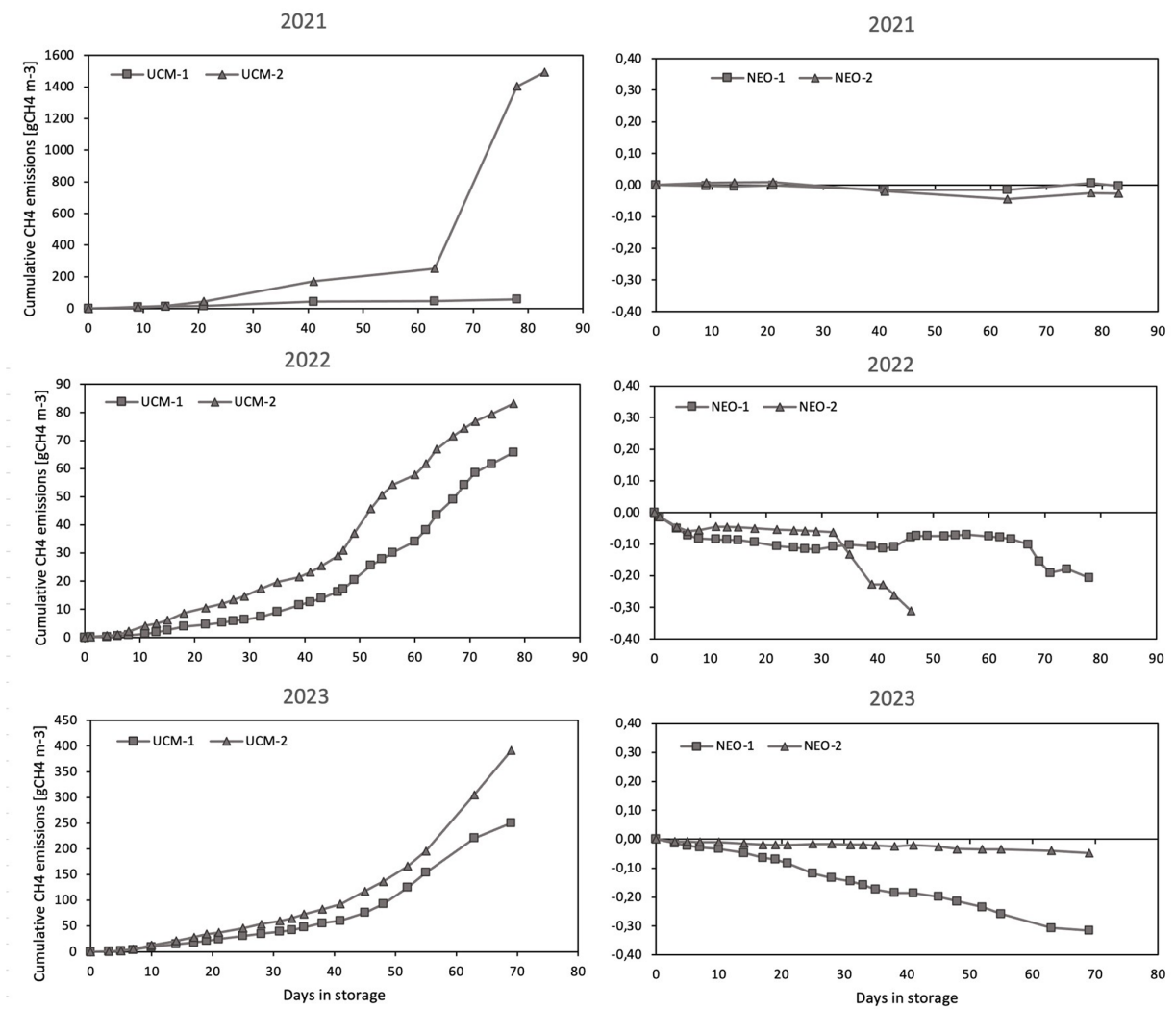


FIGURE 5
The cumulative CH₄ emissions from all tanks over the three measurement periods. The tank lid-closing mechanism malfunctioned after 48 days in NEO-2 in 2022, which is why the measurements stop.

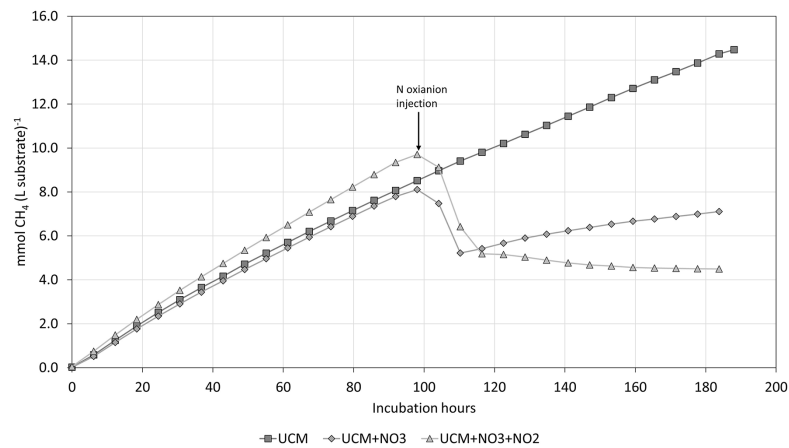


FIGURE 6
The cumulative CH₄ formation in UCM, with and without elevated N oxyanion concentrations (injected after 98 h).

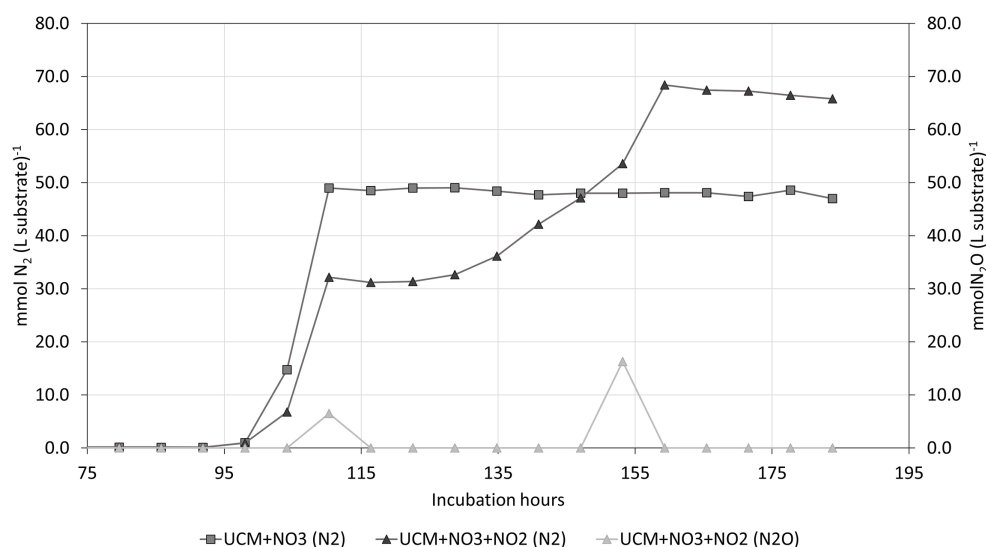


FIGURE 7

The N_2 and N_2O accumulation in the non-acidified treatments. All replicates displayed the same pattern, although shifted in time. Therefore, only one replicate of each treatment is displayed.

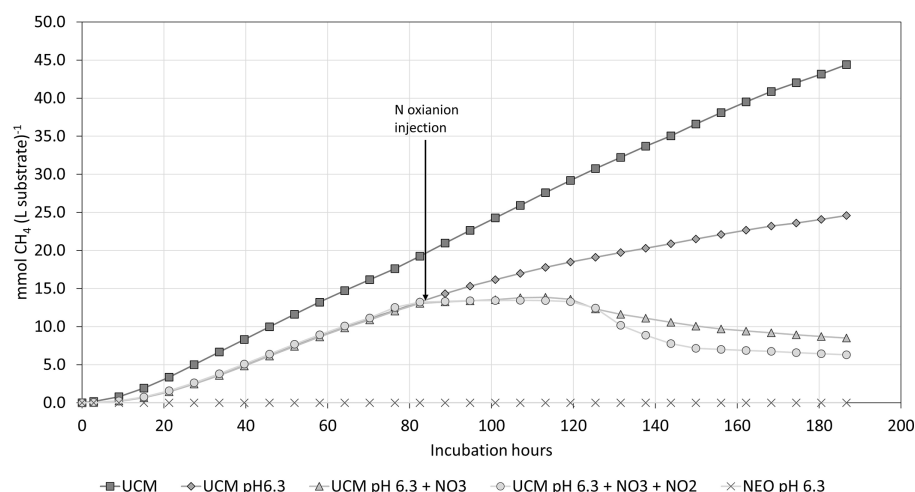


FIGURE 8

CH_4 formation in the substrate acidified with H_2SO_4 to pH 6.3 compared to a non-acidified control (UCM). Nitrogen oxanions were added after 86 h of incubation.

process, as denitrification coincides with a reduction in the CH_4 concentration that cannot be accounted for by dilution alone. Surprisingly, the NEO, even at a moderate pH of 6.3, did not result in the denitrification that occurred in its chemical imitations, suggesting that the plasma treatment itself has antimicrobial properties beyond the acidification and addition of nitrogen oxanions. One hypothesis worthwhile exploring is that the plasma treatment occurs under aerobic conditions when the facultative anaerobic denitrifiers are more likely to respire oxygen and therefore cannot easily counter the elevated levels of undissociated nitrite (HNO_2).

4 Conclusion

In this work we demonstrate that plasma-based nitrogen enrichment of cow manure inhibited methanogenesis completely in exposed outdoor storage tanks during the warm period of the year over three consecutive years. A laboratory experiment showed that the CH_4 inhibition could be achieved by reducing the slurry pH below 5, or by amending the cow manure with nitrate and nitrite. However, the latter strategy resulted in rampant denitrification, which renders plasma-based nitrogen enrichment the only method by which both methanogenesis and denitrification can be avoided at moderately low pH.

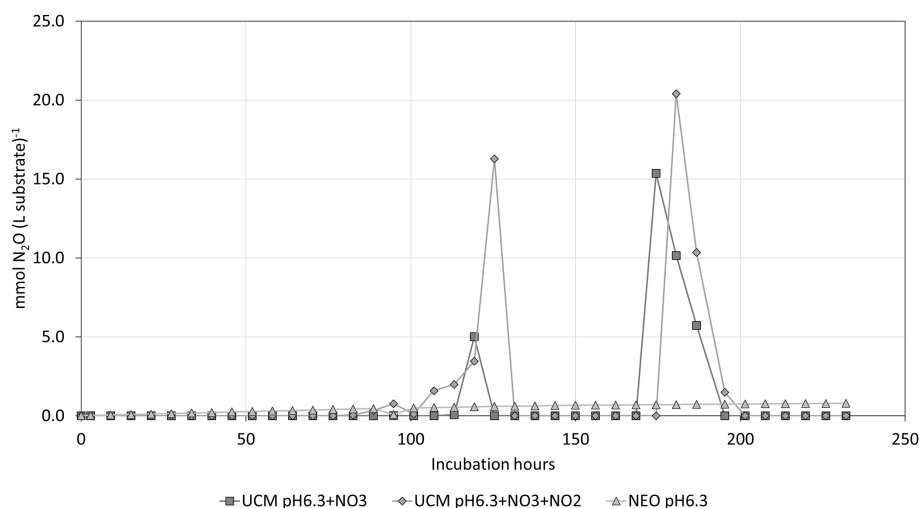


FIGURE 9

The N₂O development show abrupt peaks during microbial denitrification compared to a low but steady N₂O formation in NEO, which is likely a result of chemical interactions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MN: Writing – original draft, Writing – review & editing. PD: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The authors acknowledge the financial support from the Norwegian Research Council (grant no. 309640).

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Conflict of interest

The work was part of an industrial PhD where the MN works for a company that develops Plasma technology.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

EDITED BY

Rachel E. Thorman,
Agricultural Development Advisory Service,
United Kingdom

REVIEWED BY

John Williams,
Agricultural Development Advisory Service,
United Kingdom
Lars Stoumann Jensen,
University of Copenhagen, Denmark

*CORRESPONDENCE

Gerard L. Velthof
✉ gerard.velthof@wur.nl

RECEIVED 28 February 2024

ACCEPTED 30 April 2024

PUBLISHED 17 May 2024

CITATION

Velthof GL, Cals TCA, van 't Hull JP, Lesschen JP, Lessmann M, Porre RJ, Ros MBH, Rietra RPJJ, Schoumans OF, Veenemans L and Westerik D (2024) Managing organic resources in agriculture: future challenges from a scientific perspective. *Front. Sustain. Food Syst.* 8:1393190. doi: 10.3389/fsufs.2024.1393190

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Managing organic resources in agriculture: future challenges from a scientific perspective

Gerard L. Velthof^{1,2*}, Twan C. A. Cals¹, Jordy P. van 't Hull¹, Jan Peter Lesschen¹, Malte Lessmann^{1,3}, Rima J. Porre¹, Mart B. H. Ros¹, René P. J. J. Rietra¹, Oscar F. Schoumans¹, Lotte Veenemans¹ and Dorien Westerik¹

¹Wageningen Environmental Research, Wageningen University and Research, Wageningen, Netherlands, ²Soil Biology Group, Wageningen University and Research, Wageningen, Netherlands, ³Land Use Planning Group, Wageningen University and Research, Wageningen, Netherlands

Recycling of organic resources into agriculture has the potential to greatly increase nutrient use efficiency and improve soil carbon balance, but improper management can have adverse effects on the environment. Agriculture therefore faces large challenges to increase yields while decreasing these emissions to the environment. In this paper, we review (i) the availability and composition of organic resources, (ii) their agronomic value and risk of emissions, (iii) potential measures to reduce their emissions, and (iv) future challenges to support farmers and policy makers. The total amount of organic resource applied to soil amounted on average 41 kg nitrogen per ha agricultural land, 9 kg phosphorus per ha, and 456 kg carbon per ha in EU-27 + UK in 2017. Solid pig and cattle manures and cattle slurry are the most used organic resources. The availability of new organic resources from food processing, sewage sludge, municipal bio-wastes, and upcoming manure treatment techniques as fertilizer or soil conditioner is expected to strongly increase over the coming decade. Insight is needed into the composition of organic resources, the plant-availability of nutrients, the degradability of organic matter and the presence of contaminants. Measurement techniques become available to characterize soils, manures, crops, and emissions to the environment. However, the interpretation, and integration of data, and recommendations to farmers and policymakers using large amounts of data is expected to become more and more challenging. Many measures are available to improve nutrient and carbon management and to reduce emissions, including proper application, technological measures and structural changes in agriculture. For many measures, there is a risk of trade-offs that could lead to pollution swapping at different scales. We should focus on finding synergies between measures and no-regret management choices to develop effective mitigation strategies. The main future challenge for managing organic resources in agriculture is the development of an integrated nutrient management approach, including (i) the characterization of organic resources, their agronomic value and their environmental risks, (ii) knowledge of potential synergies and trade-offs between management measures, and (iii) implementation of this knowledge into decision support tools, models and legislation to support farmers and policy makers.

KEYWORDS

organic fertilizer, manure, compost, nitrogen, phosphorus, carbon, emission, soil

1 Introduction

Recycling of organic resources into agriculture has the potential to greatly increase nutrient use efficiency and soil quality. If done carelessly, however, it can lead to deterioration of groundwater and surface water quality through leaching and runoff of nutrients and polluting agents. Additionally, it can lead to other harmful consequences such as acidification and eutrophication of natural ecosystems resulting from ammonia (NH₃) and nitrogen oxide (NO_x) emissions and resulting N deposition, or enhanced global warming through emissions of the greenhouse gases nitrous oxide (N₂O) and methane (CH₄).

Since the 1990s, a series of policies have been implemented in the European Union (EU) to decrease agriculture-related nutrient emissions into the environment and improve the recycling of agricultural resources. Currently, emission reduction is mainly targeted by (i) the national emission ceilings for NH₃ and NO_x in the EU National Emission reduction Commitments Directive, and the United Nations (UN) Convention on Long-Range Transboundary Air Pollution, (ii) the water quality standards in the EU Water Framework Directive, and the EU Nitrates Directive, and (iii) the greenhouse gas emission reduction targets of the and the UN framework Convention on Climate Change (UNFCCC) (Oenema et al., 2011). Simultaneously, the European circular economy strategy and the Farm-to-Fork strategy aim to recycle resources, including nutrients (European Commission, 2020a). To close elemental cycles, the EU Waste Framework Directive sets ambitious targets of recycling and reuse of municipal waste to a minimum of 65% by 2035 (EU, 2018). Furthermore, with the implementation of the Fertilising Products Regulation no. 2019/1009 (FPR), the EU promotes the use of recycled and organic resources by providing harmonized conditions for the production and use of these materials (EU, 2019; Kurniawati et al., 2023).

Many measures are available to improve nutrient and carbon (C) management and to reduce emissions from organic resources used as fertilizer or soil conditioner. Measures can be categorized in management measures, technological measures, and structural changes. Management measures focus on the efficient use of nutrients and C, by incorporating composition, plant-availability, C stability, and risk of nutrients losses in fertilization strategies. Technological measures to reduce nutrient emissions include changes in livestock housing systems and manure storage, the use of manure treatment techniques, and the use of precision and low emission application techniques of manures (Chadwick, 2005; Hou et al., 2015; Bougouin et al., 2016; Jensen et al., 2020; Van der Weerden et al., 2021). Structural changes in agriculture, such as the reduction or reallocation of livestock numbers, are also options to improve nutrient and C management and reduce emissions from organic resources (Van Grinsven et al., 2018; Kim et al., 2023).

In this paper we review (i) the availability and composition of organic resources in the European Union, (ii) their agronomic value and environmental risks, (iii) the potential measures to reduce their emissions when applied to soils, and (iv) the future challenges to support farmers and policy makers.

2 Availability, composition and use of organic resources

2.1 Composition of organic resources

Organic resource composition can be variable, both within and among fertilizer types. In Table 1 the average dry matter, N, P and organic matter (OM) contents of a selection of the main organic resources used in Dutch agriculture are shown. The N:P ratio of an organic resource is an important parameter for balanced fertilization and to avoid excess application of N or P (see Section 6.1). The N:P ratio is generally higher for cattle manures than for pig and poultry manures (Table 1). The OM:nutrient ratio should be high when organic resources are mainly applied as soil improver and not as a source of nutrients. Vegetable, garden, and fruit-derived (VGF) compost has a higher OM:nutrient ratio than manures and this ratio is higher for solid manures than for slurries. Cattle slurry has the lowest N and P content on fresh product basis. Meat and bone meal is relatively rich in P and has a lower N:P ratio than the other organic resources. The UK Nutrient Management Guide (AHDB, 2023) also presents the composition of a range of organic resources. For cattle slurry, pig slurry, poultry manure, solid cattle manure and solid pig manure, they report values of 2.6; 3.6; 28; 6, and 7 g N kg⁻¹ fresh product, and 0.52; 0.65; 7.4; 1.4; 2.6 g P kg⁻¹ fresh product, respectively. Most of these values are somewhat lower than the Dutch, but the number of samples and the uncertainty level of the UK data is not available. These differences indicate however that the Dutch data -although based on a vast number of samples- may not necessarily be representative for other countries, and that average manure composition can depend on the common livestock system and level of intensity in each country.

2.2 Application of organic resources in EU-27 + UK

The MITERRA-EUROPE model was used to calculate the amount of carbon and nutrients that are applied on agricultural soils in the EU-27 + UK (Table 2). The results show that in total over 78,000 Gg of C is applied through organic resources annually in EU-27 + UK (Table 2). The total amount of organic resource applied to soil amounted 6,970 Gg N (on average 41 kg N ha⁻¹ agricultural land, based on 171.7 million ha arable and grassland), 1,593 Gg P (9 kg P ha⁻¹), and 78,294 Tg C (456 kg C ha⁻¹) in EU-27 + UK in 2017 (Table 2). The amount of N is somewhat less and that of P somewhat higher than the with MITERRA-EUROPE estimated amounts of applied mineral N and P fertilizers, i.e., on average 66 kg N and 7 kg P per ha agricultural land. Solid pig and cattle manure and cattle slurry are the most used organic resources. More than half of the C applied to soils by organic resources in the EU-27 + UK is applied as solid manures. Relatively high amounts of cattle and pig slurries are applied in countries in NW Europe such as Belgium, Denmark, Netherlands, and Germany, whereas solid manures applied are more common in France, Spain, and part of central Europe (Figure 1). The application rate of VGF compost is relatively high in Denmark, Germany, and the Netherlands and that of sewage sludge in Spain, Denmark, Germany and Italy (Figure 1).

TABLE 1 Mean ± standard deviation of dry matter (DM), N, P and organic matter (OM) content of organic resources in the Netherlands^a.

Organic resource	Content, g kg ⁻¹ product ^b				References
	DM	N	P	OM ^c	
Cattle slurry	76	4.12 ± 0.71	0.61 ± 0.15	54 ± 25	CBGV, 2023; Statistics Netherlands (CBS), 2024
Pig slurry	67	6.42 ± 1.78	1.63 ± 0.63	40 ± 38	CBGV, 2023; Statistics Netherlands (CBS), 2024
Poultry manure	561	25.0 ± 7.10	8.19 ± 2.49	478 ± 124	CBGV, 2023; Statistics Netherlands (CBS), 2024
Solid cattle manure	233	7.61 ± 3.18	1.61 ± 0.98	162 ± 68	CBGV, 2023; Statistics Netherlands (CBS), 2024
Solid pig manure	249	8.95 ± 3.39	4.18 ± 2.63	209 ± 81	CBGV, 2023; Statistics Netherlands (CBS), 2024
VGF compost ^d	681	11.8	2.79	322	CBGV, 2023
Sewage sludge ^e	230 ± 60	7.13 ± 1.79	4.14 ± 1.04	144 ± 36	Huygens et al., 2022
Meat and bone meal	94.6	9.4	5.24		Dittrich and Klose, 2008; Centraal Veevoeder Bureau, 2012

^aFor cattle, pig and poultry manure, the presented N and P values are based on a database of transported and analyzed manure samples from Dutch farms in the period 2020–2022. The number of manure samples range from 3,879 for solid pig manure to 150,362 for cattle slurry. Fewer data on OM contents of manures were available, approximately 10% of the total sample size, and a minimum of 50 individual samples per category [CBGV, 2023; Statistics Netherlands (CBS), 2024]. For VGF compost, sewage sludge and meat and bone meal the sample size is unknown, as well as the variation therein.

^bFresh weight.

^cOf which 50% is assumed to be C.

^dCompost of Vegetable, Garden, Fruit waste.

^eDewatered sewage sludge, based on 17 international peer review papers.

TABLE 2 Estimates of the amounts of organic resources applied to agricultural soil in EU-27 + UK in 2017^a.

Organic resource	N		P		C	
	Gg	% of total	Gg	% of total	Gg	% of total
Cattle slurry ^b	2,543	37%	455	29%	22,661	29%
Pig slurry	1,020	15%	267	17%	3,757	5%
Poultry manure	598	9%	229	14%	6,261	8%
Solid pig and cattle manures ^b	2,615	38%	528	33%	43,939	56%
VGF compost	22.4	0%	4.7	0%	323	1%
Sewage sludge	157	2%	106	7%	890	1%
Total	6,970		1,593		78,294	

^aSource of data: MITERRA-EUROPE, using a.o. Eurostat and National Inventory Reports data (Velthof et al., 2009; Duan et al., 2021).

^bCattle slurry includes N, P, and C excreted during grazing of cattle that produce slurry. Solid cattle and pig manure includes N, P, and C excreted during grazing of cattle that produce solid manure.

Van Dijk et al. (2016) made an assessment of the P flows in Europe in 2005. Phosphorus excreted as manure by livestock was almost fully applied to agricultural land and amounted to about 1,749 Gg P. A relatively small part of bio-waste was composted and used in agriculture in 2005 (15 Gg P), most bio-waste was lost in landfills and incineration (212 Gg P). Part of the sewage sludge in Europe was used in agriculture (137 Gg P) while a larger part was lost by landfilling and incineration (234 Gg P). On average 35% of the produced sewage sludge is applied in agriculture in the EU (Huygens et al., 2022), with very large variations between countries (European Commission, 2022). From the late 1980s onwards, the risk of prion diseases has decreased the recycling of P from animal slaughter waste in Europe. A part (22% in 2022) of the total produced animal slaughter waste has since then been labeled as high risk material (category 1 in the regulations on Animal By-products; EU, 2009; Huygens et al., 2019; Dobbelaere, 2023). This part cannot be used as feed or fertilizer, and is landfilled after incineration or used in cement kilns. Therefore, only a relatively

small portion of the waste of slaughtered animals was used as fertilizer (16 Gg P in 2005). The remaining part (281 Gg P) is lost by landfilling and incineration (Van Dijk et al., 2016), although these data could be outdated (Huygens et al., 2019). The total amount of P loss (727 Gg P) by landfilling and incineration of food waste (212 Gg P), sewage sludge (234 Gg P) and slaughtered animals (281 Gg P) in 2005, is high, i.e., more than half of the mineral P fertilizer use (Van Dijk et al., 2016). Phosphorus is a life-essential, irreplaceable element and the fossil P reserves are limited (USGS (U.S. Geological Survey), 2022). As Europe has no significant P mines, it is highly dependent on the import of phosphate ore to produce P fertilizers (De Ridder et al., 2012). Clearly, increased recycling of P from these waste streams can strongly reduce the use of mineral P fertilizers.

There is an ongoing trend in which less municipal waste is landfilled and more waste is recycled, composted and incinerated (Eurostat, 2023). The amount of food waste is currently estimated to be between 158 and 298 kg per person per year in Europe

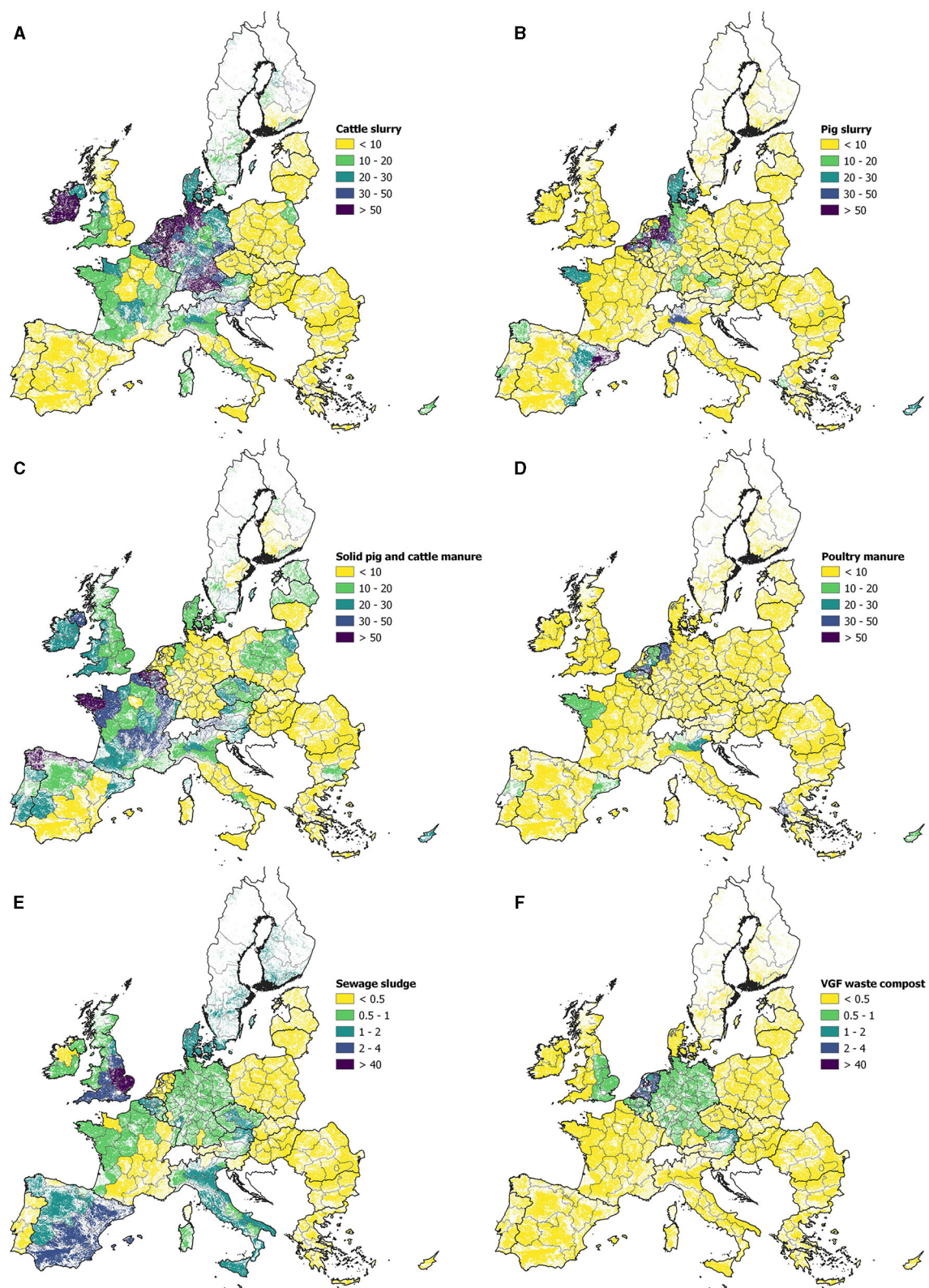


FIGURE 1

Nitrogen inputs into agricultural soils (expressed in kg N ha^{-1}) via (A) cattle slurry including slurry deposits of grazing cattle, (B) pig slurry, (C) solid pig and cattle manure including solid manure deposits of grazing cattle, (D) poultry manure, (E) sewage sludge, and (F) VGF compost to agricultural land in EU-27 and UK. Notice that the scale is different for (E, F). Average application rates are calculated at NUTS2 level using MITERRA-EUROPE (Velthof et al., 2009; Duan et al., 2021).

(Corrado and Sala, 2018), which is about 20% of the food produced (Stenmarck et al., 2016).

2.3 Developments in the availability of organic resources

The availability of organic resources for use in agriculture as fertilizer or soil conditioner from food processing, sewage sludge, and municipal bio-wastes is expected to strongly increase over the coming decade, because of the ambitious targets of recycling and reuse of municipal waste of the EU Waste Framework Directive sets (EU, 2018). The collection and recycling of municipal bio-waste is projected to increase in order to reach the EU's objective to reuse and recycle 65% of the municipal waste by 2035 (ECN, 2022). In comparison, in 2022, 48% of the municipal waste generated in the EU was recycled of which 18% organically, through composting and anaerobic digestion (Eurostat, 2023). A large fraction of the municipal waste (42%) is still incinerated or landfilled which offers potential for increasing recycling (Eurostat, 2023). The P recycling potential from waste water remains largely untapped in the EU (Serrano-Gomez et al., 2023). Some countries in Europe, such as Germany and Switzerland, have implemented measures to make the technical recovery of P from sewage sludge mandatory (Hušek et al., 2023), to ensure that P is recycled back into agricultural production. In addition, P recovery from slaughterhouse waste has become obligatory in Switzerland¹ (ESPP (European Sustainable Phosphorous Platform), 2015). Further innovations in the development of waste water management, such as the use of urine source separation technologies, will increase the availability of nutrients for use in agriculture (Larsen et al., 2021). The circular economy action plan (European Commission, 2020b) has fostered many research initiatives, exploring a broad range of nutrient valorization pathways like the resource recovery from food-processing waste waters (Durkin et al., 2022). The overall recovery potential for N and P from currently untreated or unutilized waste streams, such as food chain waste, manure and waste water has been estimated to be 4.2 Tg of N and P in the EU, which represents about 35% of the currently used mineral fertilizers in EU agriculture (Chojnacka et al., 2019). The increased recycling of organic resources will decrease the dependence on mineral N and P fertilizers in the EU.

3 Developments in measurement methods to determine agronomic value and risk of emissions

3.1 Methods to characterize composition of organic resources

Data on the composition of organic resources are very important in order to increase nutrient use efficiency and to decrease the risk of emissions to the environment. Measurement

techniques are developing, and new techniques become available to characterize soils, manures, crops, and emissions to the environment to improve nutrient management (Bühler et al., 2021; Horf et al., 2022; Reijneveld et al., 2022; Deru et al., 2023; Ge et al., 2023). These data can be used in decision support tools and fertilization recommendation to support farmers in management of nutrients (Section 7.2.1).

Analytical methods used to characterize fertilizing products have recently been standardized by the EU, as a result of the FPR that entered into force on 16 July 2022 (see CEN and ISO technical committees for the proposed and published methods).² These methods are used for samples with varying composition due to the heterogeneity of organic resources. Furthermore, Member States are allowed to have national regulations for fertilizing products based on national standards and different methods than prescribed in the FPR. Comparison of samples from similar organic resources (e.g. from cattle slurry) and developing datasets about the composition of organic resources therefore remains difficult.

New techniques to determine C, and nutrients in organic resources are near-infrared spectroscopy (NIRS), low-field nuclear magnetic resonance for (NMR) and pyrolysis (Py-GC/MS) (He et al., 2020). Moreover, sensor-based techniques can analyze the total and ammonium N-, P-, K content of organic resources on site or on-board for precision farming with fast access to data and at a low cost (Sørensen et al., 2015; Evangelista et al., 2021; Morvan et al., 2021; Feng et al., 2022; Thiessen et al., 2022; Horf et al., 2024). However, the accuracy of these techniques is often less than classical chemical methods, and depends strongly on the number of reference samples of a specific material (Derikx et al., 2021). Specific techniques for compost, for example to test the stability or biodegradability of compost, such as Oxitop[®], rottegrad (self heating test) (Binner et al., 2011), or the presence of plastic (Bläsing and Amelung, 2018), are rarely tested and used for other organic resources.

Besides analysis of their composition, properties related to decomposability of OM in organic resources are often characterized in incubation studies, in which organic resources are applied to soil. Examples are the determination of humification coefficients of organic resources (Section 4.2; Lashermes et al., 2009) and N mineralization. These incubation studies have similarities with pot and field studies to which they can be related (see Section 4.1), but also to measurements of plant-available nutrients such as citrate-extractable P (Delin, 2016), and Potential Mineralizable Nitrogen (PMN) (Westerik et al., 2023).

Many new analytical techniques are available, yet there are still many uncertainties involved in how the data obtained can be used to improve nutrient management. A combination of the aforementioned techniques can help to optimize the use of organic resources and decrease emissions to the environment. However, there are many challenges in the interpretation of the results and the handling of the large number of data generated with these techniques. Representative sampling of organic resources remains a challenge, even for these new analytical techniques.

¹ <https://phosphorusplatform.eu/scope-in-print/news/1061-switzerland-makes-phosphorus-recycling-obligatory>

² <https://standards.cencenelec.eu/dyn/www/?p=205:105:0:::>

3.2 Crop growth and yield

The effects of organic resource application on crop growth, yield, and quality are generally obtained by harvesting, weighing, and analyzing crops in a field experiment. Spectrum sensing techniques (e.g., handheld scanners using NIRS), from which information of the status of a crop in a field or field experiment can be rapidly obtained, are currently available and rapidly developing (Burkart et al., 2018; Luo et al., 2022). Crop images from satellites or unmanned aerial vehicles such as drones can also be used to get insight into the development of crops at a larger (field or farm) scale. This type of information can be used to predict crop response to (organic) fertilizers and, where necessary, adjust fertilization using precision farming techniques (Maresma et al., 2016; Zhang et al., 2020), as well as improve crop growth models (Dlamini et al., 2023). Data on crop growth and development in combination with data on nutrient input and availability of organic resources can be used in decision support tools for farmers to decide if a top dressing of a fertilizer is required to obtain the optimal yield (Section 7.2.1).

3.3 Gaseous emissions and water quality

3.3.1 Ammonia emission

There are several techniques currently used for measuring NH_3 volatilization from soils after application of (organic) fertilizers (Van Andel et al., 2017); (i) micrometeorological methods such as Eddy Covariance and the upwind-downwind method (Cassel et al., 2005), (ii) static chamber enclosure with acid soaked sponges, or other forms of acid traps, placed within the chamber, (iii) dynamic enclosure by using wind tunnels, usually in combination with an acid trap (Lockyer, 1984; Sintermann et al., 2012), and (iv) satellite instruments such as the Infrared Atmospheric Sounding Interferometer (Dammers et al., 2016; Van Damme et al., 2021). Each technique has its own advantages and disadvantages and it depends on the research question what sampling method and technique are preferred. The micrometeorological method is considered to be the most accurate and reliable, but large field sites usually limit research to only one treatment without replications. On the other hand, methods with a static or dynamic enclosure can be used to investigate several treatments and replicates on a small scale, but there are concerns in terms of the effect of the chamber and consistent underestimation due to a lack of turbulence (Smith et al., 2007; Van Andel et al., 2017). The wind tunnel technique can also be used to investigate treatments with replicates and is usually considered to be more accurate than static chamber designs, yet standardizing air displacement and the costs of production and analytical analyses limit their application in research.

3.3.2 Nitrous oxide emission

Detection techniques of N_2O emissions from the field can roughly be categorized in two groups: chamber methods and micrometeorological methods. Chamber methods are the most commonly used approach and measure the accumulation of gas in a known volume (Clough et al., 2020; Grace et al., 2020). Both manual (where the chamber is closed and opened by an operator) and

automated chambers (where the chambers are opened and closed through a pneumatic system) exist with both their advantages and disadvantages (Rapson and Dacres, 2014). The second type of sampling technique comprise micrometeorological methods. The most widely used is the Eddy Covariance method in which the emission of a gas is defined as the covariance between the gas concentration and the vertical wind (Hensen et al., 2013). Other, similar techniques are the relaxed eddy accumulation method, mass balance and plume methods, and tall tower measurements (Hensen et al., 2013; Rapson and Dacres, 2014). Analytical techniques for gas measurements include chromatographic techniques, optical techniques and amperometric techniques. Optical techniques are based on the ability of a gas to absorb infrared light (IR) at unique wavelengths. Examples of used IR detectors are Fourier transform infrared spectrometers (FTIR), photo-acoustic instruments, laser-based systems such as tunable diode laser (TDL) and quantum cascade lasers (QCL) and cavity ring down spectrometers (CRDS). The amperometric method determines N_2O concentrations by measuring the produced N_2O from the reduction of N_2O at an electrode (Hensen et al., 2013; Rapson and Dacres, 2014). The development of these different techniques have recently enabled reliable measuring N_2O concentration at extremely low concentrations. Nonetheless, each technique has its own advantages and disadvantages and it really depends on the research question and site what sampling method and analytical technique is preferred or possible.

3.3.3 Water quality

The effect of fertilization on water quality is generally assessed by the occasional sampling of groundwater or surface water, followed by chemical analysis of the water sample in a laboratory. Sensor techniques for in-field analysis are currently available and will be further developed for direct and automatic determination of concentration of nutrients in the field at a high frequency (Rode et al., 2016; Jomaa et al., 2018). These sensors, sometimes in combination with software applications, can give researchers, farmers, water authorities and regional governments continuous insight into water quality and effects of specific events, such as fertilizer application or weather events, on water quality. Insight in the temporal and spatial variability of water quality in relation to the use of organic resources can be used to derive strategies to decrease leaching to groundwater and surface water.

4 Agronomic value of organic resources

4.1 Nutrient availability

Plant nutrient availability of organic resources can be quantified by their Nitrogen or Phosphorous Fertilizer Replacement Value (NFRV or PFRV, respectively), which specifies the amount of mineral fertilizer needed for a similar crop response as the organic resource (Schröder et al., 2007; Schils et al., 2020). Accurate FRVs are required to underpin fertilizer recommendations and maximize nutrient use efficiencies. FRVs are mostly applied to N, and NFRVs can be determined in a field or pot experiment in which the rates

TABLE 3 Range (minimum–maximum) of Nitrogen Fertilizer Replacement Values (NFRVs) of different organic resources, expressed as % of mineral N fertilizer which can be replaced by N from the organic resource in the first year of application.

Organic resource	NFRV	References
	%	
Cattle slurry	35–45	Gutser et al., 2005
	13–67	Schils et al., 2020
Pig slurry	41–79	Schils et al., 2020
Poultry manure	60–85	Gutser et al., 2005
	33–42	Schils et al., 2020
Solid pig and cattle manures	10–20	Gutser et al., 2005
	14–34	Schils et al., 2020
VGF compost	0–20	Gutser et al., 2005
Sewage sludge	15–55	Gutser et al., 2005
Meat and bone meal	60–80	Gutser et al., 2005

This table is based on two literature reviews which include 32 individual studies in total.

of organic and mineral fertilizers required to obtain equal crop N uptake are compared ([Jensen, 2013](#); [Westerik et al., 2023](#)). NFRVs are commonly determined for the first year after application (i.e., short-term FRVs) and the variation in FRV is large, as is shown by two review papers in [Table 3](#). Variation can be caused by variation in organic resources composition, time and method of application, climatic conditions, soil properties, reference fertilizer and test crop ([Jensen, 2013](#)). For example, the first year NFRV of cattle slurry can increase from 36 to 53% when injected instead of surface applied, most likely through a reduction in NH_3 volatilization ([Schröder et al., 2007](#)). NFRVs are expected to increase over time because of mineralization of residual organic N in the years after application ([Gutser et al., 2005](#); [Schröder et al., 2007](#); [Hijbeek et al., 2018](#)).

Since field experiments for the determination of FRVs are time intensive and costly, attempts are being made to predict FRVs based on easily obtainable chemical properties. Total N, C:N, mineral N and Potential Mineralizable Nitrogen (PMN) appear to be good predictors for NFRV in pot and field experiments, and citrate-extractable P can predict PFRVs ([Stadler et al., 2006](#); [Delin et al., 2012](#); [Delin, 2016](#); [De Notaris et al., 2018](#); [Westerik et al., 2023](#)). NFRVs generally increase with higher total and mineral N contents, lower C:N ratios and higher PMN ([Tables 1, 3](#)), but the strength of these correlations needs to be tested under a range of field conditions. This is especially relevant with the rapid emergence of new organic resources, and the need for product-specific recommendation rates and FRVs. These new products can in theory have improved plant nutrient availability, but adequate FRVs are required to underpin fertilizer recommendations and maximize the nutrient use efficiency of these products.

NFRVs of organic resources are often used in national fertilizer recommendations and decision support tools as well as action programs of the EU Nitrates Directive. Currently, there are striking differences of assumed NFRVs between European countries, therefore large differences exist in recommended or allowed rates

of organic resources. For example, the NFRV of farm yard manure (FYM) is assumed to be 10% in the UK, whereas it is assumed to be 60% in Germany ([Jordan-Meille et al., 2022](#)). Such differences can result from alternative calculation methods, experimental setups and timeframes ([Gutser et al., 2005](#); [Jensen, 2013](#); [Schils et al., 2020](#); [Westerik et al., 2023](#)). For example, some countries (Belgium, Switzerland, Italy and Germany) take into account long term effects (>1 year) of fertilizer application on yield, whereas other countries do not. There is a reasonable agreement between countries in the order of decreasing NFRV, i.e., pig slurry > cattle slurry > poultry manure > FYM ([Webb et al., 2013](#)). However, there are also differences in NFRV among countries which are, besides the calculation method and considered period, probably also due to differences in application time and method. Standardization of calculation methods should serve to improve N use efficiency of organic resources.

Besides their supply of N and P, organic resources also supply other nutrients and trace elements ([Sager, 2007](#)). Furthermore, repeated organic resources application can improve soil properties such as bulk density, aggregate stability, infiltration capacity and water retention, although these effects are largely dependent on soil and crop type ([Fu et al., 2022](#)). Organic resource application can therefore benefit soil fertility and improve yields beyond the supply of N and P ([Hijbeek et al., 2018](#); [Kok et al., 2023](#)).

Although knowledge of NFRVs of different organic resources is rapidly emerging, insight in the chemical properties to explain and predict variability in NFRVs as well as standardization of determination methods of NFRVs is required for adequate fertilizer recommendations of new and existing products. Besides, data of supply of P, K and other macro- and micronutrients from organic resources to crops is still scarce.

4.2 Carbon stability

The addition of organic resources influences soil organic carbon (SOC) by the addition of organic material and by increasing aggregate stability and the organic C content of aggregates ([Yilmaz and Sönmez, 2017](#)). Generally, the potential to sequester C in the soil increases with increasing C:N rate of organic resources ([Hijbeek et al., 2019](#)).

The decomposability of organic resources varies largely between and within different groups of organic resources ([Lashermes et al., 2009](#); [Levvasseur et al., 2022](#)). [Levvasseur et al. \(2022\)](#) found that for example the mineralization of C from animal manures varied between 0 and 500 mg C per g added C for incubation studies lasting an equivalent of 1 year under field conditions. To predict C mineralization in the field after organic resource application, [Levvasseur et al. \(2022\)](#) recommend to calibrate models per product and not per fertilizing product group. In addition, they recommend to also include product characteristics such as the Van Soest analysis of fiber ([Van Soest and Wine, 1968](#)).

The humification coefficient (HC) is the percentage of applied OM that is not decomposed after 1 year of soil application, and is used as an indicator for OM stability of fertilizing products. HCs are used in fertilizer recommendations and in simple models for estimation of C mineralization after application of

TABLE 4 Range (minimum–maximum) of humification coefficients (HC) of different organic resources, expressed as fraction of the applied organic matter that is not decomposed within the first year after soil application.

Organic resource	HC (%)	References
Cattle slurry	23–87	De Wit and Vervuurt, 2023
Pig slurry	28–87	De Wit and Vervuurt, 2023
Poultry manure	34–55	Schoumans et al., 2023
Solid pig and cattle manures	33–90	De Wit and Vervuurt, 2023
VGF waste compost	65–93	De Wit and Vervuurt, 2023
Sewage sludge	41 ^a	Kätterer et al., 2011

The ranges include different soil types. ^aFor sewage sludge decomposition, information seems to be limited. It is reported to mineralize more than compost (i.e., have a lower HC value) in a short incubation study (Pedra et al., 2007).

OM (Janssen, 1984; De Neve et al., 2003; Egene et al., 2021). The HC is highly variable within and among different types of organic resources (Table 4). Several factors may influence the HC, such as climatic factors, soil characteristics, characteristics of the input material, and anthropogenic activities. Additionally, the relationship with initial SOC content and HC values was, based on a mathematical method, found to be non-linear: a higher SOC content leads to lower HC values (Tan et al., 2014). The wide ranges reported in Table 4 imply that using a single HC per fertilizer type for calculations of C build up, may lead to significant over- or underestimations (of up to 30%).

Other parameters used to describe C storage potential or mineralization include the indicator of remaining organic carbon (IROC) (Lashermes et al., 2009), the humus production capacity (VDLUFA, 2021), and the cumulative C-CO₂ emissions over a chosen period of time (Mondini et al., 2017). All of these terms point toward decomposability trends with C from animal by-products including meat and bone meal being most decomposable and C from compost being least decomposable. For Lashermes et al. (2009), VDLUFA (2021) as well as Mondini et al. (2017), the fact remains that these decomposability terms vary strongly within product groups.

In short, commonly, animal by-products are most easily decomposed, followed by sewage sludge, manure and compost, but the variation in C mineralization is very high within the categories.

5 Environmental consequences

5.1 Ammonia emission

Ammonia emissions after application of manures or other organic resources can constitute a major N loss pathway from animal farms. Loss of NH₃ through volatilization reduces N use efficiency of the fertilizer, contributes to the formation of airborne particulate matter, and may induce acidification, eutrophication, and indirect N₂O emissions after deposition (Behera et al.,

TABLE 5 EMEP/EEA NH₃ emission factors of different surface applied organic resources and averages (and range) for the same fertilizer types derived from the DATAMAN field database, expressed as fraction of total N applied.

	EMEP/EEA ^a	DATAMAN Field database ^b	
	Emission factor	Emission factor and range	No. studies/ observations
Cattle slurry	0.33	0.24 (0.00–0.87)	(148/484)
Pig slurry	0.28/0.20 ^c	0.28 (0.02–2.46)	(71/316)
Poultry manure	0.32	0.09 (0.02–0.21)	(8/38)
Solid pig and cattle manures	0.32/0.41 ^c	0.04 (0.00–0.18)	(26/66)
VGF compost	0.066	–	
Sewage sludge	0.111	–	

^aEEA (2023); EMEP emission factors were corrected for TAN content of the manure, EMEP tier 2 methodology does not distinguish between TAN content of liquid (slurry) and solid manures.

^bBeltran et al. (2021) and Van der Weerden (2024); only records reporting emissions after surface spreading were taken into account.

^cEmission factors for Pig slurry cover finishing pigs and sows, respectively; emission factors for solid pig and cattle manures, cover solid pig manures and solid cattle manure, respectively.

2013; Råbiger et al., 2020). Emission patterns differ for various fertilizer types. For liquid fertilizers, such as slurry and digestates, volatilization typically occurs during the first hours or days after application, whereas for solid manures, composts, and plant residues NH₃ emissions are typically lower and can be spread out over a longer timeframe, as the OM in the fertilizer breaks down. Generally, emissions are higher for liquid manures and slurries with high levels of available N, such as slurry digestates or liquid poultry manure, than for solid manures and composts (Table 5). In an elaborate screening of over 30 biobased fertilizers, Wester-Larsen et al. (2022) reported a high NH₃ volatilization potential for digestate products, and a low potential for solid products like composts.

Composition strongly affects the magnitude of NH₃ losses from organic resources, especially the total N and ammoniacal N contents and the fertilizer pH. Moreover, as NH₃ volatilization strongly depends on the chemical equilibrium between ammonium, dissolved NH₃, and gaseous NH₃, environmental factors such as temperature, wind speed, soil moisture, and contact surface between the fertilizer and air play a crucial role. Mitigation strategies can therefore be aimed either at altering the composition or chemical properties of the fertilizer, e.g., by acidifying manures (Wagner et al., 2021; Silva et al., 2022; Pedersen and Nyord, 2023) or treating them with plasma (i.e., acidifying slurry and adding NO₂ and NO₃ by N fixation) (Graves et al., 2019), or at ensuring that the environmental conditions are uncondusive for NH₃ volatilization. The latter strategy includes applying liquid fertilizers shortly before rainfall events and applying water after slurry application (Webb et al., 2014a), by injecting or incorporating fertilizers into the soil (Thompson and Meisinger, 2002; Powell et al., 2011; Dell et al., 2012; Webb et al., 2014a; Wester-Larsen et al., 2022), or by covering the fertilizer with a foamlke substance to reduce air

contact (Park et al., 2006; Lee et al., 2007; Bajagain et al., 2022). Generally, assuring quick infiltration of liquid organic resources into the soil and minimizing their exposure to air may reduce NH_3 volatilization after application. However, the aforementioned measures may in turn lead to pollution swapping to e.g., enhanced NO_3 losses or N_2O emissions (see also Section 7.1).

National inventories for reporting NH_3 emissions from agriculture often make use of emission factors that describe the cumulative emissions from a source as a fraction of total or ammoniacal N (Table 5). There are examples of national Tier 2 or Tier 3 emission calculation methodology that include emission factors for specific NH_3 abatement measures, e.g., by differentiating for various application methods in The Netherlands (Van der Zee et al., 2021), but generally national inventories do not include the option to account for these measures. Emission factors for new organic resources in combination with abatement measures are desirable for accurate calculation of NH_3 emissions and for providing farmers and governments with a means of accountability for management actions taken. Deriving these emission factors will be a major challenge, complicated by the increasing number of products and measures, and by the inherent difficulty of accurately determining NH_3 emissions. Recent efforts to synthesize information on NH_3 emission in databases with information from lab, field and model studies that describe emission data (Hafner et al., 2018; Beltran et al., 2021) can provide context for newly derived emission factors.

5.2 Nitrous oxide emission

Application of organic resources to soil can result in N_2O emissions through several processes, of which the most important are nitrification—the oxidation of NH_4^+ to NO_2^- and NO_3^- with N_2O as an intermediate product, and denitrification—the reduction of NO_3^- to N_2 with N_2O as a product of incomplete denitrification (Chadwick et al., 2011). In the last decades many studies focused on N_2O emissions following manure application (Chadwick et al., 2011; Thorman et al., 2020). The emission factors of manures (i.e., the cumulative N_2O -N loss as a proportion of total N applied to soil via manure) can range from 0.1 to 3.3% (Chadwick et al., 2011; Thorman et al., 2020; Van der Weerden et al., 2021), reflecting differences in manure type and composition, soil type, management and climate (Table 6). The emergence of manure processing techniques leads to new organic resources (Section 6.2.1). The composition of these products varies strongly (Chadwick et al., 2011), and so do the N_2O emissions following application to soil. However, many countries rely on the IPCC tier 1 default N_2O emission factors, in which the type of organic resource, soil type and application method is ignored. This leads to large uncertainties in the quantification of N_2O emission at local to global scales (Tian et al., 2020). The default aggregated IPCC-2019 N_2O emission factors are 0.3–0.4% of N applied for manures and 1.0% for other organic resources (IPCC, 2019).

Incorporation of slurry into the soil has already been shown to be an effective method to reduce NH_3 emission (Section 5.1), however some studies have shown that soil injection increases the risk of N_2O emission, pointing to a risk of pollution

swapping (Velthof and Mosquera, 2011). Others report similar N_2O emissions between broadcast and soil injection (Vallejo et al., 2005). The 4R nutrient management strategy (Section 6.1), including a lower application rate, splitting of N doses, and the application of nitrification inhibitors (Herr et al., 2020; Thorman et al., 2020) could balance the input and output of N and subsequently reduce the N_2O emission. Organic resource application should furthermore be timed to avoid application during wet conditions, actively support crop growth, and reduce excess N in the soil as it could result in large quantities of N lost by NO_3^- , which is in turn an indirect source of N_2O emission.

It is assumed that the risk of N_2O emissions from VGF compost, sewage sludge and meat- and bone meal are low, because of the generally lower mineral N content of these fertilizers.

5.3 Methane emission

Methane is produced by methanogenic bacteria under strict anaerobic conditions (Zeeman, 1991). Agricultural soils are generally well-aerated and are therefore not a source CH_4 , but can rather act as a sink of CH_4 (Hansen et al., 2024). During storage of liquid manure, however, CH_4 may be produced from easily degradable C components in the manure, such as volatile fatty acids (Kupper et al., 2020). After application to soil, part of the CH_4 produced during storage and which is dissolved in the slurry can be released (Chadwick et al., 2011). However, this is not CH_4 produced in soils. This does not hold for wetland rice soils, which are a large source of CH_4 . Application of organic resources to wetland rice may increase CH_4 emission (Hu et al., 2023). The surface area in Europe used for wetland rice production is extremely low, thus risk of high CH_4 emissions from agricultural soils in EU remains negligible.

5.4 N and P leaching and runoff

It has been hypothesized that N from organic resources has a larger risk of being lost by leaching than mineral N due to the untimely mineralization of fertilizer-derived organic N outside the growing season. This idea has likely also been taken into account when setting the specific limit for animal manure of 170 kg N ha⁻¹ in the Nitrates Directive (EEC, 1991) to prevent nitrate leaching from agriculture. However, use of labeled N shows that only a few percent of leached N in 1 year originates from the N fertilizer applied in that year, both for mineral N and organic resource N (Powelson et al., 1986; Choi et al., 2004; Frick et al., 2022). In the long term, buildup of soil organic N by crop residues and organic organic resources is the most important source of N leaching (Goulding et al., 2000). Nitrate leaching increases with long-term N application (Blicher-Mathiesen et al., 2014; Wang et al., 2019), and wrong timing of application, for example the application use of pig manure in autumn (Shepherd and Newell-Price, 2016). A meta-analysis by Ren et al. (2022) shows lower N leaching at higher SOC contents. A meta-analysis by Wei et al. (2021) shows that substitution of a part of the applied mineral N by animal manure N results in limited yield loss and decreases N leaching

TABLE 6 Average or range (minimum–maximum) of N₂O emission factors of different organic resources and application techniques, expressed as % of N applied.

Organic resource	Application technique	Grassland/arable	N ₂ O emission factor, % of N applied	References
Cattle slurry	Surface and injection	Grassland	0.31–0.50	Van der Weerden, 2024
Pig slurry	Surface and injection	Grassland	1.10	Van der Weerden, 2024
Cattle/ pig slurry/ digestate	Injection	Grass + arable	1.02 (–0.01 to 2.86) ^a	Petersen et al., 2023
Poultry manure	Incorporated	Arable	1.03–1.30	Thorman et al., 2020
	Surface	Arable	0.27–0.73	Thorman et al., 2020
	Surface	Grassland	0.05	Chadwick et al., 2000
Solid cattle manure	Surface	Grassland	0.22–0.33	Webb et al., 2004
	Ploughed	Grassland	0.01–0.02	Webb et al., 2004
	Surface	Arable	0.16	Thorman et al., 2007
	Ploughed	Arable	0.12	Thorman et al., 2007
Solid pig manure		Arable	<0.2	Nikiéma et al., 2016
	Surface	Arable	0.00	Thorman et al., 2007
	Ploughed	Arable	0.09	Thorman et al., 2007
	Surface	Grassland	<0.01	Webb et al., 2004
	Ploughed	Grassland	<0.01	Webb et al., 2004
	Surface	Grassland	0.05	Chadwick et al., 2000

^aSpring emission factor reported.

on average with 30%, while yields do decrease at large substitution rates (Ren et al., 2022). Another study showed that organic inputs in addition to mineral N did not affect leaching and runoff, but also did not improve yield (Wei et al., 2021). Application of easily available C via organic amendments can decrease N leaching by enhancing denitrification, but this might also decrease overall nutrient use efficiency (Qin et al., 2017). A meta-analysis showed a 13% decrease of nitrate leaching due to biochar addition although the mechanisms behind this remain unclear (Borchard et al., 2019).

Phosphorus leaching from soil due to various P fertilizers is mainly driven by the P surplus in the long-term and not by fertilizer type or composition (Blake et al., 2003; Eichler-Löbermann et al., 2007; Messiga et al., 2015; Lemming et al., 2019). In the case of long-term use of certain fertilizers, such as calcium-rich poultry manure (Lehmann et al., 2005), iron-rich sewage sludges, or organic P rich sources, specific P forms can be formed in soil with different leaching susceptibility (Liu et al., 2020). A long term experiment with animal manure resulted in a lower P sorption to the soil in comparison to six other types of organic resources (composts, sewage sludges) (Nest et al., 2016), implying a higher risk of P leaching of manure than other organic resources. For runoff, risk assessment tools such as P indices often rely on landscape and management factors to estimate the risk of losses (Osmond et al., 2017; Ros et al., 2020). Distance to the closest waterways, slope, the use of cover crops and the timing and method of (organic) fertilizer application are examples. Additionally, weather conditions like heavy rainfall after application of manure increases risk of surface runoff of N and P toward surface water and these events may contribute to a large extent to annual N and P leaching from applied manure (Van der Salm et al., 2012).

5.5 Contaminants

Application of biodegradable waste material is challenged with avoidance of contamination of the soil, especially with “emerging contaminants”, such as microplastics, nanoparticles and pharmaceuticals (Ng et al., 2018; Kacprzak et al., 2022). Therefore, the research on waste processing to produce safe and sustainable organic resources is still ongoing (Kurniawati et al., 2023), while at the same time recent EU legislation has banned synthetic polymer microparticles from various products (REACH EU 1907/2006) and fertilizing products (EU 2019/1009) within a transitional period of 5 years.

In Table 7 an overview of heavy metals in selected organic resources is presented. The heavy metal content is generally very low in struvite and meat and bone meal (Möller and Schultheiss, 2015), and higher in manure, compost and sewage sludge (Dach and Starmans, 2005; Smith, 2009; Kupper et al., 2014). The contents of Cu and Zn in animal manures are generally relatively high compared to those of other heavy metals. Cu and Zn in animal manure often originate from additives in feed (Adamse et al., 2017). The composition of these additives is controlled in the EU since 1970 (70/524/EEC) by limit values for Cu and Zn specific for each animal type, which have been reduced since then (EU, 2009). However, the limit values for Cu and Zn in the FPR are sometimes still exceeded, making these manures unsuitable for marketing as EU fertilizer. In contrast to Cu and Zn, the Cd and Pb contents in animal manure are low in comparison to compost and sewage sludge (Table 7).

TABLE 7 Average and/or range (minimum–maximum) of a selection of heavy metals (Cd, Cu, Pb, and Zn) of some organic resources in mg kg⁻¹ dry matter.

Organic resource	Cd	Cu	Pb	Zn	References
	mg kg ^{−1} dm				
Cattle slurry	0.40 (0.04–5.5)	42 (0.1–741)	5.6 (0.1–75)	207 (2.0–1,908)	Leclerc and Laurent, 2017
Pig slurry	0.30 (0.02–4.0)	193 (12–1,802)	3.0 (0.3–112)	934 (5.0–5,832)	Leclerc and Laurent, 2017
Poultry manure (broiler)	0.40 (0.1–1.2)	89 (8.4–760)	3.7 (1.0–24)	353 (52–790)	Leclerc and Laurent, 2017
Solid pig and cattle manures	0.30 (0.04–3.1)	23 (0.3–191)	3.8 (0.1–92)	119 (9.6–691)	Leclerc and Laurent, 2017
VGF compost ^a	0.46	47.3	62.7	181	Amlinger et al., 2004
VGF compost	0.78 (0.20–2.43)	69.3 (24.2–392)	55.5 (0.27–130)	275 (73.8–929)	Dittrich and Klose, 2008
VGF compost	0.1–1.3	15–120	5–75	0–240	Saveyn and Eder, 2014
Sewage sludge ^b	0.4–3.8	39–641	13–221	142–2,000	Amlinger et al., 2004
Sewage sludge	1.57 (0.96–3.63)	161 (99.4–234)	97.0 (26.1–285)	842 (390–1,445)	Dittrich and Klose, 2008
Meat and bone meal	0.29 (0.06–0.80) ^c	8.34 (1.66–23)	7.39 (0.01–27.7)	150 (115–174)	Dittrich and Klose, 2008
Limit PFC ^d 1 (3)	1.5 (2)	300	120	800	EU, 2019
Limit PFC 1B	3 ^e	600		1,500	

^aMedian bio-waste compost.

^bRange of means.

^cThe authors reported that for fertilizers containing > 5% P₂O₅, the Cd content is based on mg Cd kg⁻¹ phosphate.

^dProduct function category in FPR.

^eFor > 5%P₂O₅ the limit for PFC 1B (organo-mineral fertilizer) is set to 60 mg Cd kg⁻¹ P₂O₅ in EU (2019).

Levels of persistent organic contaminants, such as per- and polyfluoroalkyl substances (PFAS), polycyclic aromatic hydrocarbons (PAH), poly chlorinated biphenyls (PCB), dioxine-like PCBs (dl-PCB) and dioxines and dibenzofurans (PCDD/F), are normally very low in animal manure (Berset and Holzer, 1995; Munoz et al., 2021), but can be elevated in products such as sewage sludge (Munoz et al., 2021; Huygens et al., 2022) and compost (Brändli et al., 2007a,b; Saveyn and Eder, 2014; Huygens et al., 2020; Munoz et al., 2021). Limit values of organic contaminants have been set in the FPR for certain input categories (Component Material Categories: CMC) for the production of fertilizers (Table 8). There are concerns about the bioaccumulation and toxicity of pharmaceuticals from both manure and sewage sludge (Gworek et al., 2021). In some cases the use of pyridine herbicides that have a relative high toxicity for plants but not for grazing animals can result in herbicide levels in manure from grazing animals that are too high for use as a general fertilizer. This has resulted in proposed limit values for EU fertilizer products from animal manure for clopyralid and aminopyralid (Huygens, 2023). Some animals receive antibiotics that end up in animal manure. These may potentially leach from the soil or be harmful for soil biota (Rietra et al., 2023) if degradation is slow and sorption is low. Additionally, they may pose a threat to insects living in dung (Bruinenberg et al., 2023) and birds living on cattle farms (Buijs et al., 2022).

It has been estimated that 67 and 83% of sewage sludges (on the short- and long term, respectively) fails to comply with current limit values, if no pollution prevention is implemented (Aubain et al., 2002). For sewage sludge (ashes), techniques to extract phosphate are promoted by research (Canziani et al., 2023), which

seems especially relevant for sewage sludge that does not comply with the limit values.

In summary, many types of contaminants exist and their prevalence and concentrations vary with the type of fertilizing product, e.g., levels heavy metals and pharmaceuticals are higher in manure and other contaminants are more concentrated in e.g., sewage sludge.

6 Potential mitigation measures to reduce emissions from organic resources

6.1 Management measures

The 4R nutrient stewardship guidelines are a framework that describes practices for the application of fertilizers to increase nutrient use efficiency and reduce environmental footprints (Johnston and Bruulsema, 2014; Flis, 2017, 2018). The practices focus on fertilizer application using the Right source, the Right rate, at the Right time, in the Right place. Management of organic resources according to these principles has the potential to increase nutrient use efficiencies and mitigate emissions to the environment. According to a synthesis of several meta-analyses by Young et al. (2021), 4R strategies generally positively impacted crop yield, crop N uptake, and soil organic carbon (SOC), while reducing N₂O emissions, NH₃ emissions and N surplus. Replacing mineral fertilizers with organic resources increases the SOC content and results in reductions of N losses. Moreover, nutrient management generally has a larger effect on sustainability indicators compared to crop, soil and water measures (Young et al., 2021). The 4R

TABLE 8 Average and/or range (minimum–maximum) of a selection of organic contaminants (PCB, PAH, PFAS, PCDD/F) of some organic resources.

Organic resource	PCB ^a	PAH ^b	PFAS ^c	dl-PCB ^d	PCDD/F ^e	References
	μg kg ^{−1} DM	mg kg ^{−1} DM	μg kg ^{−1}	ng kg ^{−1} DM	ng kg ^{−1} DM	
Cattle slurry			0–1.7			Munoz et al., 2021
					0.39–20	Stevens and Jones, 2003
	< 20	0.087–0.309				Berset and Holzer, 1995
Pig slurry	< 37	0.066–0.339				Berset and Holzer, 1995
			0			Munoz et al., 2021
Poultry manure			0.4–1.9			Munoz et al., 2021
VGF compost	8.8–101.4	0.60–12.47	–	0.4–6.8	0.5–21.0	Brändli et al., 2007a,b
			2.2–225			Munoz et al., 2021
Sewage sludge			12–900			Munoz et al., 2021
					19.9–225	Stevens et al., 2001
	110–440	67–370				Stevens et al., 2003
	<400	1.7–15				Berset and Holzer, 1995
Limit CMC ^f 12-15	–	6	–		20	EU, 2019

^aSum of 7 PCBs: PCB 28, 52, 101, 118, 138, 153, 180, or sum of 11 PCBs (Brändli et al., 2007a,b).
^bSum of the 16 PAHs as mentioned in the FPR.
^cSum of the 160 PFAS compounds (see Munoz et al., 2021).
^dToxic equivalent (TEQ) of 12 dl-PCB compounds (see Brändli et al., 2007b).
^eToxic equivalent (TEQ) of 17 PCDD/F compounds as mentioned in the FPR.
^fComponent Material Categories in FPR.

nutrient stewardship guidelines can be based on a decision support tool approach using combinations of soil, crop, organic resource, and weather data (Section 7.2.1.) Decision tools can also include the risk of emissions and measures to mitigate these emissions, although currently most decision support tools only focus on fertilizer recommendations (Nicholson et al., 2020).

For animal manures and other organic resources, however, it is likely that proper 4R nutrient stewardship is more difficult to attain compared to mineral fertilizers. Animal manures, for instance, usually contain multiple nutrients in different ratios, which requires careful consideration of the right source to meet crop demand of certain nutrients without under- or overapplication of others. Herein lies an increased risk of pollution swapping, for instance between NH₃ volatilization, N leaching, and N₂O emissions, compared to mineral fertilizers.

Since nutrients are often (partly) in organic forms and mineralize over time, control of nutrient release from organic resources remains a challenge, as it is partly governed by external factors such as moisture and temperature. Repeated applications of organic resources increases N mineralization in following years (Schröder et al., 2007; Jensen, 2013; Bhogal et al., 2016; Sorensen et al., 2017). The right time of application is essential to limit the mineralization and loss of nutrients outside of the crop growing season. For manures however, the window of application may be limited by on-farm storage capacity. The right placement of organic resources (near the crop roots) can be more challenging since regulations or available techniques do not always allow this.

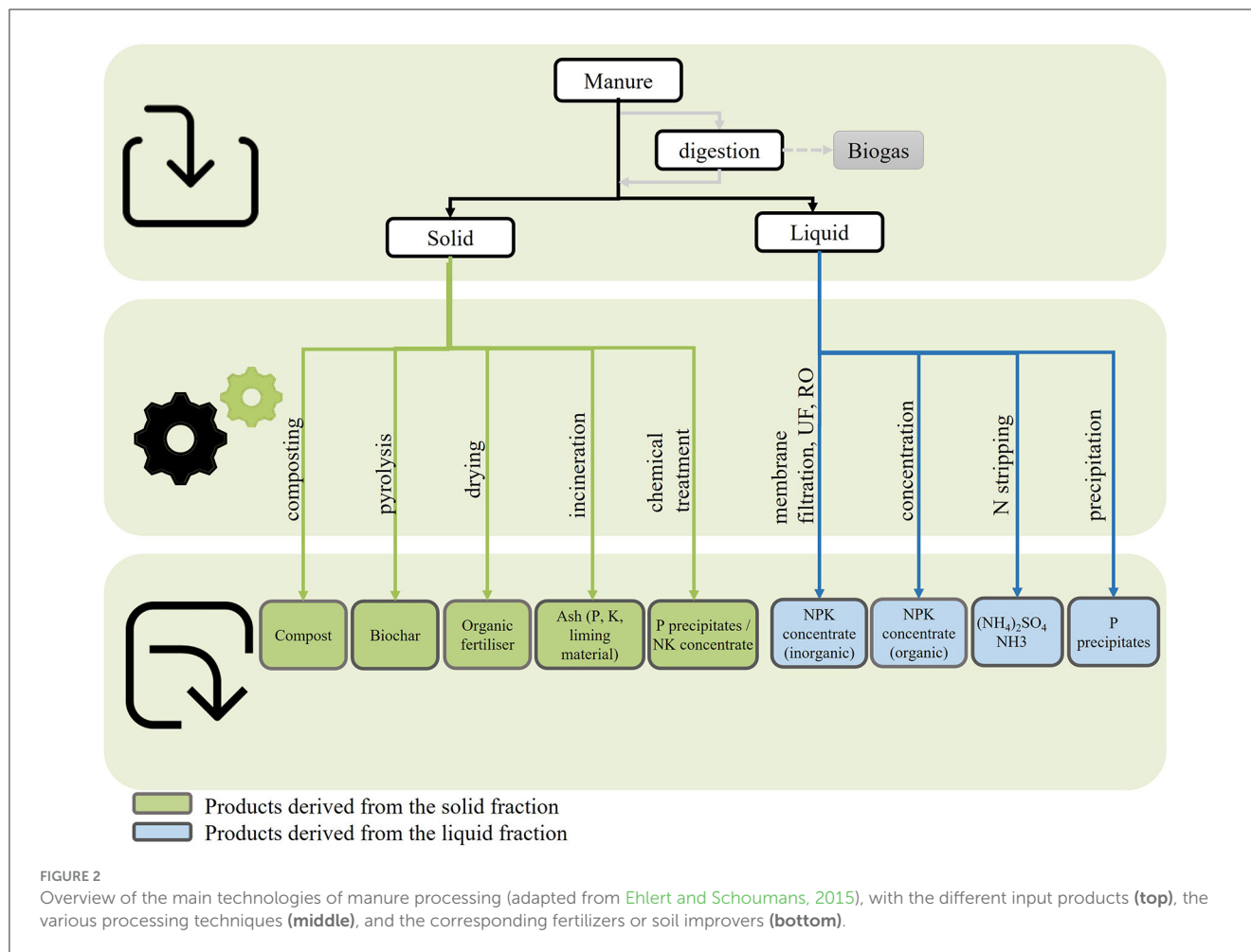
Proper implementation of 4R strategies in combination with new measurement and processing techniques have great

potential to reduce losses and increase nutrient use efficiency for organic resources, especially if they partly replace mineral fertilizers. Despite the universality of the 4R principles, optimizing strategies for application of organic resources requires tailoring them to specific environmental and management contexts (climate, soil type, cropping system, etc.). Herein still lies a big challenge.

6.2 Technological measures

6.2.1 Manure treatment

There is a wide array of available technologies to process manure (Figure 2). The development of anaerobic digestion for biogas production has given rise to digestate which is commonly used as fertilizer (Saveyn and Eder, 2014). In addition, innovative technologies were developed such as (physico-)chemical treatment, stripping, and membrane filtration to recover N and P and to produce soil conditioners (Lemmens et al., 2007; Foged et al., 2011; Ehlert and Schoumans, 2015). Separation technologies such as screw press, centrifuge, belt press with and without flocculants/polymers are used to separate manures into a liquid and solid fraction with different compositions (Aguirre-Villegas et al., 2019; Guilayn et al., 2019; Grell et al., 2023). Ammonium sulfate and ammonium nitrate are derived by stripping of ammonia with sulfuric acid or nitric acid, respectively (Lemmens et al., 2007; Foged et al., 2011; Ehlert and Schoumans, 2015). From the liquid fraction of manure, mineral NP(K) concentrates are produced using reversed osmosis technologies (Aguirre-Villegas et al., 2019; Guilayn et al., 2019; Grell et al., 2023). Evaporators



are used to produce ammonia solutions. From the solid fraction, the main recycled P products are struvite and sometimes calcium phosphate (Sigurnjak et al., 2019; Brienza et al., 2020). Recycled C-rich products such as biochars and other soil improvers are produced through drying, gasification or pyrolysis of the solid fraction (Rathnayake et al., 2023).

In addition to the aforementioned processes, innovative technologies are developed to alter the manure composition, such as permeable membranes, bipolar membrane electrodialysis, forward osmosis, biological reactors, ion exchange with selective resins and plasma technology (Graves et al., 2019). With these developments ultimately a palette of tailor-made fertilizers can be created that can be used to increase nutrient efficiency and reduce nutrient losses to the environment. Recovered ammonium nitrates and ammonium sulfates have higher NFRVs than pig manure and pig urine (Saju et al., 2023), and the P availability of separated solid fractions of manure is high as long as no metal salts such as iron salts are used during processing (Regelink et al., 2021). A constraint in the application of recovered products in EU is that according to the Nitrates Directive (EEC, 1991) all N products recovered from processed manure are still considered as manure and fall under the manure application standard of 170 kg N ha^{-1} (Huygens et al., 2020).

Table 9 shows examples of manure treatment products. The liquid fraction is a N fertilizer, low in P and OM. Reversed osmosis increases the nutrient concentration in the liquid fraction. The solid fraction is rich in OM and P, but also contains N. The fraction of total N that is present as mineral N was 71% in untreated pig slurry, 92% in the concentrated liquid fraction, and 46% in the solid fraction of this slurry in a study of Hoeksma and De Buissonjé (2020). This indicates that the N in the concentrated liquid fraction is significantly higher and more plant-available than the N of the solid fraction. Ammonium sulfate recovered via air scrubbing is a liquid N fertilizer without P and OM.

Incineration of poultry manure to generate energy produces ashes that can be used as PK fertilizer. Results of pot experiments with a crop show that in the short term the PFRV of poultry ash is about half that of regular mineral phosphate fertilizers, but that over a longer period, the value is similar to the value of regular fertilizers (Ehlert, 2020).

Biochar contains both C and nutrients. Rathnayake et al. (2023) concluded that pyrolysis of manure to create biochar would significantly reduce greenhouse gas (GHG) emissions from soils and create long-term soil C sinks. However, they also indicated that on basis of available studies it is difficult to draw conclusions about the value of biochar as a fertilizer.

TABLE 9 Examples of composition of products derived from manure treatment, as indicated in Figure 2 (incl. standard deviation).

Description of example	Content, g kg ⁻¹ product				Reference
	DM	N	P	OM	
Solid fraction	273	12.5	7.3	201	Hoeksma and De Buissonjé, 2020
Liquid fraction	2.0–7.6	32–171	2.4–17.1		Aguirre-Villegas et al., 2019
Digestate	81 ± 4	7.3 ± 0.7	1.7 ± 0.1	59 ± 3	Van Puffelen et al., 2022
Solid fraction of digestate	313 ± 3	12 ± 0.4	8.9 ± 0.8	242 ± 5	Van Puffelen et al., 2022
Liquid fraction of digestate	49 ± 2	6.8 ± 0.6	0.62 ± 0.07	32 ± 2	Van Puffelen et al., 2022
Composted solid fraction	261	8.1	1.3	205	Viaene et al., 2017
Granules	880	20.5	12.9	554	Römkens et al., 2018
Biochar from manures	400–600	6–22	10–79	50–750 ^a	Rathnayake et al., 2023
Poultry litter ash	903	0.3	52.8	33	Ehlert, 2020
Ash of solid pig and cattle manure	1,000		82–150		Møller et al., 2007
Struvite from chicken manure		18.1	70.6		Rech et al., 2020
Reverse osmosis concentrate	37 ± 5	8.1 ± 0.8	0.15 ± 0.13	14 ± 4	Van Puffelen et al., 2022
Ammonium sulfate from air scrubbing	140–330	30–86	0	0.3–0.4	Sigurnjak et al., 2019

^a Measured as C.

6.2.2 Application methods

Solid manures and composts are surface applied and are either left on top of the soil or incorporated. Livestock slurries are also surface applied to a large extent, although this results in high NH₃ losses. The risk of NH₃ losses are highest in the period shortly after surface-application of livestock manures. Surface application directly followed by plowing is an effective method to reduce emissions (Webb et al., 2014b). The longer the time between application and plowing, the larger the N loss as NH₃.

In addition to rapid incorporation after surface application, there are several application techniques available to reduce the risk of NH₃ emissions, including methods in which (i) slurries are band-placed on top of the soils, such as trailing hose and trailing show, (ii) slurries are injected a few centimeters into the soil, such as shallow or sod injection, and (iii) slurries are injected deeper below the soil surface (Bittman et al., 2014; Van der Weerden et al., 2021). Generally, the risk of NH₃ emission will decrease when slurries are injected (Section 5.1), but risk of N₂O emission may increase (Section 5.2). The N losses through NH₃ volatilization are larger than those via N₂O emission, and often also larger than those through total denitrification to N₂, implying that applying manures with low NH₃ emission techniques may lead to higher nutrient use efficiency than surface application (Huijsmans et al., 2016).

Diluted slurries or liquid fractions of treated manure can be applied via irrigation, using irrigation or fertigation systems (Misselbrook et al., 2004; Guido et al., 2020). Injection reduces the risk of NH₃ emission from application of N concentrates derived from manure treatment. However, these products have a much higher N content than livestock slurries (Table 9), which means that less volume has to be applied to obtain the required nutrient application rate. Development of new precision injection techniques, based on mineral fertilizer application techniques, is needed for adequate application of N concentrates (Van Middelkoop and Holshof, 2017). Besides increasing nutrient use

efficiency, precision application of organic resources may also reduce odor nuisance and crop contamination.

Developments in soil and crop sensing data and rapid manure analytical methods (Section 3) in combination with GPS techniques can be used in site-specific precision N fertilization techniques (e.g., Corti et al., 2023). In addition to the differentiation of rates and sources of the application of organic resources among different fields, within-field establishment of zones with different yield potential may help increase the nutrient use efficiency from organic resources (Kharel et al., 2019) if it can be combined with accurate application of the fertilizer.

6.2.3 Inhibitors and additives

Urease inhibitors (UI) delay urea hydrolysis and may therefore have the potential to reduce NH₃ emission from manure (Li et al., 2017). Yet, addition of UI shortly after excretion in livestock housing systems is practically challenging, and as most urea in manure is hydrolyzed during storage, adding UI at application is unlikely to result in large reductions in NH₃ emission and N losses from soil-applied manures. An exception may be dried poultry manures, because of the relatively large part N present as uric acid (Nicholson et al., 1996). Nitrification inhibitors (NI) inhibit the activity of nitrifying bacteria, thereby reducing the risk of both NO₃⁻ leaching and gaseous N₂O emissions. A meta-analysis has shown positive effects of inhibitors on crop yields, and a decrease in N losses of 32.9, 14.5, 37.6%, respectively by UI, NI or combinations of UI and NI, in mineral and organic resources (Sha et al., 2020). Addition of the nitrification inhibitor 3,4-dimethyl pyrazole phosphate (DMPP) to cattle slurry reduced N₂O emission with 30–40% (Dittert et al., 2001; Herr et al., 2020). Studies indicate that the inhibitors have no impact on non-target microbial composition or abundance (Duff et al., 2022), but might

affect free living N fixation (Liu et al., 2024). Studies on potential adverse effects of inhibitors on the soil microbial community are mostly short-term, and assessments over longer timeframes are still lacking.

It is often suggested that the use of biological or chemical manure additives, such as minerals, microorganisms, charcoal, or plant extracts may reduce NH_3 and GHG emissions while research often shown very limited effects (Wheeler et al., 2010), and sometime even increased emissions (Van der Stelt et al., 2007).

A meta-analysis of slurry acidification has shown that acidification can decrease emissions of NH_3 , NO_x , CH_4 by 69, 21, 86%, respectively, while various other management strategies such as biological treatment, separation strategies, different storage types can decrease NH_3 emissions but increase emissions of a least one other GHG (Emmerling et al., 2020). Trade-offs (Section 7.1.2) may occur as acidification of slurry with sulfuric acid may increase the risk of sulfate leaching to groundwater and acidification of slurry reduces NH_3 emissions but may increase risk of N_2O emission (Velthof and Oenema, 1993; Loide et al., 2020).

Clearly, there is scope to reduce N losses and increase nutrient use efficiency from organic resources by adding inhibitors and additives. These products are not always effective and may have negative side-effects that have to be considered.

6.3 Structural measures

Structural measures such as the reduction or relocation of livestock and crop production are an option for improving nutrient and C management and to reduce emissions from agriculture. In comparison to technical and management measures, structural measures generally refer to more drastic changes in the agricultural system, e.g., through transitioning toward lower livestock numbers, relocating or shifting livestock types, or changing land uses and cropping systems. Structural measures will need to be taken if large scale implementation of technical and management measures as shown in Sections 6.1 and 6.2 are not sufficient to reach the environmental targets. Besides their relevance for reaching environmental targets, structural measures will impact the nutrient demand (e.g., for fertilizing cropland) and nutrient supply (e.g., from livestock manure), which need to be considered in the future management of organic resources. The examples of studies exploring reduction potentials at the national or European scale in Table 10 show large ranges but also a high reduction potential for structural measures.

6.3.1 Changing livestock numbers

Various studies have explored the mitigation potentials of structural changes in livestock numbers. Studies on livestock relocation focused on the effects of redistributing livestock to areas where they cause lower environmental and human health impacts. Figure 1 shows large differences in livestock manure application due to variation in livestock density across the EU. In their model study, Van Grinsven et al. (2018) show that relocating the pig industry within the EU-27 can reduce emissions of NH_3 and N leaching by ~10% in source regions, but may simultaneously

increase emissions in receiving regions by 40 and 20%, respectively, for NH_3 and N leaching. Although the authors found that relocation resulted in an overall decrease of N emissions and external N costs at EU level, they also noted that relocation comes with socio-economic barriers and redistribution of pollution.

Another example of structural measures that can have a positive impact on reducing emissions is shifting livestock types. For instance, one study found that shifting from monogastric livestock to ruminants led to a decrease in N and GHGs by 2 and 5% GHG emissions given the lower demand for cropland areas and lower fertilizer inputs in ruminant production systems (Cheng et al., 2022). In contrast, other studies found that shifting away from ruminant livestock production led to reductions of GHG emissions (Aleksandrowicz et al., 2016; Grummon et al., 2023).

Larger mitigation potentials were found through measures that significantly reduce livestock numbers the livestock density in the Netherlands is high, resulting in high manure inputs (Figure 1) and resulting in a manure surplus and exceedance of environmental targets of emissions of atmospheric pollutants and water quality. Van Selin et al. (2023) estimated that reducing livestock numbers to the level that pigs, poultry and cattle can be fed with domestic feed grown in the Netherlands, would require a large reduction in livestock ranging from 29 to 100%, depending on livestock types (Table 10). This reduction would significantly reduce NH_3 and GHG emissions in the Netherlands by 47 and 27% respectively (Table 10). Gies et al. (2023) found that a reduction in Dutch dairy cattle of 7–34%, reduced the NH_3 , and GHG emissions from the agricultural sector in the Netherlands by 2–9.5%, and 4–19%, respectively.

6.3.2 Changing land use and cropping systems

A few studies investigated mitigation potential through land use changes and changes in cropping systems. One option to mitigate GHG emissions is the rewetting of peat soils, e.g., through taking peatland out of agricultural production or using shallower water tables (Boonman et al., 2022). Another structural measure is the spatial relocation of crop production, e.g., from areas where biodiversity and potential C stocks are high in order to reduce environmental impacts and biodiversity loss in more sensitive areas (Beyer et al., 2022). Furthermore, local interventions such as the introduction of buffer zones or the construction of wetlands can help prevent nutrient losses from agricultural soils to surface waters (Van den Broek et al., 2007). In the Netherlands, N application standards in sandy soil regions, prone to leaching, is stricter than in other regions (Van Grinsven et al., 2016), which may cause that some cropping systems will move from one region to another.

6.3.3 Combined interventions

Structural changes and technical improvements are not mutually exclusive and multiple studies explored their combined effects (Table 10). For instance, Van Grinsven et al. (2015) found that an extensification of the Dutch agricultural system through both reducing livestock numbers by 20–50% and reducing N fertilizer inputs by 40% led to significantly lower national ammonia emissions and nitrate leaching (Table 10). De Vries et al. (2023) showed that the combination of technical measures (e.g., low

TABLE 10 Examples of studies that examined emission reduction potentials associated with structural changes in agriculture.

Structural change	Description of measures	Overall emission reduction potential	References
Livestock relocation	Relocating pig industry within EU-27	10% NH ₃ emissions, N leaching, N runoff in source regions	Van Grinsven et al., 2018
Shifting livestock types	12% switch from monogastric to ruminant livestock at global scale	2% N emissions, 5% GHG emissions	Cheng et al., 2022
Livestock reduction	Recoupling livestock to domestic feed supply in the Netherlands resulting in reduction of dairy cattle (–29%), broiler chickens (–57%), pigs (–62%), laying hens (–67%), beef cattle (–100%), and sheep (–100%)	47% NH ₃ emissions, 27% GHG emissions	Van Selm et al., 2023
	Reducing dairy cattle to a maximum number of livestock units per hectare	2–10% NH ₃ emissions, 4–20% GHG emissions	Gies et al., 2023
Livestock reduction combined with improved management, technological measures and other structural changes	Extensification of Dutch agriculture reduction of livestock and N fertilizer inputs	37% NH ₃ emissions, 58% NO ₃ leaching	Van Grinsven et al., 2015
	Changes in diet (demitarian), only local grass and forage crops for livestock, reconnecting livestock (on average 50% less livestock), no artificial fertilizers	30–68% N flows from land to sea, 1–10% P flows from land to sea	Desmit et al., 2018
	Agro-ecological scenario for Europe with livestock by reconnection of livestock with cropping system following optimal fertilizer use	57 % cropland N soil surplus	Billen et al., 2021
	Combination of reduction in livestock numbers by 25%, improved management and technological measures	40–50% NH ₃ emissions, N leaching, N runoff, 30% GHG emissions	De Vries et al., 2023
	Combination of structural, management, technological and spatial planning measures including livestock reduction by 20%	30–34% NH ₃ emissions, 1–2% N/P runoff, 20–21% GHG emissions	Kros et al., 2024

emission housing, application techniques), improved crop, soil and nutrient management, and a reduction in livestock in the Netherlands by 25% reduced N emissions and GHG by 40–50% and 30%, respectively. A more recent study showed that reducing livestock, with additional structural measures such as buffer zones along waterways, and combined with management and technical measures, can reduce emissions by 30–40% and GHG emissions by 20% (Kros et al., 2024). The latter studies highlighted that a combination of technical and management improvements, together with livestock reduction and spatial interventions (e.g., buffer strips) are needed to significantly reduce emissions and reach environmental targets in the Netherlands. The studies also emphasized that improved management alone was not sufficient to mitigate GHG emissions and nutrient losses from livestock production in order to reach long-term climate goals which was also suggested by Weishaupt et al. (2020). Therefore, implementation of structural changes will be crucial in order to reach emission targets and effectively mitigate agricultural pollution.

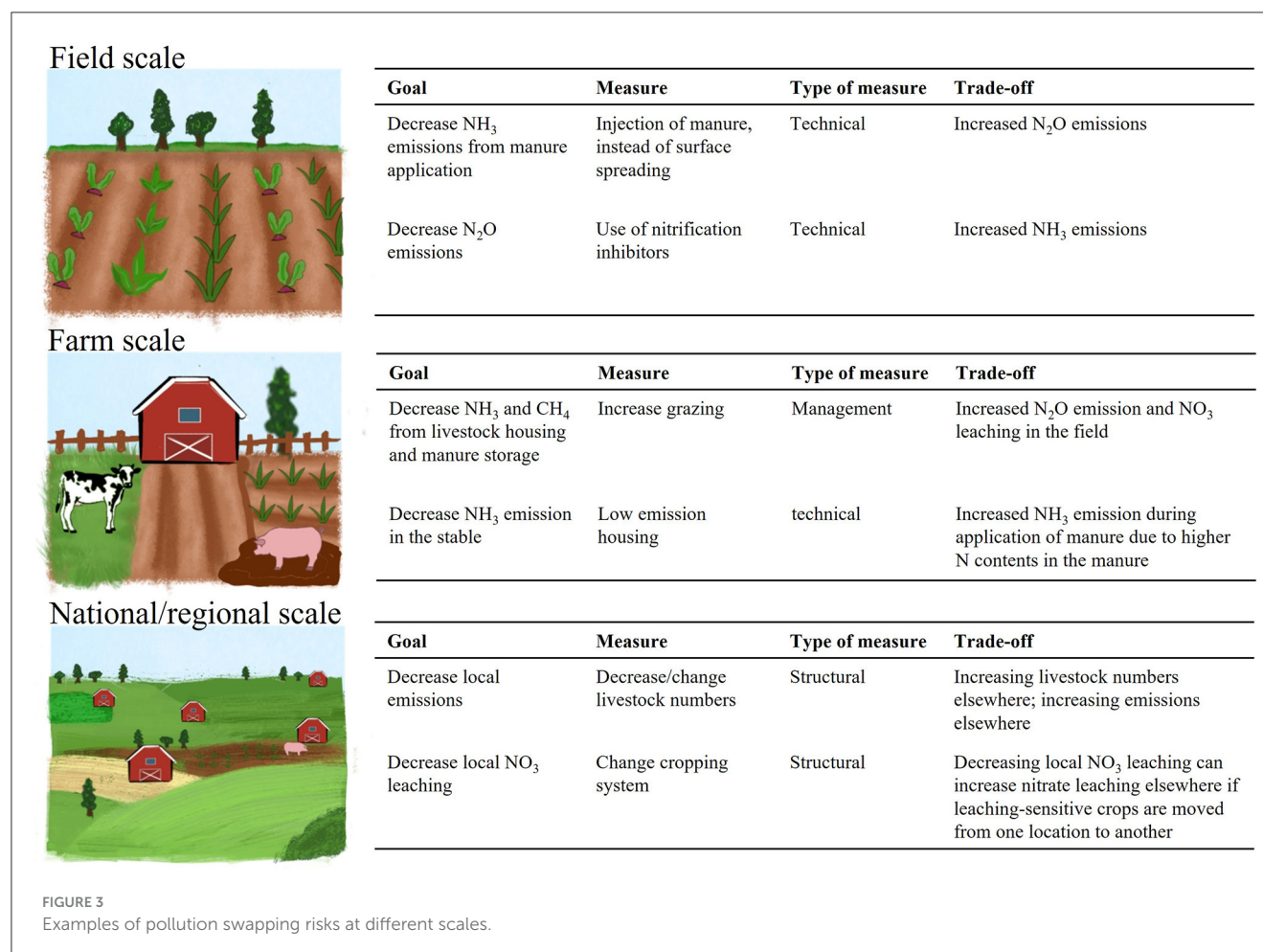
These examples show that in regions with a high livestock density and manure surplus, the implementation of structural changes next to technological and management measures will be needed in order to effectively mitigate agricultural pollution in Europe.

7 Future challenges to support farmers and policy makers

7.1 Risk of trade-offs between measures

A combination of measures have to be taken to increase nutrient use efficiency, improve soil quality and reduce gaseous emissions and leaching from organic resources so that the targets of the different environmental policies can be met. Combinations of measures may have synergies and strengthen their effects; however there is also a risk of trade-offs or pollution swapping. Different types of pollution swapping between measures can take place (Figure 3). Examples of the different types of pollution swapping risks are:

- Between management strategies, e.g., grazing decreases the risk of NH₃ and CH₄ emissions, but increases risk on NO₃ leaching and N₂O emission (Bussink, 1994; Velthof et al., 1996; Corré et al., 2014);
- Between technical measures; e.g., slurry injection reduces NH₃ but increases N₂O emission (Velthof and Mosquera, 2011; Goedhart et al., 2020) and acidification of slurry reduces NH₃ emissions but may increase sulfate leaching or N₂O emission (Velthof and Oenema, 1993; Loide et al., 2020);



- Within a farm; e.g., low NH₃ emission housing increases the ammoniacal N content in manures and because of that increases the risk of NH₃ emission when the manure is applied (Bittman et al., 2014);
- Within a country; e.g., in the sandy regions in the South of the Netherlands strict N application standards are applied for crops with a high nitrate leaching risk (Van Grinsven et al., 2016). This may stimulate the growth of these crops in the sandy regions in the North with higher N application rates and because that may increase leaching in these regions;
- Between countries; e.g., high environmental pressure and strict environmental rules for pig production in one country (e.g., the Netherlands) may increase the number of pig farms in another countries (e.g., Spain).

Clearly, avoiding pollution swapping and finding synergies in the implementation of combinations of measures is challenging. If sufficient information on organic resources is available, potential trade-offs can be taken into account. For example, Velthof and Mosquera (2011) calculated the total effect of application method on N₂O emissions, taking into account indirect emissions from NH₃ volatilization and the need for extra mineral fertilizers due to loss in NFRV after surface application. Based on their results and IPCC emission factors, they showed that, although

injection of cattle slurry on grassland can lead to higher direct N₂O emissions than surface spreading, the indirect emissions from NH₃ volatilization and the additional mineral fertilizer need result in higher total N₂O emissions for surface application (Velthof and Mosquera, 2011). This effect was not the same for pig slurry on arable land due to the high emission factor for injection of pig slurry, which shows that trade-offs are often heavily context dependent. In-depth knowledge on the composition, agronomic value and risks on the emissions of organic resources to the environment is therefore needed to derive effective nutrient management strategies. This especially holds for new waste products from the circular economy and new products from manure treatment (Figure 2), as the agronomic and environmental consequences of the use of many of these products is not yet clear.

7.2 Challenges for farmers

Fertilizer recommendations and decision support tools are important sources of information for farmers to increase nutrient use efficiency and decrease nutrient losses. Recent papers show that there is a large diversity in recommendations and tools between countries in the EU and that harmonization, shared

learning, and a collective approach is recommended to tackle environmental problems (Nicholson et al., 2020; Higgins et al., 2023). The EU FAST tool (<https://fastplatform.eu/>) is developed for European Commission's DG Agriculture and Rural Development as nutrient management tool to support EU farmers, farm advisors, developers of digital solutions, and policy makers. This tool contains different modules, including a fertilizer module. Besides nutrient management tools, tools are also developed to calculate soil C sequestration and GHG emissions from farms (Arulnathan et al., 2020; Alexandropoulos et al., 2023), such as the Cool Farm Tool (Hillier et al., 2011) (<https://coolfarm.org/>). There is also an increasing interest of the food industry in these type of tools, as many industries have made commitments to decrease their GHG emissions. In the coming decade, farmers might be subject to an increasing level of accountability for the environmental footprint of their farm and stimulated to use aforementioned tools, by both governments and industry partners. This can give rise to new challenges because of the extra administration and record keeping these tools demand or because of management changes required to achieve set targets.

Fertilizer recommendations and decision support tools require information about the composition (Sections 2.1 and 3.1), the plant-availability of nutrients (Section 4.1) and degradability of OM of organic resources (Section 4.2). Ideally, analyses of the composition and nutrient availability of organic resources in combination with (real-time) data on soils, crop and weather are used, but at least standard values for a range of different organic resources have to be available for reliable recommendations (Nicholson et al., 2013). If tools are also used for estimating the risk of gaseous emissions and leaching, information on emission factors of NH₃ and GHG for organic resources and coefficients for calculation of leaching are required (Section 5). Emission factors can be derived from default values of the EMEP/EEA air pollutant emission inventory guidebook, and IPPC guidelines, or from country specific emission factors. Farmers should also be aware about the potential risks of contaminants present in the organic resources, especially when they use these fertilizers frequently (Section 5.5). The use of farm specific emission factors and coefficients would improve the accuracy of emission calculations, using for example process-based soils models (Heinen et al., 2020).

The rapid development of analytical techniques for composition of organic resources, soils, crops, weather, and emissions to the environment, will further improve farm specific nutrient management strategies and DSS-tools. However, there are large challenges regarding the interpretation and integration of the huge amount of data that will be derived from these new techniques. Ultimately, they have to lead to scientifically underpinned and clear recommendations to farmers about daily field operations.

Lastly, the implementation of measures, that for example promote manure treatment or change of animal housing systems, is challenged with financial and practical barriers. Building manure processing installations or new stables is expensive and requires permits; all in all, it usually takes several years before such a change is implemented. For the biogas sector the key barriers are the lack

of financial incentives and policy frameworks that ensure profitable operation until at least the investment is paid back (Kampman et al., 2017).

7.3 Challenges for policy makers

7.3.1 Monitoring of emissions

EU countries are obliged to report gaseous emissions of atmospheric pollutants and GHGs to the European Commission and United Nations. Emissions of NH₃, NO_x, fine particles and non-methane volatile organic compounds (NMVOC) have to be reported to the EU (NEC; EU, 2016) and United Nations [UNECE; Convention on long-range transboundary air pollution/Gothenburg protocol (UN, 2013)]. The emission of GHGs have to be reported as part of the Paris Climate Agreement to the United Nations Framework Convention on Climate Change (UNFCCC; UN, 2015). Moreover, EU countries have to write a progress report about implementation of the Nitrates Directive every 4 years, which includes data on water quality and nutrient use, and implementation of nutrient management measures.

The calculation methods for monitoring of atmospheric pollutants are presented in the EMEP/EEA air pollutant emission inventory guidebook (EEA, 2023). The calculation methods of GHG emissions are presented in the IPCC guidelines (IPCC, 2019). Both guidelines include different methodologies for calculation of emissions: Tier 1 (default method and emission factors), Tier 2 (default method and country specific or technology-specific emission factors), and Tier 3 (country specific calculation method). The EMEP/EEA guidebook differentiates Tier 1 emission factors of NH₃ and NO_x for application of manures, sewage sludge and other organic N resources (including composts and digestates). The Tier 1 and Tier 2 emission factors for NH₃ are differentiated for livestock and manure types (in total 20 categories). The IPPC guidelines include one default aggregated N₂O emission factor for all N sources and disaggregated emission factors for all organic N fertilizers. For accurate emission estimates and effective mitigation, country-specific, management-specific, and organic resource-specific emission factors for production, storage and application are needed. Studies aiming to derive emission factors for soil application have to follow protocols or general rules accepted by scientists, e.g., the VERA protocol for NH₃ and the Global Research Alliance N₂O chamber methodology guidelines (Vera, 2009; De Klein et al., 2020). There are large challenges to obtain emission factors for organic resources. These challenges will further increase because of the increasing number of organic resource types from manure treatment and new waste streams, with different product-specific emissions.

Combining data sources of different countries in large databases will improve estimates of emission factors (Beltran et al., 2021; Van der Weerden et al., 2021). Moreover, such databases create perspectives for the derivation of emission factors that take more controlling factors into account than the current simple emission factors based on N input and can be used to improve emission estimates from organic resources with models, such as the FAN, ALFAM-2, and DNDC (Li et al., 2012; Hafner et al., 2019; Vira et al., 2020).

7.3.2 New organic resources

It is expected that (new) organic resources from manure treatment, food processing, sewage sludge, and municipal bio-wastes will become available for use in agriculture as fertilizer or soil conditioner. Similar approaches to those described in the previous section to obtain emission factors for gaseous emissions are also needed for agronomic value of fertilizers, e.g., the NFRV (Section 4.1) and HC (Section 4.2). Policies often use these indicators for measures, such as the N application standards in the Nitrates Directive or calculations for carbon farming. These indicators were also used for setting up criteria for the use of treated manure above the “170 kg N per ha” threshold for manure application in the Nitrates Directive (Huygens et al., 2020).

Besides the agronomic value and risk of emissions of new organic resources, it is also very important to get insight into the possible presence of contaminants in these fertilizers to avoid soil pollution. The EU FPR provides rules for the use of new fertilizers.

8 Conclusions

There are huge challenges in maximizing the use of organic resources and meanwhile meeting all the targets on emissions of atmospheric pollutants, GHGs, water quality, and contaminants. In EU27+UK, nutrient (N, P) inputs in soil through organic resources roughly equal those of mineral fertilizers, but there are large regional differences in the EU in use of these fertilizers (Figure 1). The availability of organic resources from food processing, sewage sludge, and municipal bio-wastes is expected to strongly increase over the coming decade (Section 6.2.1). Also manure treatment will further develop, resulting in organic resources with different agronomic value and environmental impact (Section 6.2.1).

The rapid development of analytical techniques can be applied to enhance the knowledge of the use of organic resources and the presence of contaminants (Sections 3 and 5.5). There are, however, many challenges in the accurate sampling of the fertilizers and the handling/interpretation data generated. There is a need to link easily obtainable parameters to agronomic performance (FRVs and HCs) and environmental risk of losses. Insight in the chemical properties to explain and predict variability in the coefficients as well as standardization of determination methods is required for adequate fertilizer recommendations of new and existing products (Sections 4.1 and 4.2).

Both effective management and technological measures are available and often tested (Sections 6.1 and 6.2). Structural measures will have a large social and economic impact on farmers and regional communities, but can solve environmental issues at local and regional scales (Section 6.3). Synergies and trade-offs between measures may occur at different scale and between different types of emissions (Figure 3).

There are many EU policies dealing with the use of organic resources, but with a different focus, i.e., on atmospheric pollutants, GHGs, water quality, and contaminants. The implementation of these fragmented policies on national to local scale is often only partly effective. An integrated nutrient management approach addressing all the objectives of the single policies is needed. Such an approach (Integrated Nutrient Management Action Plan; INMAP) is already proposed by the European Commission, as part of the Farm-to-Fork strategy.

The main future challenge for organic resources in agriculture is an integrated nutrient management approach, including (i) the characterization of organic resources, their agronomic value and their environmental risks, (ii) knowledge of potential synergies and the risk of trade-offs between nutrient management measures, and (iii) implementation of this knowledge into (emission) models, inventories, and legislation to support farmers and policy makers.

Author contributions

GV: Conceptualization, Writing—original draft, Writing—review & editing. TC: Writing—original draft. JH: Writing—original draft. JL: Software, Writing—review & editing. ML: Writing—original draft. RP: Writing—original draft. MR: Writing—original draft. RR: Writing—original draft. OS: Writing—original draft. LV: Visualization, Writing—original draft. DW: Writing—original draft.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

EDITED BY

David Robert Chadwick,
Bangor University, United Kingdom

REVIEWED BY

Arturo F. Chica,
University of Cordoba, Spain
Inga Grinfelde,
Latvia University of Agriculture, Latvia

*CORRESPONDENCE

María Pilar Bernal
✉ pbernal@cebas.csic.es

RECEIVED 27 February 2024

ACCEPTED 10 May 2024

PUBLISHED 27 May 2024

CITATION

Bernal MP, Álvarez-Robles MJ,
Bernal-Molina P and Clemente R (2024)
Bottom ash from combustion of chicken
manure as a fertiliser material.
Front. Sustain. Food Syst. 8:1392445.
doi: 10.3389/fsufs.2024.1392445

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Bottom ash from combustion of chicken manure as a fertiliser material

María Pilar Bernal*, María José Álvarez-Robles,
Paula Bernal-Molina and Rafael Clemente

Centro de Edafología y Biología Aplicada del Segura, CSIC, Murcia, Spain

The accumulation of excess manure and the energy costs of the buildings housing the animals are the most important problems that chicken farms have to face nowadays. The combustion of chicken manure to obtain thermal energy for animal holdings has been demonstrated through the research project AVIENERGY, in Spain. To ensure the circularity of the process, the combustion by-product, bottom ash, needs to be recycled and used. This work studies the agricultural use of ash from chicken manure combustion as a fertiliser product and determines its capacity for mineral fertiliser substitution. Ashes from co-combustion of chicken (broiler) manure with wood at different proportions were chemically analysed to determine their nutrient concentrations and availabilities. Then, one ash sample was used as a fertiliser for lettuce cultivation in a pot experiment. Different application rates were tested according to the phosphorus (P) content of the ash, being equivalent to 50, 65, 80 and 100% of the standard inorganic P fertilisation. The results indicate that the ash from combustion of chicken manure meets the requirements for inclusion in the fertiliser component material category 'CMC 13', and can be used for the preparation of fertiliser 'PFC 1(C)(I)(a)(ii): Compound Solid Inorganic Macronutrient Fertiliser (multi-nutrients)', and also possesses characteristics of "PFC 2: Liming Material" according to the EU legislation for fertiliser products. Although plant production was slightly reduced with ash fertilisation, the ashes reached a fertiliser capacity able to substitute 80% of the mineral P fertiliser, with a liming capacity of 30%. Hence, the ash from chicken manure combustion could be applied to soil, especially if acidic, in partial substitution of mineral fertilisers, to supply P.

KEYWORDS

ash nutrients, fertiliser efficiency, mineral fertiliser substitution, neutralising capacity, phosphorus, plant nutrition, recovery efficiency

1 Introduction

The poultry sector is the largest meat producer in the world, with more than 127 mT/y, followed by pork with more than 120 mT/y (FaoStat, 2021). However, in Europe the largest meat production is of pork (30.8 mT/y), followed by poultry (21.5 mT/y), which has experienced the greatest growth in recent years, due to the increase in demand for chicken and turkey meat as well as eggs (FaoStat, 2021). This has triggered the problems associated with the accumulation of animal manure in farms, which entails a serious environmental and economic challenge for the farms. As a consequence, poultry farmers are increasingly forced to optimise manure treatment practices based on circular economy criteria. Poor manure management practices can trigger harmful effects on the environment (contamination of soils

and aquifers, and gas emissions; Burton and Turner, 2003). Furthermore, feed production is considered the main factor responsible for greenhouse gas (GHG) emissions in the poultry meat sector, together with changes in land use, energy consumption and manure management and storage (Zisis et al., 2023). Manure management, therefore, represents one of the most relevant problems in the poultry sector nowadays. Energy use is also an important source of GHG emissions in poultry production, both for eggs and meat (Zisis et al., 2023), as it is required for ventilation, feeding, lighting, egg collection, sorting, heating and operation of the mechanical equipment.

The direct application to agricultural soils is the most frequent use of animal manure, which, when well managed, is a correct way to recycle organic matter and nutrients in the soil–plant system. The use of organic wastes, such as manure, as a source of organic matter for soils acts on the soil–plant system, both directly stimulating plant development and improving plant nutrition. It also indirectly increases the water retention capacity and decreases the risk of erosion of the soil. However, without adequate management, pollution and environmental impact problems may occur (e.g., salinity, nutrient imbalances, dispersion of pathogens, odour and gas emissions, etc.; Burton and Turner, 2003). Animal manure is not considered an equilibrated fertilising material in terms of nutrient composition, and excess P can be applied to the soil when poultry manure is applied based on crop N requirements (Bolan et al., 2010). To address all these issues, the Spanish Operational Group AVIENERGY aimed to enable an efficient use of manure generated in poultry farming, through the implementation of a thermochemical combustion process for the production of thermal energy on farms. The objective was to improve the competitiveness and reduce the environmental impact of the poultry sector in areas with high livestock loads, where manure production exceeds greatly the capacity of the agricultural soil for its use. To ensure the circularity of the process, the combustion by-product, bottom ash, needs to be recycled and used.

According to the European List of Wastes (European Commission, 2000, 2014), bottom ash generated from biomass combustion is identified as a solid waste (code 100101). The biomass ash can be used in the construction sector as a cement replacement in mortars (Modolo et al., 2017), and for soil stabilisation, as a structural filler and additive in synthetic aggregates (Gomez-Barea et al., 2009). Also, ash can be used in agriculture as a nutrient source or liming material (James et al., 2012; Silva et al., 2019), or even as a buffer for control of pH in anaerobic digestion (Novais et al., 2018). The application of ash to agricultural soils allows the recycling of essential nutrients in the soil–plant system (Schiemenz and Eichler-Löbermann, 2010; Omil et al., 2011). In addition, the direct application of ashes to soil can be an easy form to supply nutrients, such as P from ash, instead of using more complicated methods such as their extraction with acid solutions (Zeng et al., 2021). However, several concerns have been highlighted regarding the application of combustion ash to agricultural soils, such as the presence of metals, organic contaminants, water soluble salts (that can cause a potential increase in soil salinity) and potentially toxic elements such as chloride (Demeyer et al., 2001; Vassilev et al., 2013; Huygens et al., 2019; Silva et al., 2019).

The European legislation for fertiliser products (EC Regulation, 2009) has recently added the materials from thermal oxidation to the list for component material category CMC 13 for EU fertiliser products (EU Commission, 2021), with specific quality requirements. Ash materials

obtained through thermochemical conversion under non-oxygen-limiting conditions from Category 2 of animal by-products, such as animal manure (EC Regulation, 2009), can be included in EU fertilising products. However, the characteristics of the combustion ashes are not homogeneous and depend on the input material and the combustion conditions (Demirbas, 2007).

In the present study, the starting hypothesis was that the ash material remaining after combustion of chicken manure can be a valuable fertiliser material for farmers, to substitute for mineral fertilisers. Then, the objective of this work was to determine the characteristics and the capacity for mineral fertiliser substitution of the bottom ash obtained from the combustion of chicken manure at the farm level, in order to establish its possible use as a fertiliser product.

2 Materials and methods

2.1 Ash samples collection and analysis

Ash samples from co-combustion of chicken (broiler) manure with wood chips at different proportions were chemically analysed to determine their nutrient concentrations. Ash samples were obtained directly from the combustion system built in a chicken farm to obtain thermal energy. The burner (50 kW) was adapted to the starting material with a fixed grill, but with a mobile cleaning system to avoid the accumulation of the combustion material at the entry area and to facilitate the correct distribution of the aeration throughout the grill. Mixtures with different proportions of chicken manure and wood chips were burnt in the farm system. The proportions were (by weight): manure at 100%, manure:wood at 50:50 and 60:40 and wood at 100%. The resulting bottom ashes were sampled. An initial ash sample of the 50:50 manure:wood mixture was obtained during the optimisation of the burning system, while the rest of the ash samples were obtained after equipment optimisation. The ash samples were fractionated into three particle sizes: > 4 mm, 2–4 mm and < 2 mm. The >4 mm fraction was discarded due to the presence of impurities, mainly from unburned material. The 2–4 mm fraction was ground to <2 mm and incorporated into the smaller fraction, to obtain a homogeneous sample.

The phytotoxicity of the ashes was determined by a germination test with seeds of *Lepidium sativum*, using 1:10 (w:v) water extracts and dilutions with distilled water to obtain extract concentrations of 12.5, 25, 50 and 75%, in addition to the undiluted extracts (100%). Ten seeds were placed in a Petri dish (11 cm in diameter) containing filter paper with 2 mL of the corresponding extract or distilled water (control); ten Petri dishes were used for each extract concentration and the control. After 48 h of incubation in the dark at 25°C, the germinated seeds were counted and the root length of each germinated seed measured. The germination index was calculated by multiplying the percentages of root length and germination, both with respect to the control (Zucconi et al., 1981).

2.2 Pot experiment

Ashes from the 50:50 manure/wood mixtures were used in a pot experiment as a P fertiliser. The selection was based on the difficulties

found for the combustion of 100% manure associated to the low bulk density, making the mixture with wood more feasible from a practical point of view. Pots (14.5 cm in diameter) were filled with 1.5 kg of an agricultural soil. The soil was a sandy loam of pH 7.5 with 7.8% CaCO_3 , electrical conductivity of 0.230 dS m^{-1} , 6.7% organic matter, 3.9 g kg^{-1} total organic carbon (TOC), 2.03 g kg^{-1} total-N and 15.8 mg kg^{-1} available-P. Six treatments were run: non-fertilised control soil, mineral fertiliser and four different application rates of ash, equivalent to 50, 65, 80 and 100% of the phosphorus (P) supplied in the mineral fertiliser treatment; these doses were equivalent to 262, 350, 437 and 525 kg of ash per ha, respectively. The mineral fertiliser treatment comprised a 15:15:15 N:P:K fertiliser and KNO_3 , equivalent to 130 kg N/ha , $40 \text{ kg P}_2\text{O}_5/\text{ha}$ and $205 \text{ kg K}_2\text{O/ha}$ (García-Serrano et al., 2010). The ash treatments were complemented with NH_4NO_3 and KNO_3 , to achieve the same N and K fertilisation as the mineral fertiliser treatment. Four pots per treatment were prepared and in each pot three plants of lettuce were grown: plantlets of variety Almadra baby (1 month-old), acquired commercially, were transplanted from germination trays. The experiment was run for 60 days in a growth chamber under controlled conditions of temperature, humidity and light (12 h photoperiod, $25/18^\circ\text{C}$ day/night and 60/70% humidity day/night). The soils were watered at least twice per week using tap water. The above-ground parts of the plants were harvested, weighed fresh, washed with distilled water and dried at 60°C to determine the dry weight. The plant material was then ground to 0.5 mm for chemical analysis. Soil samples were taken from each pot at harvesting, air-dried and sieved to 2 mm for analysis.

From the results for the nutrient concentrations in the plants and their production (plant biomass as dry weight) in each treatment and in the unfertilised control, the efficiency in the use of the nutrients was determined. The apparent recovery efficiency (RE) of P was calculated as Fixen et al. (2015):

$$\text{RE} = (\text{Uptake}_{\text{treatment}} - \text{Uptake}_{\text{control}}) / \text{P}_{\text{applied}}$$

Where $\text{Uptake}_{\text{treatment}}$ is the plant uptake of P in the treatment ($[\text{P}] \times \text{dry weight}$), $\text{Uptake}_{\text{control}}$ is the plant uptake of P in the unfertilised control and $\text{P}_{\text{applied}}$ is the amount of P applied (all in g per pot).

The equivalent fertiliser efficiency (EFE) of the ash at the different application rates was calculated as the percentage of the apparent recovery efficiency of the mineral fertiliser.

$$\text{EFE} = (\text{RE}_{\text{ash}} / \text{RE}_{\text{fert}}) \times 100$$

Where RE_{ash} is the apparent recovery efficiency of the ash treatment and RE_{fert} the apparent recovery efficiency of the mineral fertiliser treatment.

2.3 Analytical methods

The ash and soil samples were analysed for pH (Crison Basic 20 pH meter) in a water saturated paste for soil and in a 1:5 water extract (w:v) for ash, electrical conductivity (EC) (Crison GLP 31 Conductimeter) in a 1:5 water extract (w:v) and TOC and total nitrogen (also for plants) using an automatic elemental microanalyser (EuroVector elemental

analyser, Milan, Italy). The total concentrations of macronutrients, micronutrients and heavy metals were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; ICAP 6500DUO ONE FAST, Thermo Scientific, Waltham, MA, United States) after microwave (Ethos-1, Milestone, Sorisole, Italy) acid digestion using $\text{HNO}_3 + \text{HCl}$ for ash samples and $\text{H}_2\text{O}_2 + \text{HNO}_3$ for plant samples. The soluble cations (K, Na, Ca and Mg) and anions (chloride, nitrite, nitrate, phosphates and sulphates) in ash were determined in water extracts (1:10 w:v), by ICP-OES and ion chromatography (IC), respectively. In soils, nitrate was determined using a selective electrode in 1:5 water extracts (USEPA, 2007), available-P was measured colorimetrically in 0.5 M NaHCO_3 extracts (Watanabe and Olsen, 1965) and available-K was measured by flame photometry after extraction with 1 N ammonium acetate at pH 7 (Kudsen et al., 1985). Available micronutrients and heavy metals in the soil were analysed by ICP-OES after extraction with 0.005 M DTPA + 0.01 M CaCl_2 + 0.1 M triethanolamine (pH 7.3; 1:5 w/v; 1 h) (Lindsay and Norvell, 1978). The availability of P and K in the ash samples was analysed both in water extracts (room temperature and 65°C) and in ammonium citrate extracts (UNE EN 15957). The neutralising capacity of the ashes was determined by titration according to UNE-EN 12945. All results for soil and ash refer to dry matter (105°C , 24 h).

2.4 Statistical analysis

The results were evaluated by one-way ANOVA and the differences between means were determined by the post-hoc Tukey's test, at a level of significance of $p < 0.05$. The data were tested for normality using the Shapiro–Wilk test. The statistical software IBM SPSS Statistics version 26 for Windows was used for the statistical analysis.

3 Results

3.1 Characteristics of the different ash samples collected

The ash samples showed high pH and EC values (Table 1), with the initial sample (the one taken during the stabilisation of the combustion system) having the highest EC. The concentrations of TOC and TN were low in all samples, which indicates the loss of organic material during the combustion process; the highest values were again observed in the initial sample, while there were no significant differences among the rest of the samples. Contrastingly, the concentration of nitrate ($\text{NO}_3\text{-N}$), which is directly available to plants, was lower in the initial sample than in the rest of the ashes. A different situation occurred with the concentration of total P, which was highest in the initial sample. Despite the ash from 100% manure was the second richest in total-P, it showed the highest concentration of soluble phosphates ($> 330 \text{ mg kg}^{-1}$), which was unexpected and possibly a consequence of the peculiar characteristics of that particular manure sample. Generally, P showed rather low solubility in water (below 1.5% of total P), whereas the concentration of available-P (extracted in ammonium citrate) reached 4.15% of total-P in the sample from 100% manure, and less than 1% in the rest of the samples (data not shown).

TABLE 1 Composition of the combustion ash from chicken manure (M) and wood (W) mixtures.

Ash	pH	EC (dS m ⁻¹)	TOC (g kg ⁻¹)	Total-N (g kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	Total-P (g kg ⁻¹)	PO ₄ ³⁻ -P (mg kg ⁻¹)	Total-K (g kg ⁻¹)	Soluble-K ⁺ (g kg ⁻¹)	Total-Na (g kg ⁻¹)	Soluble-Na ⁺ (g kg ⁻¹)
Initial M + W 50:50	11.6 ± 0.3ab	20.4 ± 0.3a	168 ± 6.0a	11.1 ± 0.4a	292 ± 19d	32.4 ± 0.7a	3.7 ± 0.1b	111 ± 2a	nd	15.8 ± 0.2a	nd
Manure 100	11.1 ± 0.1b	18.1 ± 0.3b	56.9 ± 2.9b	3.7 ± 0.2b	582 ± 8cd	24.4 ± 0.1b	336 ± 2.9a	88.4 ± 0.3b	45.1 ± 0.9a	13.7 ± 0.3b	5.2 ± 0.1a
M + W 50:50	11.9 ± 0.1a	16.5 ± 0.2c	38.5 ± 2.2b	2.4 ± 0. bc	895 ± 69a	11.1 ± 0.2c	5.5 ± 0.2b	68.2 ± 1.5bc	32.0 ± 0.6b	8.5 ± 0.2c	3.0 ± 0.1b
M + W 60:40	11.9 ± 0.1a	17.3 ± 0.2bc	40.5 ± 2.5b	2.6 ± 0.3bc	816 ± 8b	11.2 ± 0.6c	5.1 ± 0.1b	66.6 ± 1.1c	33.0 ± 0.0b	8.5 ± 0.2c	3.2 ± 0.1b
Wood 100	11.8 ± 0.1a	16.2 ± 0.1c	37.3 ± 1.9b	2.0 ± 0.2c	823 ± 20b	11.2 ± 0.3c	6.5 ± 0.2b	62.3 ± 2.8c	32.6 ± 0.1b	7.9 ± 0.3c	3.2 ± 0.1b
ANOVA	*	***	***	***	***	***	***	***	***	***	**

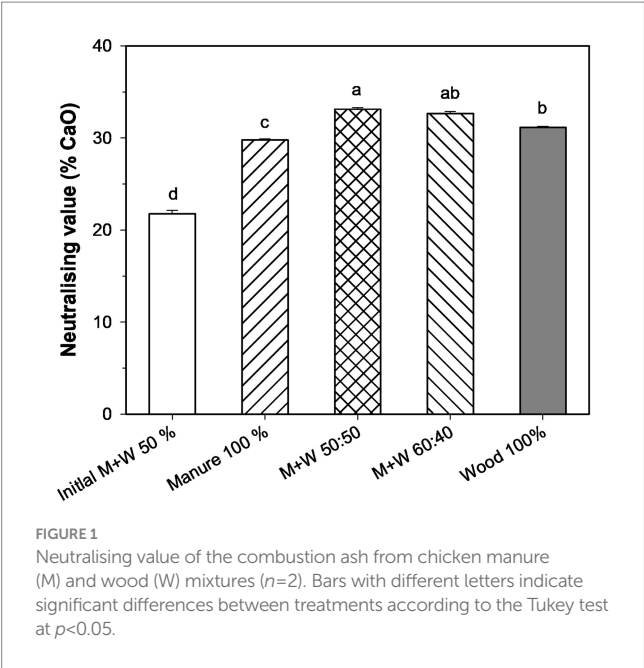
EC, electrical conductivity; TOC, total organic carbon; TN, total nitrogen. Total elements and water-soluble forms for anions (NO₃⁻-N and PO₄³⁻-P) and cations (K⁺ and Na⁺). Results refer to dry weight (mean ± SE; n = 2). Values followed by different letters indicate significant differences between treatments according to the Tukey test at *p* < 0.05; *, **, ***, ****; Significant at *p* < 0.05, 0.01, 0.001, 0.0001. nd: not determined.

The main plant nutrients that could be provided by the ashes were P (as indicated) and K (Table 1). In contrast to P, almost 50% of total K was water-soluble (at room temperature) and directly available to plants. Potassium solubility was higher in both hot water and ammonium citrate extracts, with values reaching 80.1 > 73.8 > 64.4 ≥ 63.9% of total-K in hot water extracts for ashes from 100% manure, 100% wood and the manure:wood 60:40 and 50:50 mixtures, respectively, and 94.9 > 87.7 > 84.3 > 74.8% of total-K, respectively, in ammonium citrate extracts. The high soluble K and Na concentrations were mainly responsible for the high EC of the ashes. Sodium can be considered a potentially toxic element when it reaches high levels, causing toxicity in plants. However, the concentration of total-K was 7.4 times higher (on average) than that of Na, which may prevent Na toxicity in plants. Furthermore, there is a predominance of soluble K over soluble Na (Table 1), so the risk of Na toxicity is very low, although the high EC may cause phytotoxicity problems due to osmotic stress in plants.

Other main elements present in the ashes include Ca and Mg, with very similar values for all samples, the ranges being 126–146 g kg⁻¹ for Ca and 36–38 g kg⁻¹ for Mg. These elements can contribute significantly to the high pH values of the ashes, and may also be responsible for their high neutralising capacity, mainly regarding those derived from mixtures of chicken manure and wood chips (Figure 1). These data indicate that combustion ash has a CaO substitution capacity of 34% by weight, which can be very relevant for the use of ash in acidic soils, partially replacing the lime required for pH correction.

The ash materials were rich in essential plant micronutrients (Fe, Cu, Mn and Zn). Small differences in the concentrations of these elements were observed between samples (Table 2); the greatest differences occurred for Zn, with the initial M + W 50:50 sample having the highest value, followed by ash from 100% manure. This may indicate the possible transfer of this element from the metallic part of the burner to ash prepared of the system, but could be associated also with the presence of Zn in the chicken manure. Potentially toxic elements, such as heavy metals and metalloids (As, Cd, Cr, Ni and Pb), were all low, with the only exception of Pb in the initial sample, that reached 133 mg kg⁻¹, again probably due to an early contamination with this element, coming from the burner system during its optimisation period. In agreement with this, the concentration of Pb in the rest of the samples was low. The ash from chicken manure combustion had the lowest concentrations of potentially toxic elements such as Cr, Tl and V (Table 2).

The most relevant issue regarding the agricultural use of the combustion ash samples analysed was their high salt content, as shown by their very high EC values. The potential toxicity associated with the salinity of the ashes was determined in a germination test (Figure 2). Water extracts of the ashes, whether pure (100%) or partially diluted (75%), were highly phytotoxic and completely inhibited seed germination (germination index (GI) value of zero). Greater dilution of the extracts (50%) allowed the germination of some seeds, but the GI was still very low (<4%). Further dilution was necessary to obtain an adequate number of germinated seeds and the GI increased as the concentration of the extract decreased: 81 ± 7.8% for a dilution of the extract to 25% and 102 ± 6.9% for a dilution to 12.5%. Then, it was necessary to dilute the extract to 25% to obtain adequate germination without symptoms of toxicity (> 80%). The 12.5% extract may even have been beneficial for germination, probably due to the supply of nutrients from the ashes.



3.2 Pot experiment

The plant growth was adequate with all ash treatments; it was lowest in the control without fertilisation and highest with the mineral fertiliser, followed by the ash that supplied 100% P (Figure 3). The treatments with ash at 50 to 80% P produced similar plant yields, greater than the control but lower than for the mineral fertiliser treatment. The concentration of N in the plants was highest in the mineral fertiliser and ash at 100% P treatments, being lowest for the control soil without fertilisation; the rest of the treatments with ash gave a N concentration in the plants that was significantly lower than for the mineral fertiliser (Table 3). However, the P concentration in the plants was not significantly different among the different fertilising treatments (with mineral fertiliser or with ash), but all showed higher values than the unfertilised control. These results indicate that the ash can supply enough P to plants for their growth.

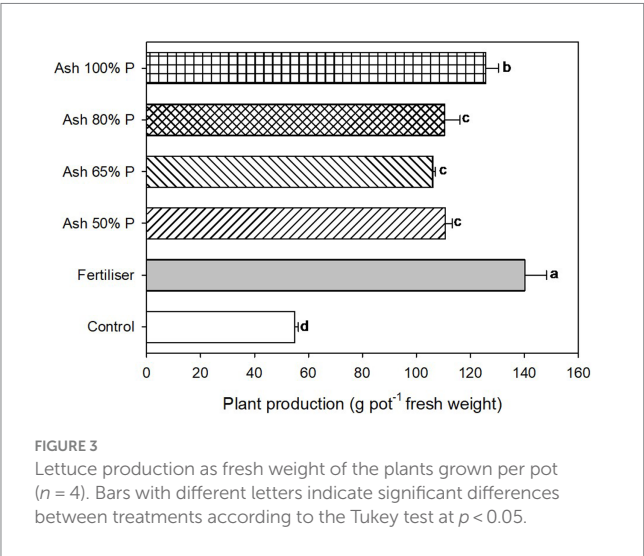
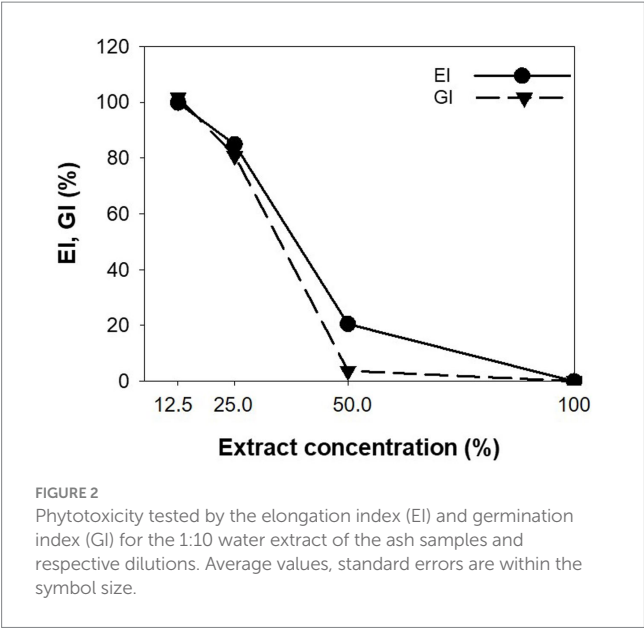
The concentration of K was highest in the plants receiving the mineral fertiliser and lowest in those grown in the control soil. Ash treated plants showed intermediate K concentrations, with no significant differences among them (Table 3). Contrastingly, Na levels in the plants were not affected by the addition of ash, in agreement with the fact that the total and soluble concentrations of this element were lower than those of K in the ashes (Table 1). The concentrations of the different micronutrients in the plants were all very similar for all treatments—Fe and Zn showed no statistically significant differences, while small differences were observed for Cu and Mn between treatments (Table 3) – and all can be considered normal for plant growth. No accumulation of heavy metals was observed in the plants after the use of combustion ash as a fertiliser substitute (data not shown).

The most relevant changes in the characteristics of the soil were found for the pH values at plant harvest, with a concomitant increase as the ash rate increased, from 7.7 in the control to 8.0 in the treatment with the highest ash rate (Table 4). This effect clearly indicates the

TABLE 2 Concentrations of micronutrients and potentially toxic elements in the combustion ash from chicken manure (M) and wood (W) mixtures.

Ash	Fe (g kg ⁻¹)	Mn (g kg ⁻¹)	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cd (mg kg ⁻¹)	Cr (mg kg ⁻¹)	Ni (mg kg ⁻¹)	Pb (mg kg ⁻¹)	Tl (mg kg ⁻¹)	V (mg kg ⁻¹)
Initial M+W 50:50	7.06±0.3b	4.37±0.05c	481±14	1940±4a	3.51±0.01a	62.2±1.1ab	32.2±3.2c	133±1.0a	nd	19.0±0.8b
Manure 100	12.3±0.6ab	7.00±0.05b	462±3	1,231±10b	0.66±0.03c	48.7±4.7c	48.3±0.8b	30.2±5.8b	0.16±0.01b	24.7±0.7ab
M+W 50:50	14.2±0.4ab	8.67±0.23a	415±15	713±36c	0.86±0.09b	71.1±4.0a	59.7±1.5a	55.8±7.9b	0.30±0.02a	25.2±0.5a
M+W 60:40	15.1±2.5a	8.15±0.14a	388±7	712±14c	1.07±0.01b	60.0±1.1abc	51.4±0.3d	48.2±3.0b	0.31±0.02a	25.0±1.1ab
Wood 100	13.7±0.9a	7.81±0.39a	444±69	710±17c	1.09±0.09b	50.6±0.1bc	58.6±1.2a	47.9±8.6b	0.32±0.02a	24.5±1.9ab
ANOVA	*	***	NS	***	***	**	**	***	**	*

Results refer to dry weight (mean ± SE; $n=2$). Values followed by different letters indicate significant differences between treatments according to the Tukey test at $p<0.05$; *, **, ***, Significant at $p<0.05$, 0.01, 0.001; NS, not significant. nd, not determined.



usefulness of ash to regulate the pH in acidic soils, as it would decrease the amount of lime required for soil pH adjustment.

Despite the high EC of the ash, this parameter did not increase significantly in the soil (Table 4). Parameters such as the concentrations of TOC and total N (and also nitrate) in the soil showed only very small differences among the treatments, which could be associated with their low concentrations in the ash, and also to the fact that N was supplied at the same rate to all treatments (except the control). The concentration of available P in the soil was increased significantly in the treatment with the highest ash rate, followed by the treatment with the lowest rate of ash and the mineral fertiliser. These results might be showing that the P potentially provided by the ash in excess of the plant requirements was fixed in the soil, due to its calcareous character and the low solubility of this element in the ash, as previously discussed. Furthermore, the concentration of available K in the soil was significantly lower in all the ash treatments than for the mineral fertiliser and the unfertilised control (Table 4), which suggests that the

TABLE 3 Composition of the plants regarding the main macro- and micro-nutrients and sodium under the different fertiliser treatments.

Treatment	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Na (g kg^{-1})
Control	1.18 \pm 0.05c	2.43 \pm 0.16b	19.8 \pm 1.1c	8.54 \pm 0.61	3.19 \pm 0.16a	41.3 \pm 3.2	3.2 \pm 0.2c	76.7 \pm 1.0a	8.0 \pm 0.8	1.3 \pm 0.07
Fertiliser	2.57 \pm 0.16a	4.68 \pm 0.52a	34.5 \pm 1.3a	7.57 \pm 0.75	2.73 \pm 0.18abc	63.6 \pm 7.1	5.9 \pm 0.5a	58.0 \pm 4.1ab	10.9 \pm 0.9	1.1 \pm 0.09
Ash 50% P	2.21 \pm 0.10b	4.44 \pm 0.35a	29.2 \pm 1.8ab	6.92 \pm 0.19	2.61 \pm 0.09abc	46.8 \pm 2.8	4.6 \pm 0.3b	56.7 \pm 3.6b	9.1 \pm 0.9	1.3 \pm 0.08
Ash 65% P	2.00 \pm 0.10b	4.15 \pm 0.26a	23.1 \pm 1.2bc	5.89 \pm 0.57	2.40 \pm 0.17c	52.7 \pm 2.8	3.6 \pm 0.2ab	51.3 \pm 5.0b	8.8 \pm 0.4	1.3 \pm 0.08
Ash 80% P	2.01 \pm 0.13b	4.33 \pm 0.28a	27.0 \pm 1.7b	7.60 \pm 0.72	3.08 \pm 0.26ab	53.9 \pm 4.0	4.2 \pm 0.2ab	63.4 \pm 3.8ab	10.7 \pm 0.9	1.3 \pm 0.09
Ash 100% P	2.24 \pm 0.16ab	4.17 \pm 0.23a	25.7 \pm 1.1bc	6.68 \pm 0.47	2.52 \pm 0.18bc	51.5 \pm 5.3	4.1 \pm 0.1ab	58.2 \pm 6.1ab	8.38 \pm 0.4	1.5 \pm 0.14
ANOVA *	***	**	***	NS	*	NS	***	**	NS	NS

Values followed by different letters indicate significant differences between treatments according to the Tukey test at $p < 0.05$; *, **, ***, Significant at $p < 0.05$, 0.01, 0.001; NS, not significant. Cd and Pb $< 1.0 \text{ mg kg}^{-1}$.

TABLE 4 Characteristics of the soil after lettuce harvesting, for the different treatments.

Treatments	pH	EC (dS m ⁻¹)	TOC (g kg ⁻¹)	Total-N (g kg ⁻¹)	Available-P (mg kg ⁻¹)	Available-K (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)
Control	7.7 ± 0.02d	0.229 ± 0.003a	8.46 ± 0.38ab	0.91 ± 0.03abc	15.5 ± 0.5bc	338 ± 11a	8.1 ± 0.5
Fertiliser	7.7 ± 0.02 cd	0.231 ± 0.004a	7.45 ± 0.22b	0.83 ± 0.01c	17.1 ± 0.2ab	311 ± 13a	8.2 ± 0.9
Ash 50% P	7.8 ± 0.03 cd	0.196 ± 0.008b	7.28 ± 0.31b	0.83 ± 0.03c	17.8 ± 0.4ab	229 ± 8b	7.0 ± 0.1
Ash 65% P	7.9 ± 0.01b	0.226 ± 0.008a	8.27 ± 0.60ab	0.85 ± 0.01bc	13.8 ± 0.2c	175 ± 6 cd	7.9 ± 0.3
Ash 80% P	7.9 ± 0.01ab	0.241 ± 0.005a	8.80 ± 0.11a	0.98 ± 0.00abc	14.8 ± 0.3c	164 ± 5d	7.1 ± 0.1
Ash 100% P	8.0 ± 0.01a	0.216 ± 0.004ab	8.47 ± 0.32ab	0.95 ± 0.03ab	18.2 ± 0.8a	205 ± 9bc	8.6 ± 0.2
ANOVA*	***	***	NS	***	***	***	NS

EC, electrical conductivity; TOC, total organic carbon; TN, total nitrogen (mean ± SE; *n* = 4). Results refer to dry weight. Values followed by different letters indicate significant differences between treatments according to the Tukey test at *p* < 0.05; *, **, ***: significant at *p* < 0.05, 0.01, 0.001; NS: not significant.

K provided by the ashes and not assimilated by the plants (if any) did not remain in the soil in plant available forms.

Despite the presence of certain heavy metals in the ashes, their available concentrations (extractable with DTPA) in the soil were all quite low (the highest being 2 mg kg⁻¹, for Pb), which indicates a scarce risk of metal accumulation in the soil or plant toxicity. In fact, the total amount of heavy metals added to the soil with the ash at the highest application rate was (mg kg⁻¹): 1.12 Zn, 0.28 Cu and <0.1 for the rest of heavy metals (Cd, Co, Cr, Ni, Pb), which support the assessment of scarce risk of metal accumulation. These values are far below the limit values for amounts of heavy metals which may be added annually to agricultural land, based on a 10-year average (Cd 0.15, Cu 12, Ni 3, Pb 15 and Zn 30 mg kg⁻¹) according to the European legislation (Council Directive, 1986).

Using the values of the concentration of P in the plants, the plant biomass (the dry weight of the aerial part) and the amount of P added in each treatment, the use of the P applied through fertilisation was calculated as its apparent RE. The apparent RE of P from the mineral fertiliser was 72.8% of the applied P, greater than for the ash treatment at 100% P (61%). The values diminished as the application rate of ash increased, following the order 125 > 69 > 64 ≈ 61% in the ash treatments at 50, 65, 80 and 100% P, respectively. The high P efficiency at the lowest ash rate indicates that the amount of P added was not enough for the plant requirements, and the plants had to take up part of the P already available in the soil.

The fertilisation efficiency of the ash treatments in comparison with the mineral fertiliser indicates the equivalent fertiliser efficiency and therefore the fertiliser replacement value (Figure 2). The ash treatments were able to replace 79% of the mineral P fertiliser when the ash was applied at the same P rate as the mineral fertiliser (ash treatment 100% P), with greater values for 80 and 65% P application by ash (82 and 90% of the mineral P fertiliser, respectively). All this indicates that the ash P fertilising capacity is capable of providing 80% of the P supplied by the mineral fertiliser for the P nutrition of the plants.

4 Discussion

The characteristics of combustion ashes depend both on the biomass properties and on the combustion system used (Demirbas, 2007). The chemical reactions that take place during biomass combustion mostly oxidise organic carbon to carbon dioxide and

hydrogen to water, while nitrogen is transformed to N₂ and emitted to the atmosphere. However, N₂O can be formed as a result of the partial oxidation of the NH₃ present in animal manures (such as chicken manure), from the partial reduction of NO_x by NH₃, or by the decomposition of NH₄NO₃ (Billen et al., 2015). Most of the inorganic components of the biomass will remain in the resulting ash, mainly in the fly ash (Knapp and Insam, 2011). The different characteristics of the initial ash sample taken during the optimisation of the burning system, such as the greater TOC, TN and EC values, as well as the high soluble NO₃-N concentrations, clearly indicate a deficiency in the combustion process (Knapp and Insam, 2011). The homogeneous values found for the rest of the samples demonstrate the effective optimisation of the combustion system. The P and K concentrations in the ashes were lower than the average values found in ashes derived from other poultry manures (Lanzerstorfer, 2017; Huygens et al., 2019), but in general were higher than the values obtained for wood. Once the combustion system was optimised, the ash from 100% manure had the highest concentration of P, related to the high concentration of this element in chicken manure (Alvarenga et al., 2020). In addition, the conditions of the combustion system could also affect the concentration of soluble-P in the ash. Combustion of manure with wood could increase the flame temperature due the greater higher heating value (HHV) of wood (19.59 MJ kg⁻¹) than poultry manure (16.18 MJ kg⁻¹) and the increase in the amount of volatile matter in mixtures with wood (Turzyński et al., 2022). These could affect the degree of crystallinity of the ash and P availability, as the lower the fraction of crystalline P-phases was, the higher the bioavailability of the material (Nordin et al., 2020). According to Nordin et al. (2020) the chemical compounds found in bottom ash during combustion depends on the fuel and affected by temperature reached; for instance, hydroxyapatite (Ca₅(PO₄)₃OH) was the more common compound found in bottom ash from woody-type biomass, while AlPO₄ survives the combustion of sewage sludge and, on co-combustion with wood, it breaks and reacts with Ca and Mg with the formation of new compounds (Ca and Mg phosphates). These changes in phosphate composition can alter P plant availability in bottom ash and may account, at least in part, for the high soluble phosphate concentrations found in the 100% manure sample ashes. However, P was generally poorly soluble in water, the highest proportion of soluble-P occurred in ash from 100% manure, with 1.38% of total P as water soluble PO₄³⁻-P. Other authors have also found a low proportion of water-soluble P in combustion ash (Choudhury et al., 2020). However, the solubility of P from the ashes in ammonium citrate, indicating the plant available P concentration (Bougnom et al., 2011; Omil et al., 2011;

Schiemenz et al., 2011), was greater, in agreement with the results obtained for soil available-P in the pot experiment. The abundant basic cations (Ca, Mg and K) present in the ashes were likely responsible for their alkaline pH and their high neutralising capacity (Omil et al., 2011). These elements are transformed into oxides at high temperature, and then hydrated and carbonated during the combustion process (Demeyer et al., 2001). The values found for chicken manure ashes are within the range for wood ash (20–90% CaCO₃, equivalent to 11–50.4% CaO) according to Bougnom et al. (2011), which is mainly due to their content of hydroxides and carbonates of calcium, magnesium and K. For this reason, the use of combustion ash has traditionally been considered to be useful in acid and forest soils (Bougnom et al., 2011; Omil et al., 2011).

Although the utilisation of waste materials as fuels can be considered as a positive environmental approach for the production of renewable energy, thereby decreasing the use of fossil fuels, the presence of certain elements can cause problems in the combustion system and may also result in environmental risks after soil application of the ash. Chlorine plays a major role in ash formation as it facilitates the mobility of many inorganic compounds (Demirbas, 2007). Sodium and K lower the melting point of ash and, hence, can increase ash deposition and fouling. In addition, the content of heavy metals and soluble salts in the combustion ash can lead to negative effects in the soil after repeated ash applications. In fact, the high phytotoxicity of the ash was associated with its high salinity (high EC). Consideration should be given to this before its potential agricultural use; its use with young plants or non-germinated seeds should be avoided, and its application to the soil well before seeding or transplanting is recommended.

The EU fertiliser legislation includes, in the component materials category CMC 13, the “thermal oxidation materials and their derivatives” (EU Commission, 2021). However, to protect plants and soils, the regulation limits the total concentrations of potentially toxic elements in combustion ash to Cr < 400, Tl < 2, V < 600 (all in mg kg⁻¹) and Cl⁻ < 30 g kg⁻¹. The ash from chicken manure studied here did not exceed these limit values (Table 2), guaranteeing the safe use of the bottom ash generated from chicken manure as a fertiliser material or as a component material of fertilisers. The characteristics of the ash samples can be compared with the requirements for fertiliser products according to the European legislation (EU Regulation, 2019). These materials can be included in some product functions categories (PFCs) such as “Solid Inorganic Macronutrient Fertiliser” (multi-nutrients) (PFC 1), “Liming Material” (PFC 2) and “Inorganic Soil Improvers” (PFC3) (EU Regulation, 2019). The multi-nutrients option ‘PFC 1(C)(I)(a)(ii): Compound Solid Inorganic Macronutrient Fertiliser (multi-nutrients)’ requires a minimum content of 3% P₂O₅ plus one of the other considered plant macronutrients, at a concentration above 3% for K₂O and above 1.5% for CaO, MgO, SO₃, or Na₂O. The bottom ash from chicken manure, alone or mixed with wood, of the present experiment, obtained after the optimisation of the combustion system, complied with these nutrient requirements, with average values of (% ± SE): P₂O₅ = 3.1 ± 0.76; K₂O = 8.78 ± 0.63; CaO = 19.83 ± 0.69; MgO = 6.16 ± 0.08; SO₃ = 2.11 ± 0.16 and Na₂O = 1.66 ± 0.22 (below the maximum value of 40%). The P₂O₅ concentration of the ash was very close to the limit required; then, the addition of other source of P₂O₅ may be required to obtain a multi-nutrients option ‘PFC 1(C)(I)(a)(ii)’. The sum of the nutrients is above the minimum (18%) established in this European legislation (EU Regulation, 2019). The neutralising

value of the ash was above the 15% CaO equivalence required for a liming material (PFC 2). In addition, the concentrations of all inorganic contaminants in the ash were below the maximum limit values for PFC 1 (with the exception of the sample taken during system optimisation), but values higher than the limit for PFC 2 and PFC 3 could occur for Cu and Zn. Thus, special attention should be paid to the presence of Cu and Zn in chicken manure destined for combustion, in order to obtain ash useful as a liming material or an inorganic soil improver in line with the European legislation.

The results of the pot experiment indicate that the amount of nutrients supplied to the plants when using ash was enough for their growth. In fact, the application of ash at a rate equivalent to 100% of the P supplied by the mineral fertiliser gave a plant production and P concentration in the plants that were very close to those in the fertiliser treatment. Although the apparent RE for P was slightly lower for ash than for mineral fertiliser, a fertiliser replacement value of 80% can be estimated for the ash used (i.e., the combustion ash can replace 80% of the inorganic P fertiliser). This is highly relevant in terms of reducing the use of P extracted from rock phosphate, which can be considered a strategic resource; in fact, the reserves of P are expected to have fallen by 25% in 2100 based on current demand (Malingreau et al., 2012). The use of ash as a fertiliser material has a clear advantage over that of fresh manure, since (as the N has been removed) it can be applied based on crop P requirements, which would reduce the potential P over-application, P run-off and soil P accumulation that could result from the application of the manure on a N requirement basis for the crop (Choudhury et al., 2020). In spite of the low solubility of the ash P in water, this material is an effective P fertiliser for crops, as indicated by the enhanced extractability of P in ammonium citrate. In addition, this low percentage of water-soluble P could prevent any loss by run-off after soil application (Codling, 2006; Choudhury et al., 2020).

5 Conclusion

From the results of the present work, the following conclusions can be drawn:

- The ash materials derived from combined chicken manure and wood chips combustion are rich in essential nutrients, mainly P and K. When chicken manure was burnt alone (without wood biomass) the concentration of P in the ashes was highest. Phosphorus was generally poorly soluble in water, while K was highly soluble in warm (65°C) water.
- The combustion ash can replace 80% of the inorganic P fertiliser and its low percentage of water-soluble P could prevent any P-loss by run-off after soil application.
- The high pH value and the elevated concentrations of Ca and Mg give chicken manure ashes high neutralising values of up to 30% of CaO eq., which can be relevant regarding their use as amendments in acid soils in partial substitution of the usual liming materials.
- The high salinity of the ash, with a much greater abundance of K relative to Na, should not cause problems in the development of the crop or in the soil, if applied at agronomic rates based on the crop P requirement. However, phytotoxicity due to salinity may occur, which implies that agricultural use is not recommended

for non-germinated seeds or young plants; ash should be applied to soils at low application rates and well before sowing.

- The ash from combustion of chicken manure satisfies the requirements for inclusion in the fertiliser component material category CMC 13, with total nutrient concentration close or above the limits established for PFC 1(C)(I)(a)(ii): Compound Solid Inorganic Macronutrient Fertiliser (multi-nutrients), and also has characteristics that allow its inclusion in PFC 2: liming material.
- However, the combustion process must be efficient enough to reduce the TOC concentration in ash to below 3%, while the Cu and Zn in the input manure need to be controlled so that their concentrations in the ash do not exceed the limits for liming materials and soil improvers.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

MPB: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. MÁ-R: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. PB-M: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. RC: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing.

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Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The Project AVIENERGY has been co-financed by the European Agricultural Fund for Rural Development (EAFRD, FEADER) of the European Union (80%) and by the Spanish Ministry of Agriculture, Fisheries and Food (20%), within the framework of the National Rural Development Program 2014–2020 (ref. O00000226e2000044442). Budget: €582,960.04. Total grant: €565,160.04. The General Directorate for Rural Development, Innovation and Agri-Food Training (DGDRIFA) is the authority for applying the grants.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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OPEN ACCESS

EDITED BY

José Luis García-Morales,
University of Cádiz, Spain

REVIEWED BY

Stefan Hörtenhuber,
University of Natural Resources and Life
Sciences Vienna, Austria
José Manuel Rato Nunes,
Polytechnic Institute of Portalegre, Portugal

*CORRESPONDENCE

Mario Álvarez Salas
✉ mario.alvarez@usys.ethz.ch

RECEIVED 15 February 2024

ACCEPTED 17 June 2024

PUBLISHED 08 July 2024

CITATION

Álvarez Salas M, Sica P, Rydgård M,
Sitzmann TJ, Nyang'au JO, El Mahdi J,
Moshkin E, de Castro e Silva HL,
Chrysanthopoulos S, Kopp C, Wali K,
Zireeni Y, Ural-Janssen A, El Hajj Hassan S,
Kebalo LF, Chadwick D and Jensen LS (2024)
Current challenges on the widespread
adoption of new bio-based fertilizers: insights
to move forward toward more circular food
systems.
Front. Sustain. Food Syst. 8:1386680.
doi: 10.3389/fsufs.2024.1386680

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Sitzmann, Nyang'au, El Mahdi, Moshkin,
de Castro e Silva, Chrysanthopoulos, Kopp,
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Kebalo, Chadwick and Jensen. This is an
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Current challenges on the widespread adoption of new bio-based fertilizers: insights to move forward toward more circular food systems

Mario Álvarez Salas^{1*}, Pietro Sica², Maja Rydgård²,
Tomas Javier Sitzmann³, Jared Onyango Nyang'au⁴,
Jihane El Mahdi⁵, Egor Moshkin⁶, Hellen Luisa de Castro e Silva⁶,
Stamatis Chrysanthopoulos⁷, Clara Kopp², Khan Wali⁵,
Yusra Zireeni⁸, Aslihan Ural-Janssen⁵, Samaya El Hajj Hassan⁶,
Lelenda Florent Kebalo⁹, David Chadwick⁸ and
Lars Stoumann Jensen²

¹Institute of Agricultural Sciences, ETH Zürich, Lindau, Switzerland, ²Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark, ³Department of Agricultural, Forest and Food Sciences, University of Turin, Turin, Italy, ⁴Department of Agroecology, Aarhus University, Aarhus, Denmark, ⁵Department of Plant Sciences, Wageningen University, Wageningen, Netherlands, ⁶Department of Green Chemistry and Technology, Ghent University, Ghent, Belgium, ⁷Instituto Superior de Agronomia, University of Lisbon, Lisbon, Portugal, ⁸School of Environmental and Natural Sciences, Bangor University, Bangor, United Kingdom, ⁹National Research Institute for Agriculture, Food and the Environment (INRAE), Paris, France

To meet global food demands sustainably, it is necessary to safeguard finite natural resources and reduce harmful emissions to the environment. Nutrients in biowastes are often not managed appropriately. Instead, they can be recovered, recycled into bio-based fertilizers (BBFs) and reincorporated into food production systems. This review addresses three critical issues for developing and adopting new BBFs, focusing on the European context: (1) BBFs should match the agronomic efficiency of mineral fertilizers. We propose that the agronomic efficiency of BBFs can be increased through pre-treating the inputs in biowaste transformation processes (e.g., anaerobic digestion), chemical treatments of existing BBFs, organo-mineral combinations, and soil placement strategies. (2) Production and use of new BBFs is not free of environmental impacts, and these are influenced by regional conditions. (3) Public perception and end-user preferences play a significant role in the adoption of BBFs. Therefore, it is vital to address the requirements of end-users of BBFs. Our findings indicate that for widespread adoption, BBFs need sufficient and reliable nutrient amounts and crop-adequate ratios, as well as competitive pricing. A key advantage of BBFs over mineral fertilizers is their ability to improve soil fertility. However, farmers also require fertilizers that can be handled and applied with existing machinery and offer the practicality of commercial products. Another important aspect is the willingness of consumers to buy products fertilized with BBFs. Designing and promoting BBFs requires a careful assessment of environmental impacts and regional conditions, as the sustainability of BBFs depends on factors like energy sources and biowaste transport distances. Ultimately, the goal is to promote a circular economy and not just to substitute mineral fertilizers with new products. This review aims to guide researchers, policymakers, and stakeholders by highlighting key innovations and addressing critical barriers.

KEYWORDS

sewage sludge, bio-based fertilizer, compost, circular economy, fertilizer

1 Introduction

An increase in agricultural productivity is necessary to properly feed a growing human population. However, improving the sustainability of global food production is also a priority. Agriculture is responsible for 17 % of global greenhouse gas (GHG) emissions (FAO, 2021). Additionally, current agriculture significantly contributes to the degradation of ecosystems and causes the depletion of freshwater and nutrient reserves (Foley et al., 2011; Poore and Nemecek, 2018). Making agriculture more sustainable can be achieved through recovering and recycling nutrients into bio-based fertilizers (BBFs), which can be reincorporated into food production systems.

The concept of planetary boundaries (Rockström et al., 2009) defines the limits of nine key systems crucial for maintaining Earth's safety and habitability. One of these key systems is the biogeochemical flow of phosphorus (P) and nitrogen (N). Currently, 22.6 Tg of P and 190 Tg of N are emitted yearly, doubling and tripling, respectively, the planetary boundaries for each nutrient (Richardson et al., 2023). The main driver of exceeding the system's boundaries is the continuous and excessive P and N inputs in agriculture (Sandström et al., 2023). Correcting this issue requires recycling, improving retention, and utilizing nutrients more efficiently (Carpenter and Bennett, 2011; Sandström et al., 2023).

Fertilizer nutrient use occurs mostly within an inefficient linear economy: a take-make-dispose approach (Chojnacka et al., 2020; Donner et al., 2020). *Take* refers to mineral fertilizers, which are derived, *taken*, from phosphate rock deposits or synthesized through energy-intensive industrial processes, as in the case of Haber-Bosch N. Then, to *make* food and grow crops, mineral fertilizers are applied to agricultural land. As consumption of agricultural products is centralized in urban areas, nutrients concentrate in urban wastewater and end up being *lost* to the atmosphere, aquatic environments or *disposed* in landfills (Witek-Krowiak et al., 2022). This perpetuates the inefficient system as new mineral fertilizers need to be continually produced to maintain agricultural outputs.

On a global scale, P fertilizer use efficiency is between 9 and 12% for cereal crops (Yu et al., 2021), while N fertilizer use efficiency averages between 48 and 78% in croplands (You et al., 2023). In Europe, P fertilizer use efficiency is 57% (Schoumans et al., 2015). Despite improvements in recent decades, significant P and N surpluses persist, leading to eutrophication and other forms of ecosystem damage (Bouwman et al., 2013; Reid et al., 2018; Ural-Janssen et al., 2023; Muntwyler et al., 2024). For example, extracting mineral P generates hazardous mine tailings (Silva et al., 2022). Production of synthetic Haber-Bosch N is responsible for *ca.* 2% of carbon emissions globally and relies on non-renewable natural gas (Osorio-Tejada et al., 2022). These problems highlight an urgent need to shift to circular nutrient economies (Foley et al., 2011).

A circular economy is a system where materials are kept inside, instead of disposed, thus minimizing external resource usage and energy requirements (Ritzén and Sandström, 2017). Achieving this involves reducing nutrient losses during food production and consumption and recovering and reutilizing nutrients (Harder et al.,

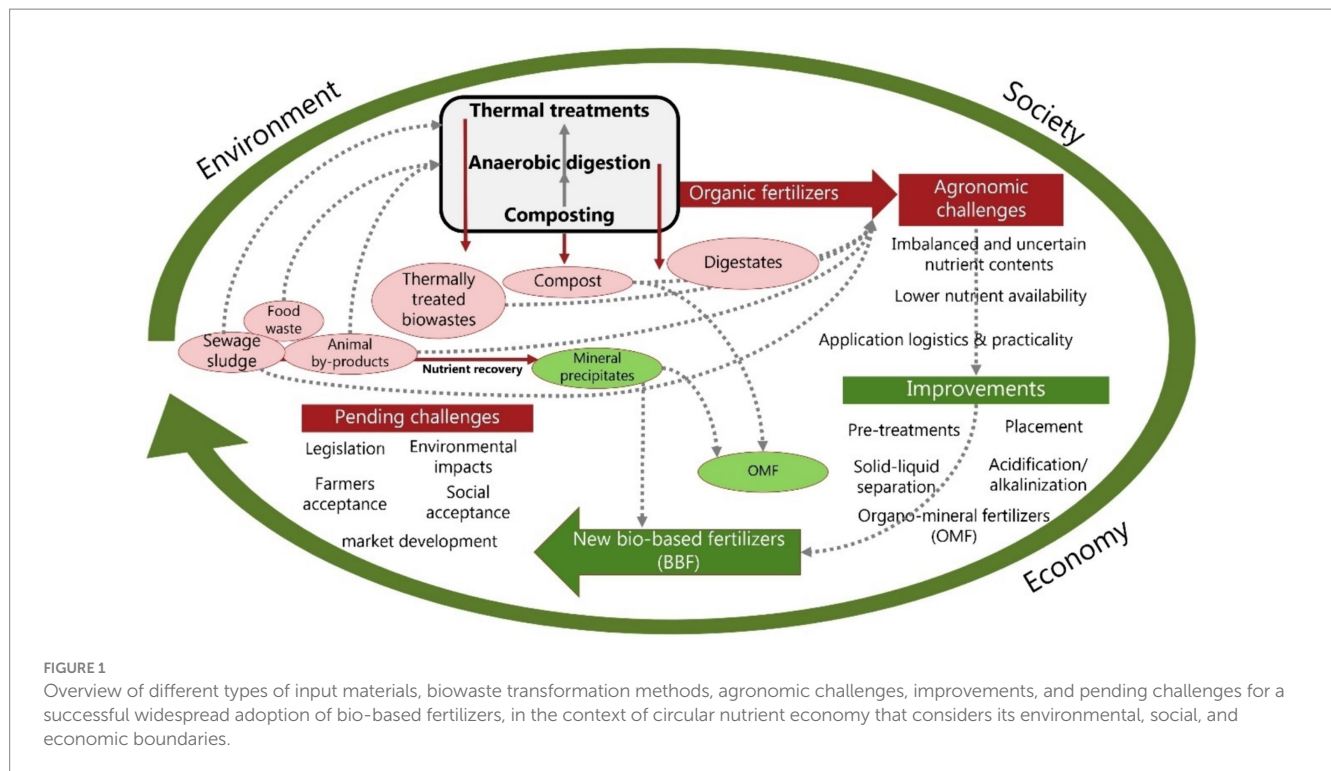
2021). Sourcing fertilizers from P and N that otherwise would be wasted, significantly contributes to shaping circular nutrient economies, as opposed to mined P or synthetic N.

The concept of Doughnut Economics (Raworth, 2017) emphasizes that circular economies should exist within the boundaries of meeting human economic and social needs, while safeguarding the planet's ecosystems. In consequence, the production and use of fertilizers with recovered nutrients must develop in conjunction with the agricultural, economic, and social needs of the specific context in which it takes place, involving stakeholders in the decision-making process (Schengel and Goehlich, 2024). Furthermore, it is essential to consider the available P and N sources, the existing infrastructure, and the regulatory and market conditions (Moshkin et al., 2023; Garmendia-Lemus et al., 2024).

In the European Union (EU), approximately 1 Tg of mineral P fertilizers and 10 Tg of synthetic N fertilizers are applied annually (Eurostats, 2023). Meanwhile, considerable sources of P and N, such as sewage sludge, are underutilized compared to the United States and Australia (Marchuk et al., 2023). Therefore, the EU is attempting to transition to a circular economy and maximize the recovery of nutrients (Sporchia and Caro, 2023). At the forefront of the EU's efforts is the European Green Deal, a set of policy initiatives with the goal of making the EU climate neutral by 2050 (European Commission, 2019). The food system and agricultural core of the European Green Deal is the Farm to Fork Strategy, which aims to overhaul policy and regulation to create a more circular European food system. From an agricultural perspective, one of the central aims of the Farm to Fork Strategy is to reduce nutrient losses by at least 50% (Heyl et al., 2023).

The required changes are gradually being incorporated into policy and legislation, reflected in the Common Agricultural Policy reforms, which now promote a precision approach to plant nutrition to improve nutrient use efficiency (Heyl et al., 2023). The Circular Economy Action Plan (European Commission, 2020) has underlined the need to foster nutrient recycling and facilitate the reincorporation of waste streams into agriculture. Reincorporation and upcycling/recycling of nutrient-rich wastes into new circular fertilizer products replacing synthetic fertilizers has been supported by the recent updates in the Fertilizing Product Regulation (Regulation (EU) 2019/2009). These policy and regulation changes have caused a renewed interest in approaches for incorporating waste-derived nutrients into fertilizers.

In this context, the EU has committed significant research efforts through initiatives such as Horizon 2020 and Horizon Europe. Among these initiatives is the EU-funded FertiCycle project, a Marie Skłodowska-Curie Actions Innovative Training Network, in which all authors of this review paper were actively involved. The FertiCycle project aimed at proposing solutions for nutrient recovery and recycling within the European framework, addressing agronomical, environmental, and economic challenges associated with the development and adoption of improved and new bio-based fertilizers, and thus contributing to a more circular nutrient economy in the EU. The aim of this review is to present the contributions of the FertiCycle project within the context of critical challenges and current state-of-the-art literature on BBFs in the EU, guiding researchers,



policymakers, and stakeholders by highlighting key innovations and addressing critical barriers for BBF adoption. Figure 1 provides an overview of the agronomic challenges, improvements, and pending challenges presented and discussed in this review.

2 What is a bio-based fertilizer?

The use of the term bio-based fertilizer or BBF has increased lately in academic publications for describing a broad category of materials of biological origin used to supply nutrients to crops (Chojnacka et al., 2020; Wester-Larsen et al., 2022; Rodríguez-Alegre et al., 2023; Kurniawati et al., 2023a). However, distinguishing BBFs from organic fertilizers and biowastes presents a challenge.

The term “biowaste” encompasses materials of biological origin discarded as wastes, including byproducts from agriculture, food production, animal husbandry, and human life. Nutrient-rich biowastes, when applied to soils to provide nutrients for plants, are included under the category of organic fertilizers (Pain and Menzi, 2011). In everyday language, a distinction is made between a general biowaste and an organic fertilizer, only based on their actual usage. Manure, defined by the Oxford English Dictionary as “Dung, excrement or compost, esp. as spread over or mixed with soil to fertilize it” is a prevalent example.

The term “animal manure” describes a mixture of animal feces, urine and bedding material that is utilized as a fertilizer (Shober and Maguire, 2018). Animal manures often undergo some degree of processing, such as anaerobic storage, addition of water or homogenization to facilitate its application to the field. However, the term BBF is employed in literature advocating alternatives to mineral fertilizers or describing materials of enhanced fertilizer value, implying a more technologically advanced material than a basic, raw organic fertilizer such as an animal manure (Luo et al., 2022; Egas

et al., 2023; Moshkin et al., 2023; Garmendia-Lemus et al., 2024). Therefore, it is arguable that an animal manure is not a BBF, since it had no treatment specifically to improve its fertilizing qualities.

The core value of a BBF lies in its circularity (Chojnacka et al., 2020). Bio-based fertilizers are intended to allow the reincorporation of nutrients that otherwise would be wasted, have an improved nutrient use efficiency compared to a less processed organic fertilizer, or decrease possible negative environmental impacts compared to its organic or mineral fertilizer counterparts. Furthermore, organic fertilizers can be associated with containing organic matter and being capable of, e.g., improving soil structure or being a source of organic carbon. However, a BBF can also include nutrients derived from biomass, in the absence of organic matter, and resemble mineral fertilizers in their chemical and physical forms, as long as the nutrients are from biological origin.

The concept of biological origin is one of the main points of discussion for recognizing a fertilizer as bio-based according to the European Sustainable Phosphorus Platform (ESPP, 2023). In the context of bioplastics, the European Standard EN 16575 (August 2014) recognizes the biological origin, when the material is wholly or partly derived from biomass renewable during human lifespans, i.e., excluding materials fossilized and embedded in geological formations.

The European Union updated Fertilizing Product Regulation (FPR, regulation EU 2019/1009) has not yet established a precise definition for BBFs. However, it distinguishes between materials of inorganic and organic origin and introduces categories for nutrients of biological origin. The FPR incorporates so-called product function categories (PFC) into legislation, which categorize different types of fertilizing products based on their primary purpose. Products within these categories need to be composed of materials recognized within specific component material categories (CMCs). Therefore, for a bio-based product to be recognized as a fertilizer, or be included as a part of one, its components need to fit within a CMC. Relevant PFCs

TABLE 1 Product function categories (PFC) and sub-categories from the updated fertilizing product regulation (Regulation (EU) 2019/1009) pertinent to new bio-based fertilizers.

Category	Description	Sub-categories	Component material categories (CMCs)
PFC 1	Fertilizers - Products with the primary function of supplying nutrients to plants or mushrooms	PFC 1 (A): Organic Fertilizers PFC 1 (B): Organo-Mineral Fertilizers PFC 1 (C): Inorganic Fertilizers	CMC 2: Plants or Plant Extracts CMC 3: Compost CMC 4: Fresh Crop Digestate CMC 5: Non-Fresh Crop Digestate CMC 6: Food Industry By-products CMC 12: Precipitated phosphate salts & derivatives (e.g., struvite) CMC 13: Thermal oxidation materials & derivatives (e.g., ashes) CMC 14: Pyrolysis & gasification materials CMC 15: Recovered high purity materials (e.g., ammonium salts, purity >95%)
PFC 7	Product Blends - Products that fit into two PFCs, e.g., a product designed to supply nutrients to plants but also acts as a soil improver		

and CMCs are summarized in Table 1. Main novelties include CMC 12, which regulates the use of recovered phosphate from, e.g., wastewater treatment plants. This category sets limits of tolerance for chemical impurities and pathogens, and allows materials of less purity than those classified under CMC 15. Animal by-products are still outside the scope of the FPR (EC, 2022), and regulated under the Animal By-products Regulation EC 1069/2009 (EC, 2022). However, this will change once the European Commission establishes endpoints in the processing chain for individual by-products. Currently, there is a draft proposal for the inclusion of animal by-products in the FPR.¹

The clear definition of the term BBF is challenging and still debatable. One definition that has been proposed is that BBFs are materials or products derived from materials of biological origin, with a content of bioavailable nutrients high enough to be used as a fertilizer (Wester-Larsen et al., 2022). On the other hand, Rodríguez-Alegre et al. (2023) proposed that waste streams, i.e., manure, sewage sludge and food processing wastes, which can be valorized, through processing, into a fertilizer product should be termed BBFs. Both definitions indicate that some degree of processing is necessary for a biowaste or an organic fertilizer to acquire BBF status. Moreover, literature also has assigned bio-based status to fertilizers where the nutrients are recovered (e.g., via ammonia stripping or P precipitation) from biowastes, or when they are contained in new fertilizing products combining synthetic nutrients with those of biological origin.

It is not our goal to replace the term “organic fertilizer” with BBF. It is rather to provide an overview of the challenges and opportunities for reincorporating nutrients from biowastes into the food system and improving the agronomic efficiency of existing organic and bio-based fertilizers. Therefore, creating new, improved BBFs. For the purpose of this review, we will consider a BBF, any material of biological origin (e.g., a biowaste), that has been treated with the purpose of enhancing its fertilizing properties (e.g., an acidified slurry); as well as fertilizing products, where a significant proportion of its nutrients is of biological origin, for example, an

organo-mineral fertilizer containing a combination of mined rock phosphate and ammonium nitrate gained through ammonia stripping of manure (Wester-Larsen et al., 2022).

3 From biowastes to new bio-based fertilizers

The importance of BBFs in a more sustainable agriculture is underscored by the abundance of waste materials such as agricultural and food waste (e.g., olive pomace), sewage sludge and animal excreta (Kummu et al., 2012; Mateo-Sagasta et al., 2015; Shober and Maguire, 2018). These biowastes contain carbon (C), P and N, making them valuable but potentially harmful for the environment if improperly handled (Chojnacka et al., 2020).

One of the main challenges in utilizing biowastes and organic fertilizers as nutrient sources is maximizing the availability of the contained P and N, which are often only partly or slowly available (Chojnacka et al., 2020). Raw biowastes also pose challenges such as nutrient imbalances relative to crop demand, pathogen contents and high volumes, requiring chemical and physical modifications (Overmeyer et al., 2023; Zireeni et al., 2023; Kopp et al., 2023b). Biowaste transformations (e.g., composting, anaerobic digestion, pyrolysis) designed to tackle logistical or environmental challenges can negatively impact the nutrient availability (Jameson et al., 2016).

Biowastes undergo several treatments depending on legal, economic, or environmental requirements. For example, cattle excreta are composted, or subjected to ammonia stripping; sewage sludge can be anaerobically digested, composted, dewatered, or incinerated (Kanteraki et al., 2022). An overview of available input materials for new BBFs is given in Table 2.

3.1 Phosphorus and nitrogen in raw materials for new bio-based fertilizers

3.1.1 Phosphorus

Increasing the P fertilizer value in biowastes is challenging, due to the diverse chemical forms of inorganic P (Pi) and organic P (Po), influenced by the biowaste source and processing (Meyer et al., 2018).

¹ The latest version of the draft amendment of the FPR was elaborated on October 2, 2023, and can be consulted under the document name Ares(2023)6629522.

TABLE 2 Types of potential main input materials for new bio-based fertilizers.

Material	Description	Examples of transformation methods into BBF	References
Animal by-products	E.g. Raw animal excreta, meat, and bone meal	Acidification, alkalinization	Cao et al. (2020), Chrysanthopoulos et al. (2024), Fangueiro et al. (2015), Sica et al. (2023), and Zireeni et al. (2023)
Compost	Biowastes that went through a composting process	Incorporation into organo-mineral fertilizers	Sitzmann et al. (2024)
Digestates	Solid or liquid output of the anaerobic digestion process of biowastes	Biomass pre-treatments Electrokinetic pre-treatment Ensiling pre-treatment Re-digestion after solid-liquid separation	Carlsson et al. (2012) Nyang'au et al. (2023a) Nyang'au et al. (2023b) Aguirre-Villegas et al. (2019)
Mineral precipitates or concentrates	Mineral compounds extracted, separated, or precipitated from biowastes (e.g., struvite, ammonium sulfate)	Incorporation into organo-mineral fertilizers and direct application	Fangueiro et al. (2017), Hušek et al. (2022), Sena et al. (2021), Zabaleta and Rodic (2015)
Plant based materials	Plant juices, extracts, and solids, olive pomace	Composting, anaerobic digestion, solid-liquid separation	Ameziane et al. (2020), Muscolo et al. (2019) and Sorensen and Thorup-Kristensen (2011)
Sewage sludge	By-product of wastewater treatment plants that concentrates solids of sewage.	Acidification, alkalinization Incorporation into organo-mineral fertilizers	Sica et al. (2023) Deeks et al. (2013)
Thermally treated biowastes	Biowastes converted into ash or biochars via incineration or pyrolysis, respectively.	Pre-treatments	Kopp et al. (2023a)

List was originally categorized by Wester-Larsen et al. (2022).

We added sewage sludge, due to the different legislation and fertilization requirements: sewage sludge is excluded from the FPR and most European countries have stricter regulations on land application of sewage sludges compared to other biowastes (Gianico et al., 2021). Some European countries ban the land application of sewage sludge entirely, as in the case of Switzerland (Verordnung Über Umweltgefährdende Stoffe, 2002). Another introduced category was “thermally treated materials”.

Phosphorus is plant available only in the form of dissolved inorganic orthophosphates (Tate, 1984).

Most of P in manure and feces is water-soluble Pi, whereas Po varies based on the feed and animal and type (Barnett, 1994; Poulsen and Kristensen, 1998). Dairy cattle manures contain between 6 g·kg⁻¹ and 16 g·kg⁻¹ of total P, mostly Pi (Barnett, 1994). Poultry manures contain between 13 g·kg⁻¹ and 23 g·kg⁻¹ of total P, have variable proportions of Pi and Po, and are rich in calcium-bound Pi forms (Barnett, 1994; Neijjat et al., 2011). One of the most important challenges regarding manures are their imbalanced P to N ratios, that may result in excessive P application when fertilization aims to meet plant N demand (Sharpley, 1996). High concentrations of water-soluble Pi in and organic compounds have been linked to high P leaching in soils treated with manure (Glæsner et al., 2011). The transformation of manure with composting significantly concentrates the contained P due to dry matter loss, and reduces P solubility (Zhang et al., 2023).

Phosphorus in composts is predominantly inorganic (Frossard et al., 2002; Zhang et al., 2023). In an evaluation of 16 samples of composted Swiss solid urban biowastes and woody wastes, Frossard et al. (2002) measured P concentrations between 2 g·kg⁻¹ and 7 g·kg⁻¹, mostly in the form of Pi. Phosphorus in compost included both water-soluble Pi as well as relatively insoluble calcium phosphates like apatites or octacalcium phosphates. Sewage sludges contain Po and Pi forms of low solubility [e.g., associated to iron (Fe), aluminum (Al) or calcium (Ca)] influenced by the diversity of P removal techniques (e.g., flocculation with iron or aluminum salts or biological P removal) used in wastewater treatment plants (Frossard et al., 1997; Wang et al., 2022). Anaerobic digestion (AD) of sludges can increase the crystallinity of Pi forms and leave behind only recalcitrant Po (Wang et al., 2022). Alkalinization by liming, done for sanitization purposes

(Anderson et al., 2015; Malinowska, 2017), produces the formation of calcium phosphates that can become plant available in acidic soil conditions (Lindsay, 1972; Meyer et al., 2018). Solubility of P in sewage sludge is strongly decreased during thermal treatments for stabilization or volume reduction (e.g., pyrolysis or incineration), causing sewage sludge ashes to contain little available Pi due to the formation of stable P mineral forms (Nanzer et al., 2014; Lemming et al., 2017). Sewage sludge ashes and meat and bone meal have low mineral P fertilizer equivalencies, of 30 and 40%, respectively; untreated sewage sludge typically ranges from 60 to 90%, depending on the method used for P removal (Möller et al., 2018). The P in meat and bone meal is mainly in the form of hydroxyapatite (Jeng et al., 2007).

3.1.2 Nitrogen

The challenges associated with the N fertilizer value from biowastes include the diversity of N forms in biowastes and the losses through collection, treatment, storage, and soil application (Lassaletta et al., 2014; Fangueiro et al., 2015; Nigussie et al., 2017). In biowastes, N is usually contained in organic forms (urea and other amides, amino acids, undigested feed and food protein residues, nucleic acids) with variable amounts of inorganic plant available forms like ammonium (NH₄⁺) and nitrate (NO₃) (Langmeier et al., 2002; de Guardia et al., 2010; Bosshard et al., 2011). Human and animal urine contain most N in the form of urea, which is enzymatically transformed shortly after excretion into NH₄⁺. Feces contain more complex N forms, for example, bacterial or endogenous debris for which availability will depend on mineralization processes (Dion et al., 2020).

Anaerobic digestion of manures and sewage sludges increases the inorganic N content (Hafner and Bisogni, 2009; Jiang et al., 2022). During AD, mineralization of N forms takes place (Möller and Müller,

TABLE 3 Aspects regarding the form and contents of phosphorus and nitrogen and agronomic challenges of biowastes and potential input materials for new bio-based fertilizers.

Biowaste	Phosphorus	Nitrogen	Agronomic challenges	References
Cattle manure	6–16 g·kg ⁻¹ total P, mostly soluble Pi	Organic N forms (urea, amino acids), mineral N (NH ₄ ⁺ -N)	Imbalanced P to N ratios, potential excessive P application, risk of P leaching.	Barnett (1994) and Poulsen and Kristensen (1998)
Poultry manure	13–23 g·kg ⁻¹ total P, variable Pi and Po, rich in calcium-bound Pi	Organic N forms (urea, amino acids)	Variable P and N content, calcium-bound Pi, insoluble at neutral to alkaline pH.	Barnett (1994) and Neijat et al. (2011)
Sewage sludge	Very rich in P (up to 40 g·kg ⁻¹)	Dissolved organic N	Impact of P flocculation or removal technique on P availability.	Frossard et al. (1997) and Wang et al. (2022)
Sewage sludge ashes	Pi of very low availability	Low quantity due to thermal treatment	Low P solubility due to formation of crystalline forms.	Lemming et al. (2017) and Nanzer et al. (2019)
Olive Pomace	Raw pomace is poor in available P (<0.02–0.04%)	High C:N ratio	Low pH, high content of organic components that can be phytotoxic, low P and N contents.	Ameziane et al. (2020) and Muscolo et al. (2019)
Meat and bone meal	20–40 g·kg ⁻¹ total P, mainly hydroxyapatite derived from bones	Proteins and amino acids derived from the meat and blood.	Low P solubility due to stable mineral forms, having a low availability to plants, especially in soils with neutral and alkaline pH.	Brod et al. (2015) and Christiansen et al. (2020)
Mineral precipitates or concentrates, e.g., struvite:	Struvite: > 12.5 % total P (Mg associated P)	E.g. Ammoniacal form when ammonia stripping is used	Although it is already produced commercially the precipitation and recovery processes are costly, making it an expensive fertilizer.	Christiansen et al. (2020) and Li et al. (2019)
Organo-mineral fertilizers (OMF)	>2% P ₂ O ₅ – P that can come from mineral fertilizer (e.g., diammonium phosphate) and/or from P contained in the organic fraction	>2% N, minimum 0.5% organic N (if OMF contains P and/or K). >2.5% N, minimum 1% organic N (OMF with only N as macronutrient). N can come from mineral fertilizer and from the organic fraction	OMF are already commercialized, generally more expensive than equivalent mineral fertilizers. Peat is still the main organic matrix used in Europe. If nutrients come from the organic fraction, challenges are typical of organic fertilizers (low solubility and high variability among batches).	EC (2019), Paré et al. (2010) and Rodrigues et al. (2021)
Composts	2–7 g·kg ⁻¹ total P, mostly Pi, rich in calcium-bound Pi	Inorganic N (NH ₄ ⁺ , NO ₃ ⁻) and organic N forms	Dry matter loss reduces P availability.	Frossard et al. (2002) and Zhang et al. (2023)
Digestates	Depends on the biowaste. Improved (decreased) C:P ratios in, e.g., olive pomace	High inorganic N content, low organic N, decreased C:N ratio	N losses during digestion are lower than composting, but increased crystallinity reduces P availability, high N losses when surface applied.	Hafner and Bisogni (2009), Jiang et al. (2022) and Pedersen et al. (2021)

Nutrient concentrations reported in percentage or g·kg⁻¹ dry matter.

2012). Nitrogen losses during the digestion process are significantly smaller compared to composting or thermal treatments (Chojnacka et al., 2020). Anaerobic digestion leads to decreased C to N ratios in the digestate effluent (Möller and Müller, 2012) as C is converted to biomethane gas (mixture of CO₂ and CH₄), while N is conserved in the digestate. Mechanical separation of solid–liquid phases from the anaerobic digestate concentrates directly available inorganic N (as dissolved ions) in the liquid fraction and slowly available organic N in the solid fraction (Zabaleta and Rodic, 2015; Chojnacka et al., 2020).

Composting of household waste, pig slaughterhouse sludge, and green algae resulted in losses of 36–66% of total N to the atmosphere (de Guardia et al., 2010), predominantly caused by ammonia (NH₃) volatilization (Chowdhury et al., 2014). Volatility and speciation of ammoniacal-N in biowastes is influenced by the pH (Moraes et al., 2017). Acidification of animal manures prevents N emissions during processing, storage and land spreading and reduces NH₃ volatilization by shifting the equilibrium toward the non-volatile

NH₄⁺ (Fangueiro et al., 2015). Similarly, during storage of biowastes or composting, acidification and pH management minimize N losses (Chowdhury et al., 2014; Cao et al., 2020; Kupper et al., 2020). Delayed applications of N-rich materials during composting can also reduce N losses (Nigussie et al., 2017).

Much of the existing waste management infrastructure prioritizes sanitation and emission reduction rather than recycling nutrients (Magid et al., 2006). For instance, wastewater treatment plants emit substantial amounts of N into the atmosphere through nitrification–denitrification processes aiming to reduce wastewater N (Marchuk et al., 2023). The goal of current and future waste management is to transform these facilities into nutrient recovery centers (Marchuk et al., 2023). The application of chemical and physical treatments to the outputs of waste management infrastructure, coupled with enhancements in existing processes are a step in that direction. Table 3 includes an overview of P and N forms in biowastes, as well as related agronomic challenges.

3.2 Treatments to enhance the P and N fertilizer value of bio-based fertilizers

3.2.1 Pre-treatment of inputs in anaerobic digestion

Anaerobic digestion improves the N fertilizer value of digestates compared to untreated inputs. Anaerobic digestions mineralize decomposable organic matter in slurries, reduce the C to N by converting C to biogas, and increase the $\text{NH}_4\text{-N}$ to total N ratio through organic N mineralization (Sørensen and Møller, 2009). However, with an increasing shift toward using high solid co-substrates such as lignocellulosic agricultural wastes (e.g., straw and other crop residues) with short hydraulic retention times, a smaller proportion of the organic matter (40–60 %) is degraded into biogas and the rest remains in digestates (Romio et al., 2021). Resulting digestates have reduced fertilizer values due to their high dry matter contents, higher C to N ratios and lower infiltration rates enhancing the risk of NH_3 loss (Møller et al., 2022; Pedersen and Hafner, 2023). Biogas plants prioritize biogas yields over digestate quality (Logan and Visvanathan, 2019). This is driven by the higher value of biogas compared to digestate and incentives such as renewable energy targets.

Optimization of AD for enhanced biogas production and also nutrient availability can be achieved through biomass pre-treatments. Biomass pre-treatment techniques, classified as physical, chemical, biological, or a combination of these, have variable effects on biomass utilization in the AD process, dependent on the pre-treatment mechanism and feedstock characteristics. Biomass containing lignin or bacterial cells are the most affected during pre-treatment for enhanced AD process (Carlsson et al., 2012).

Nyang'au et al. (2023a) investigated the effects of electrokinetic and ultrasonication pre-treatments of biowastes in a two-step AD process on nitrogen fertilizer replacement value of digestates obtained from two biogas plants. The electrokinetic pre-treatment step significantly increased the ratio of ammonium-N to total N in the digestates before the second AD step. However, the effect leveled off after the secondary digestion step. The study demonstrated how integrating pre-treatment technologies into biogas plants could improve the fertilizing properties of the digestates.

Another study by Nyang'au et al. (2023b) highlighted the use of ensiling as a biological pre-treatment method to enhance biogas yield and improve the fertilizer value of the digestates. Ensiling significantly impacted physico-chemical properties of straw, increased methane yield by 4 to 14 %, and increased net inorganic N and S release in the soil compared to non-ensiled straw. They attributed the positive effect to enhanced substrate biodegradation during the ensiling, which increased biochemical accessibility and nutrient solubilization during AD.

Pre-treatments can be effective measures to increase the fertilizer value of digestates. However, the selection of a pre-treatment technique should consider net effects, including cost and energy consumption (Meegoda et al., 2018). Many studies solely evaluate pre-treatment benefits by comparing extra energy output against energy consumption, overlooking other potential advantages such as increased digestate fertilizing value.

3.2.2 Acidification of slurries

Emissions derived from barns and slurry storage represent 80% of agricultural NH_3 emissions, which can be mitigated by acidification

to a pH in the range of 4.5–6.8 (Fangueiro et al., 2015). Moreover, utilization of untreated animal slurry in horticulture poses potential risks in terms of food safety. Slurry acidification can be employed to address both NH_3 emissions and food safety risks (Fangueiro et al., 2015; J. Rodrigues et al., 2021).

Utilization of sulfuric acid for slurry acidification represents an addition of available sulfur that increases the fertilizer value of the slurry (Zireeni et al., 2023). Acidification increased the concentration of water-soluble P in 20–65% compared to raw slurry (Regueiro et al., 2020). Moreover, there are benefits associated with the soil application of acidified slurry. In the study conducted by Schreiber et al. (2023) where acidified slurry (pH 5.5) was applied to a Haplic Cambisol (pH 7), a significant increase of 38% in N use efficiency was observed in plant biomass. Zireeni et al. (2023) found that the application of acidified slurry (pH 5.5) to a Cambisol (pH 6.8) transitorily reduced soil pH by at least 0.4 units for up to two months, before the pH went back to its baseline. A transitory acidification of soil can be beneficial in soil conditions where P is a limiting factor.

For slurry acidification, sulfuric acid, and to a lesser extent, nitric and hydrochloric acids are utilized (Fangueiro et al., 2015). However, other additives and processes for bio-acidification involving agro-industrial by-products were tested by Chrysanthopoulos et al. (2024) for acidifying pig slurries. It was shown that bio-acidification of pig slurries through fermentation is possible when the fermentation substrate contains sufficient organic C (Chrysanthopoulos et al., 2024). Moreover, slurry bio-acidification using rice bran, a biowaste rich in N, as a fermentation substrate significantly increased the total N content compared to untreated slurry (Prado et al., 2020). Conclusively, acidification of slurries can be used beyond addressing hygienic concerns and mitigating NH_3 emissions. Acidification can be a way to utilize agro-industrial by products and increase the fertilizer value of slurries.

3.2.3 Acidification and alkalization of sewage sludge, sewage sludge ashes and meat and bone meal

Sica et al. (2023) evaluated the impact of acidification and alkalization pre-treatments on the P solubility of sewage sludge, sewage sludge ashes and meat and bone meal. For acidification, a 2 to 1 biowaste to solution ratio was utilized, with sulfuric acid concentrations ranging from 0.25 M to 10 M. Alkalization was carried out using sodium hydroxide concentrations ranging from 1 M to 2.5 M. For alkalization with lime $[\text{Ca}(\text{OH})_2]$, lime quantities equivalent to 10 to 40% of the fresh weight of the biowaste were added. Acidification resulted in a decrease in the pH of the biowastes to a range between 1 and 4, while alkalization treatments raised the pH of the biowastes between 8 and 12. It was observed that acidification significantly increased the solubility of P, leading to a greater release of P into the soil and an increase in soil water-extractable P. Water-extractable P in the sewage sludge ashes increased up to 60 times. In meat and bone meal, water-extractable P rose from 4% to more than 80% of the total P when pH dropped below 4. Alkalization with sodium hydroxide was found effective in increasing soil P availability in sewage sludge, and potentially providing similar sanitation effects to lime. However, plant trials were not conducted by Sica et al. and they highlighted that the higher costs of sodium hydroxide compared to lime may limit the large-scale application of this pretreatment.

Similarly, Keskinen et al. (2023) used residual organic acids from cellulose extraction to acidify sewage sludge to both pH 7 and 4.5. They observed a significant increase in P solubility at pH 4.5, but it did not impact P uptake by ryegrass. It is important to mention that their results demonstrated that the acidification of sewage sludge solubilizes other elements, including harmful metals, which may harm plant growth (Imadi et al., 2016).

3.2.4 Chemical modifications of thermally treated biowastes to enhance P availability

In the Netherlands, Switzerland, and Belgium, mono-incineration has emerged as the predominant practice (Mininni et al., 2015). Recent legislative changes in Sweden, the Czech Republic, and Denmark have made possible utilizing sewage sludge biochar for agricultural purposes.

Both incineration and pyrolysis offer advantages such as volume reduction, increased P concentration, pathogen elimination or reduction, and potential energy recovery. Additionally, pyrolysis forms recalcitrant C and contributes to soil C sequestration and climate change mitigation (Smith, 2016). However, as previously discussed, thermal treatment decreases the P availability.

The use of additives before pyrolysis can enhance P availability in biochar. The addition of magnesium hydroxide alters the resulting P forms after pyrolysis, mainly by avoiding the formation of crystalline Ca-P minerals in favor of amorphous and more soluble Mg-P forms (e.g., MgNH_4PO_4 and $\text{Mg}_3(\text{PO}_4)_2$), which has resulted in a 20% increase in the P availability of poultry litter biochar (Zwetsloot et al., 2015; Leite et al., 2023). The addition of calcium oxide (CaO) in thermal treatment may offer similar benefits. Liu et al. (2019) found

that addition of 10% CaO converted non-apatite inorganic P forms into apatitic forms, (e.g., $\text{Ca}_3(\text{PO}_4)_2$ and $\text{Ca}_3\text{Mg}_3(\text{PO}_4)_4$). This increased the sewage sludge biochar P availability and promoted plant growth in acidic soils.

Another alternative is offered by pH modification treatments after pyrolysis or incineration. The effect of acidification with sulfuric acid was assessed in three ashes (sewage sludge, poultry litter, digestate solids) and four biochars (digestate solids, sewage sludge, meat and bone meal, insect frass) (Kopp et al., 2023b). While the P availability from untreated ashes and biochars was very low, the acidification significantly increased the total plant P uptake from all materials from 2 to 35 times. Acidification solubilized mainly Ca-P and did not increase heavy metal uptake nor strongly affected soil pH which remained at 6.4. A synthesis of the effectiveness of alkalization and acidification treatments for P solubilization is provided in Figure 2.

3.2.5 Combined treatments

Integrated approaches throughout manure management chains elevate the fertilizer value and nutrient concentration and mitigate trade-offs between NH_3 and GHG emissions (Aguirre-Villegas et al., 2019). One such effective integrated approach involves coupling anaerobic digestion of biomass with solid-liquid separation of the resulting digestate. In this strategy, the solid fraction is re-digested to enhance further biogas and nutrient recovery. The liquid fraction is then used as a fertilizer, since it is enriched with a higher proportion of NH_4^+ and overall higher N. Alternatively, the solid fraction, rich in P, may be utilized to fertilize P-deficient soil or transformed into value-added products like biochar, contributing to soil C retention (Fangueiro et al., 2015), or used as livestock bedding material.

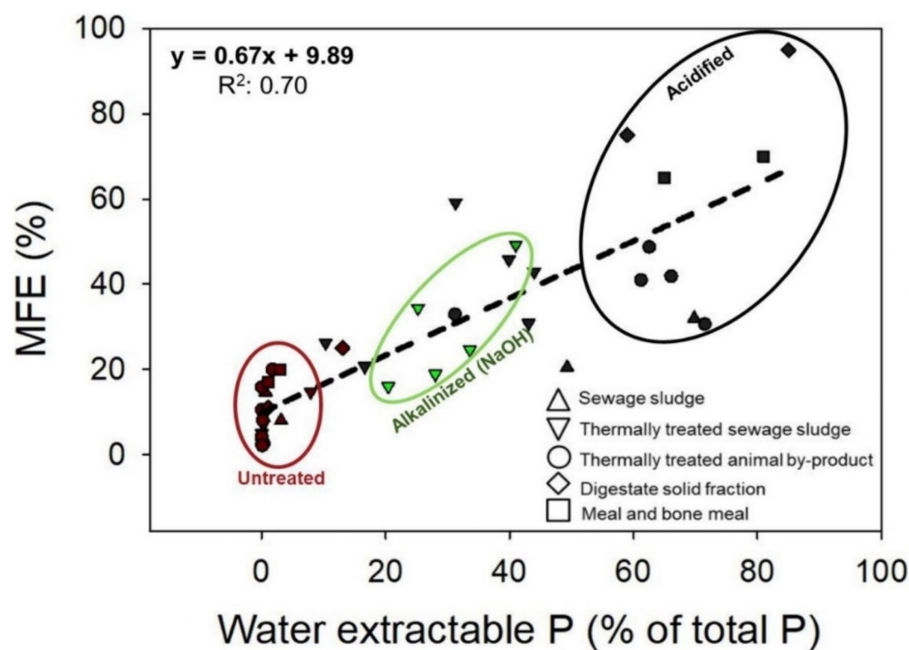


FIGURE 2

Correlation between the P solubility (water extractable P) and the mineral fertilizer equivalent of untreated, alkalized, and acidified biowastes (Sica et al., 2023; Kopp et al., 2023a,b). The figure shows that biowastes with initially low water-soluble P (<5% of total P content) significantly increased this proportion after acidification, while thermally treated sewage sludge also did so after alkalization. Furthermore, a correlation ($R^2 = 0.7$) between the water-extractable P content of these materials and their mineral fertilizer equivalent values was observed.

Other integrated approaches that could improve the fertilizer value of digestates include AD followed by acidification of the digestates, source segregation of manure followed by anaerobic digestion and AD followed by plasma treatment of digestates. Plasma treatment of digestates fixes reactive N from the atmosphere to the slurries in nitrite and nitrate forms (Graves et al., 2019). The nitrite and nitrate fixation forms nitric acid (HNO_3) and nitrous acid (HNO_2), which lowers the slurry pH and produces the previously mentioned benefits (Winter and Chen, 2021). Plasma treatment of slurry has shown a potential to replace more mineral fertilizer and increase yields compared to untreated slurry (Cottis et al., 2023). Moreover, this treatment reduces both methane (CH_4) and NH_3 emissions during storage and field applications, although the risk of increased losses via nitrous oxide (N_2O) emissions and nitrate leaching needs to be assessed (Cottis et al., 2023). Integrated approaches offer a comprehensive solution for optimizing fertilizer values and contribute to sustainable agricultural practices by minimizing environmental impacts.

3.3 Organo-mineral combinations

Organo-mineral fertilizers (OMFs) are a mixture of materials of biological origin with one or more mineral fertilizers (Smith et al., 2020). This allows the use of BBFs rich in organic C as the organic matrix, such as compost. The use of OMF has been reported to have some advantages over mineral fertilizers alone, as addition of organic C can reduce N losses and increase N use efficiency (Richards et al., 1993; Antille et al., 2014; Florio et al., 2016). Similarly, OMFs increase plant P use efficiency by causing a prolonged release of plant available P in soil (Antille et al., 2013).

The enhanced nutrient use efficiency can be linked to various processes. For instance, there is an electrostatic attraction of phosphates to organic materials, reducing the P mobility in soil that could be otherwise fixed to clay minerals (Gwenzi et al., 2018; Luo et al., 2021). The chemical interactions between the organic matrix and mineral fertilizer can result in the formation of insoluble compounds that precipitate out of the soil solution (Mazeika et al., 2016; Carneiro et al., 2021; Luo et al., 2021). Additionally, the organic material acts as a physical barrier between the mineral fertilizer granule and the soil solution, thereby decreasing the solubility of the fertilizer granule (Limwikran et al., 2018). Lastly, the stimulation of soil microbial activity leads to the immobilization of nutrients into the microbial biomass and a subsequent gradual N release that reduces leaching (Richards et al., 1993; Mandal et al., 2007).

The FPR establishes that at least 3% of the total mass of an OMF must be composed of organic C if it is a liquid OMF, and at least 7.5% on a solid OMF, while the sum of macronutrients needs to be 6% or 8%, respectively (EC, 2019). This broad definition allows the production of OMFs with varying characteristics.

One type of OMF is where the organic matrix is used as the main source of one or more nutrients and mineral fertilizer is added to produce a specific nutrient ratio that will depend on specific soil and plant nutrient requirements (Rady, 2012; Antille et al., 2013; Anetor and Omuetti, 2014). This type of solid OMF, in addition to supplying macro and micronutrients, can be used as a soil amendment to improve physical properties due to its large organic C content (Babalola et al., 2007). On the flip side, OMFs rich in organic C require

the application of large quantities to the soil - in the order of tons per hectare- to adequately fulfill crop nutrient requirements (Antille et al., 2013; Mazeika et al., 2016). Such large required application volumes could result impractical.

A second group of solid OMFs has a small proportion of organic C from a material of biological origin. This material acts mainly as a protecting matrix for the mineral nutrients and not as a relevant nutrient source. Low organic C OMFs are designed to produce a slow release of mineral nutrients into the soil solution increasing the nutrient use efficiency (Richards et al., 1993). However, although the use of low organic C OMFs has the advantage of increasing the efficiency of the mineral fertilizer – and therefore reducing its application volume – they have the disadvantage of negligibly contributing with organic C to the soil.

There is a significant tradeoff between OMFs with high and low organic C. In those with low organic C, biowastes are not a significant source of nutrients. Therefore, their potential contribution to biowaste recycling is limited. If the mineral nutrients in the OMF are not from biological origin, it is advisable not to consider them a bio-based fertilizer (ESPP, 2023). Despite this limitation, biowastes in low organic C OMF play a crucial role in enhancing the nutrient use efficiency of mineral fertilizers, which is a significant benefit.

3.4 Application methods and strategies to improve the fertilizer value of bio-based fertilizers

Various studies highlight the importance of fertilizer P placement for enhancing the fertilizer value (Quinn et al., 2020; Freiling et al., 2022). Applying mineral P fertilizers by placement close to the seeds maximizes crop P uptake in the early growth stages (Grant et al., 2001; Grant and Flaten, 2019). Another strategy is subsurface band application (10 cm to 20 cm depth). This is mainly adopted in tropical countries with highly sorbing P soils that are susceptible to long drought periods. Subsurface band application creates P-rich bands with high available P contents, that are less susceptible to the soil surface drying (Meyer et al., 2023). However, the placement of P-rich fertilizers, as would be the case with some BBFs, is not as effective as the placement of mineral P fertilizers. Two possible explanations are the lower P solubility in BBFs compared to mineral fertilizers; and nutrient imbalances, leading to the over application of elements that may be toxic to the plant (Lemming et al., 2016).

Placement of sewage sludge increased root proliferation in the placement zone but did not enhance plant growth and total P uptake (Lemming et al., 2016). It significantly reduced P uptake from the soil compared to a treatment where the fertilizer was completely mixed with the soil. Regarding sewage sludge ash, Lemming et al. (2016) observed that placement did not attract root growth to the placement zone and significantly reduced both P uptake and plant growth. Building up on that, Sica et al. (2023) suggested that biowastes may have sufficient soluble P to attract roots to the placement zone, however, available P may not be sufficient to sustain plant growth for longer periods. Therefore, treatments to increase the P solubility of bio-based fertilizers are needed, along with placement strategies.

Fertilization with organic fertilizers may lead to the overapplication of organic N in the placement zone. Accumulated

TABLE 4 Negative environmental impacts of BBFs, mitigation strategies and relevant literature.

Negative impact	Mitigation strategy	References
Overapplication of nutrients due to non-optimized nutrient ratios	Optimize N to P ratio through solid–liquid separation or source segregation; use organo-mineral combinations	Deeks et al. (2013) and Sharpley (1996)
Presence of heavy metals in sewage sludges and other BBFs	Thorough monitoring and management of biowastes; use of conversion factors to predict heavy metal concentration	de Castro et al. (2023), Geng et al. (2020), Kupper et al. (2014), and Lu et al. (2012)
Presence of microplastics and organic pollutants in soil from sewage sludge	Monitoring; improving wastewater treatment processes; pyrolysis and thermal treatments	Corradini et al. (2019), Crossman et al. (2020), and Kanteraki et al. (2022)
Emission of GHGs from BBF production and soil application	Use energy from renewable sources for BBF production, placement and seasonal fertilization strategies; use locally sourced biowastes; use of nitrification inhibitors	Kar et al. (2023) and Meneses-Quelal and Velázquez-Martí (2020)
Emissions of NH ₃	Storage with reduced exposed surface area; injection of digestates into soil rather than surface applications; use acidifying agents;	Álvarez-Gaitan et al. (2016) and Maris et al. (2021)

organic N is mineralized over time, releasing high amounts of NH₃ into the soil (Chaves et al., 2004). Plants can suffer NH₃ toxicity symptoms, such as inhibiting root growth at concentrations above 100 mg·kg⁻¹ (Nkebiwe et al., 2016). To reduce NH₃ toxicity effects, placement should be done more than 10 cm from the seeds, and/or days before sowing, allowing NH₃ to be nitrified thus lowering its concentration over time, while the plant is starting to develop (Delin et al., 2018; Baral et al., 2021). Acidification may also reduce the negative effects of NH₃ toxicity (Pedersen et al., 2017).

4 Environmental impacts of bio-based fertilizers

4.1 Environmental benefits of bio-based fertilizers production and use

Recycling P reduces phosphate rock mining, thereby avoiding the associated environmental impacts such as landscape degradation, contamination of water bodies, and emission of GHGs associated with transport (Higgins, 2001; Fayiga and Nwoke, 2016). Moreover, using phosphate rock and derivatives for fertilization can result in the contamination of water bodies and soils with heavy metals and hazardous elements like cadmium, uranium and arsenic (Fayiga and Nwoke, 2016). Similarly, reducing the reliance on synthetic N contributes to lowering net GHG emissions and energy consumption, as Haber-Bosch synthesis of NH₃ is one of the major fossil energy consuming processes worldwide (Osorio-Tejada et al., 2022; Gao and Cabrera Serrenho, 2023).

Beyond the substitution of mineral fertilizers, bio-based fertilizers offer additional environmental benefits. For example, by offering a slower release of nutrients compared to mineral fertilizers, nutrient leaching is reduced (Mandal et al., 2007). Bio-based fertilizers containing organic C enhance soil structure by increasing the organic matter content which also may be beneficial for microbial communities (Mayer et al., 2022). In soils in a Danish long-term field experiment, soils treated with compost significantly increased their organic C content compared to the mineral NPK treatment by up to 3%. Moreover, they significantly reduced their bulk density from 1.6 kg·l⁻¹ down to 1.2 kg·l⁻¹, and improved their soil structure, thus requiring less energy consumption for tillage (Peltre et al., 2015).

4.2 Negative environmental impacts associated with bio-based fertilizer production and use, and mitigation strategies

Due to non-optimized nutrient ratios relative to crop demand in many BBFs compared to mineral fertilizers, fertilization with BBF and organic fertilizers could lead to overapplication of nutrients. An overview of environmental impacts associated with BBFs is available in Table 4. For instance, fertilization with manures can lead to overapplication of P when aiming to meet crop N demand (Sharpley, 1996). Similar problems arise when using digestates (Kadam et al., 2022), sewage sludge and other biowastes (Deeks et al., 2013; Lemming et al., 2019). Mitigation strategies encompass minimizing N losses during production, storage, and application of the BBF, thus maximizing the N content and its use efficiency, for which Ndegwa et al. (2008) and Pedersen and Hafner (2023) offer extensive reviews. The N to P ratio of some BBFs such as those derived from animal manures can be optimized by separating N from P through solid–liquid separation, or through segregation at-source of feces and urine before any processing step (Hjorth et al., 2009; Vu et al., 2016). Another strategy is using an organo-mineral combination, that provides the desired N to P ratio and corrects for the nutrient imbalance (Deeks et al., 2013).

The presence of contaminants could be a problem for some BBFs. Sewage sludges can contain cadmium, lead, and other heavy metals that may accumulate in soils after fertilization (Lu et al., 2012). In a study in Chile, significant amounts of microplastics accumulated in the soil after a decade of fertilization with sewage sludge (Corradini et al., 2019). The presence of persistent organic compounds like polychlorinated biphenyls, polycyclic aromatic hydrocarbons, traces of pharmaceutical compounds and hormones in sewage sludges, is discussed by Kanteraki et al. (2022). An overview of pollutant removal techniques for sewage sludge is provided by Geng et al. (2020). Olive pomaces contain phytotoxic phenolic compounds, lipids, and organic acids. Composting pomaces can help mitigate their toxicity by degrading some of the harmful compounds and increasing their fertilizer value (Muscolo et al., 2019; Ameziane et al., 2020).

Heavy metals are of potential concern in digestates and composts depending on the local input materials (Kupper et al., 2014; Kadam et al., 2022). Therefore, there is a pressing need for thorough monitoring and management of these biowastes. A breakthrough for

the simplification of monitoring on a local scale was the obtention of conversion factors to predict the concentration of heavy metals in Belgian manure-derived digestates (de Castro et al., 2023). These conversion factors were based on process parameters of the AD processes, allowing the prediction of the concentrations of aluminum, chromium, copper, iron, manganese and zinc based on the dry matter and the biodegradable fraction content of digestates.

Emissions of GHGs can result from both the production of BBFs and their soil application. In the production stage, mineral precipitates and thermally treated biowastes specifically raise concern as they require energy-intensive treatments. Thermal treatments and separation processes such as ammonia stripping, membrane electrodialysis, ion-exchange and struvite precipitation may emit less C to the atmosphere compared to traditional mineral fertilizer synthesis. However, their operational processes also demand substantial energy consumptions (Meneses-Quelal and Velázquez-Martí, 2020; Kar et al., 2023). Therefore, the source of the utilized energy will influence the environmental impacts of such treatments.

During soil application, NH_3 emissions from manure digestates are often higher than from untreated manure (Holly et al., 2017; Emmerling et al., 2020). Furthermore, using ammonium sulphate to target sulfur fertilization has been linked with increased and overlooked NH_3 emissions in neutral to alkaline soils (Powlson and Dawson, 2022). This not only emits NH_3 , contributing to undesired N deposition in natural ecosystems, but also results in losses of recovered N and the energy invested in its recovery. Likewise, fertilization with sewage sludge causes emissions of N_2O and CH_4 (Álvarez-Gaitan et al., 2016).

Implementing mitigation strategies is essential to minimize emissions from BBFs. These strategies include selecting appropriate application timing and methods, managing soils to maximize C retention, and using stabilizing additives like nitrification inhibitors (Severin et al., 2016; Tariq et al., 2022). For instance, injecting digestates into the soil reduces NH_3 emissions compared to surface applications (Hou et al., 2015). Similarly, applying slurry to grasslands during spring rather than autumn is linked to less N_2O emissions (Maris et al., 2021). Soil management practices such as minimizing or eliminating tillage and implementing crop rotations and leys, maximize the amount of C retained in agricultural soils (Jarecki et al., 2003). Acidifying digestates with, e.g., sulfuric acid can significantly reduce NH_3 emissions (Pedersen and Nyord, 2023).

4.3 Assessment of environmental impacts from bio-based fertilizers

Single mitigation strategies can potentially result in pollution swapping. Therefore, understanding the interaction of all processes involved and the impact of specific local conditions is necessary. A comprehensive and integrated approach such as a life cycle assessment (LCA) is optimal to assess the potential environmental impacts of fertilization with BBFs (Jensen et al., 2020; Egas et al., 2023).

For example, Styles et al. (2018) used a LCA to assess BBF production from the liquid fraction of digestate of food waste in Sweden, applying NH_3 stripping and struvite precipitation. The BBF production and field application was compared to the conventional management of digestate's liquid fraction, including storage and field application. They concluded that producing BBF from liquid digestate results in significant

environmental benefits due to the avoidance of CH_4 , N_2O and NH_3 emissions compared to the conventional management of liquid digestate. Moreover, application of that BBF enhanced the substitution of synthetic fertilizer due to the targeted use of nutrients. Another LCA on digestate utilization compared four alternative BBF production scenarios in relation to mineral fertilizer production (Alengebawry et al., 2022). This study included two technologies for nutrient extraction from the solid fraction and two more for the liquid fraction. Results showed that in all scenarios, BBFs constituted environmentally beneficial alternatives compared to mineral fertilizers, provided the digestate was pretreated to remove pollutants and pathogens.

Several studies have applied LCA to assess different aspects of fertilization with sewage sludge (Yoshida et al., 2018; Ding et al., 2021). For example, Yoshida et al. (2018) assessed the long-term impacts after field application of sewage sludge by using emission factors calculated by Bruun et al. (2016). Emission factors for sewage sludges were calculated considering sewage sludges with different properties, applied to three soil types, using three precipitation regimes and varying application amounts. This approach enabled the use of region-specific emission factors in the LCA (Yoshida et al., 2018). Normalizing the LCA results to yearly *per capita* emissions showed that human toxicity and ecotoxicity impacts were of greatest concern, largely due to the zinc and copper content in sewage sludge (Yoshida et al., 2018).

Ultimately, regional and local conditions may determine the sustainability and feasibility of using one BBF over another. Walling and Vaneekhaute (2020) reviewed emission factors on organic and inorganic fertilizer production and use. This study recommended that emission factors should be estimated based on case-specific data due to the high variation in emissions depending on the composition of the fertilizer and the impact of local conditions like soil type and climate. In the LCA study of Beyers et al. (2022), the environmental impact of pig slurry acidification was assessed for the climatic, agronomic, and legislative conditions of Denmark, Spain, and the Netherlands. Slurry acidification reduced the environmental impacts related to emissions of GHG and NH_3 . However, the acquisition of energy and materials for the acidification process led to increased off-farm impacts in some categories, including fossil resource depletion and human toxicity. Furthermore, the effectiveness of acidification to reduce environmental impacts varied between countries due to differences in legislative requirements and energy sources. Thus, specific regional conditions (soil, climate, legislation, and farming practices) are crucial for the overall environmental sustainability of fertilization with specific BBFs. The PLCI 2.0 model has been developed to account for regional differences in LCAs of P-containing BBFs applied in European regions (Rydgård et al., 2024). This model incorporates factors such as regional soil P concentrations, soil erosion rates and distribution of crop types. Furthermore, it enables the modeling of the impact of different fertilization practices on P losses, harvesting of P in crops and the substitution of mineral P fertilizer.

5 Bio-based fertilizer market developments – social acceptance and economic drivers

Concerns regarding biosafety and environmental impact raised in recent decades, particularly for sewage sludge, have cast a negative

perception on fertilization with some biowastes (Ekane et al., 2021). There is still a significant gap between the EU and other regions in the West in biosolids (treated sewage sludge) adoption. For instance, on average, only 35% of produced sewage sludge is reincorporated into agriculture in the EU (Hušek et al., 2022), compared to 55% in the United States and Canada, and more than 70% in Australia (Marchuk et al., 2023). However, the European average varies significantly by nation; for example, it is 0% in the Netherlands and Slovakia, but as high as 80% in Ireland (Hudcová et al., 2019). A comprehensive understanding of regulations, as well as preferences and needs of end-users and major stakeholders is necessary for a better acceptance and adoption of BBFs (Goldstein and Beecher, 2007; Ekane et al., 2021).

5.1 Stakeholders in the BBF market

In the BBF market, primary stakeholders include livestock farmers with a surplus of manure for export/processing, and crop farmers who are potential end-users of BBFs (Jensen et al., 2017; Kurniawati et al., 2023a). Other important players are food and pharmaceutical industries, waste management companies, recycling fertilizer companies and farmers with crop residues on the supplier side, as well as garden owners and horticultural producers on the end-user's side (Jensen et al., 2017; Venegas et al., 2021). Governments, public institutions (local/national/EU), civil society, non-governmental organizations (NGO), the food industry, investors, media, and scientists are also stakeholders in the European BBF market (Nedelciu et al., 2019).

The influence of primary stakeholders (farmers) on the supply and demand of BBFs varies. While livestock farmers are incentivized to utilize surplus manure; crop farmers exert more power in accepting or declining recycled products based on their perceptions and preferences (Case et al., 2017). Thus, defining and addressing stakeholder requirements and preferences is crucial for BBF adoption.

5.2 Attributes of bio-based fertilizers that influence acceptance and perception

The acceptance of BBFs among farmers in Europe is mainly influenced by four attributes: known nutrient contents, organic matter contents, cost and ease of application (Egan et al., 2022). Negative perceptions often arise from uncertainty regarding the N, P and K contents in BBFs (Tur-Cardona et al., 2018; Egan et al., 2022), contrasting with the precision offered by mineral fertilizers. Farmers are aware of the uncertainties in nutrient contents in organic fertilizers, and they prefer BBFs with nutrient ratios that fit crop demands (Egan et al., 2022). Therefore, reliable and known amounts and ratios of nutrients are essential for facilitating adoption.

A strength of BBFs is their organic matter content, and their perceived capacity to enhance soil structure, improve soil productivity, and increase water retention capacity of soils (Case et al., 2017; Gwara et al., 2021). These benefits are well-recognized by farmers (Egan et al., 2022). However, these advantages alone are not enough to completely substitute mineral fertilizers with BBFs and need to be accompanied by the other three attributes.

The cost of BBFs and their fertilizing properties relative to mineral fertilizers play a pivotal role in social acceptance. Another advantage of BBFs is their perceived low-cost and high nutrient content (Case

et al., 2017). Farmers are more likely to adopt BBFs if they are competitively priced (Egan et al., 2022). Logistical costs and perceived higher overall expenses may deter adoption, necessitating a cost at least half that of mineral fertilizers for widespread acceptance (Tur-Cardona et al., 2018). Despite potential cost differences, the ecological co-benefits of BBFs mentioned before, could sway farmers toward their adoption with proper awareness (Egan et al., 2022). We discuss such potential in the next section.

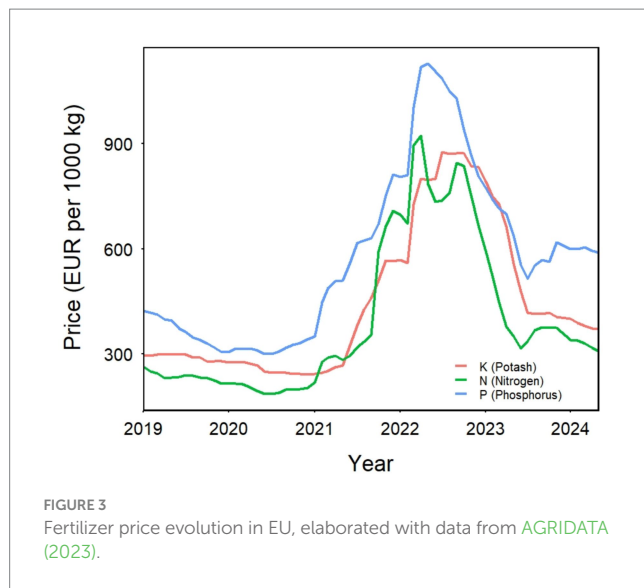
The ease of application and the practicality of BBFs also play a major role. The form of the BBF (solid, semi-solid, liquid, or granulated) significantly influences farmer preferences and acceptance (Tur-Cardona et al., 2018; Egan et al., 2022). Solid and semi-solid forms are favored over liquids due to ease of application with existing farm machinery and improved forms, such as pellets, can enhance acceptance and willingness to pay (Hills et al., 2021). Thus, farmers prefer BBFs to be granular like mineral fertilizers.

Farmers may be reluctant to utilize BBFs due to the potential negative perception from clients and consumers of their products (Simha et al., 2017). In a Polish survey, an important source of negative perception stemmed from concerns about potential health risks associated with fertilization using nutrient-rich biowastes (Smol, 2021). Consumer acceptance of products fertilized with BBFs is greater for ornamental plants than for horticultural crops for consumption (Segrè Cohen et al., 2020). This indicates that final consumers of produce fertilized with BBFs may hold negative perceptions. Media and NGOs also influence consumer opinion and their perceptions of BBF products (Jensen et al., 2017). Even if farmers are willing to use recycled nutrients, the reluctance of final consumers and the food industry to consume products fertilized with BBFs may prevent them from doing so (Barquet et al., 2020; McConville et al., 2023). Therefore, it is important to raise awareness among produce consumers and inform them about the environmental advantages of BBFs.

5.3 Current bio-based fertilizer market

Although precise estimations of the European BBF market size are not available, it is evident that the BBF market is smaller compared to the conventional mineral fertilizer market. Approximately half of the EU's fertilizer inputs for P come from mineral P, and about two-thirds of N fertilizers are produced through the Haber-Bosch process (Schoumans et al., 2015; Einarsson et al., 2021). Both studies recognize animal manures as the most significant alternative sources of P and N aside from mineral fertilizers. Therefore, the BBF market is still in early stages, requiring substantial changes in product availability, quality, legislation, competition dynamics, and stakeholder perceptions (Kvakkestad et al., 2023).

A favorable landscape is currently emerging for the development of the BBF market. Legislative developments, as outlined in section 2, have established a legal framework for BBF products in the European market, providing a conducive environment for market growth. However, a crucial question arises: where should the focus be directed? In the previous section, we outlined five criteria important for BBF adoption. Additionally, literature has emphasized that BBFs should resemble mineral fertilizers in consistent supply, allowing for a seamless transition for farmers accustomed to using commercially available mineral fertilizers (Gregson et al., 2015; Case et al., 2017; Buysse and Cardona, 2020; Kvakkestad et al., 2023).



Buyse and Cardona (2020) suggested that rising prices of mineral fertilizers would create an opportunity for the BBF industry to develop, given the higher production costs of BBFs. The fertilizer market has experienced significant volatility (Figure 3) during the period from 2021 to 2023, particularly due to rising geopolitical tensions and the war in Ukraine, leading to several increases in mineral fertilizer prices (AGRIDATA, 2023). The price increase in late 2021 and 2022 created a new reality for BBF producers, making more nutrient recovery technologies economically feasible (Hermann and Hermann, 2021). Since early 2023, fertilizer prices have been decreasing and stabilizing. At the time of writing this article, mineral fertilizer prices have returned at the levels of mid-2021. However, despite the prices being lower than in 2022, they remain higher than previous pre-2021 levels. Given the ongoing geopolitical pressures on the European Union, it is unlikely that the prices will decrease further in the foreseeable future (Alexander et al., 2022; Brownlie et al., 2023; Rabbi et al., 2023).

The pricing and competitive position of BBF compared to mineral fertilizers are influenced by many factors: demand, availability of adequate BBFs, logistics, local legislation, and regional fertilization practices (Case et al., 2017; Kvakkestad et al., 2023). Previous studies recommended pricing of BBF significantly lower than mineral fertilizers (Case et al., 2017; Tur-Cardona et al., 2018). However, Moshkin et al. (2023) considered the willingness-to-pay and perceptions of potential users in an EU farmer survey and suggested that BBFs should be priced equivalently to mineral fertilizers. While willingness-to-pay may still be lower for BBFs compared to mineral fertilizers, marketing strategies could help justify higher prices and contribute to the development of the BBF industry. Moreover, branding of BBFs plays an important role in their adoption. For example, using the term “biosolids” instead of “treated sewage sludge” positively impacts consumer attitudes and acceptance (Lu et al., 2012).

6 Discussion

The EU’s initiatives like the Farm to Fork strategy and the new EU Fertilizer Regulation signal a shift toward a more circular nutrient

economy, with BBFs playing a crucial role. However, agronomic and environmental challenges persist for BBFs. The main challenge identified is the uncertainty surrounding the quantity and availability of nutrients, as well as the lack of adequate nutrient ratios in biowastes (Egan et al., 2022).

Many biowastes have deficiencies in their nutrient compositions when used as organic fertilizers, and these deficiencies can be exacerbated by transformation treatments applied to biowastes. However, deficiencies can be corrected through chemical treatments or pre-treatments that enhance the nutritional properties of the transformation outputs (e.g., digestate). Despite these improvements, many BBFs still have limitations compared to the practicality of more concentrated and stable mineral fertilizers. To ensure adequate nutrient quantities and ratios, organo-mineral combinations are a promising alternative (Deeks et al., 2013; Sitzmann et al., 2024), as well as improved processes of nutrient recovery in waste management facilities (Marchuk et al., 2023). To maximize the agricultural efficiency of BBFs, including organo-mineral combinations, it is essential to use them alongside appropriate placement strategies. This approach ensures that the benefits of both organic and mineral components are fully realized, promoting sustainable and effective fertilizations.

The current economic conditions, marked by high prices of mineral fertilizers and changes to the EU Fertilizer Regulation, present an unique opportunity for the growth of the BBF market. However, barriers such as negative perceptions of BBF and logistical issues hinder their adoption by farmers. Improving perception could be achieved by offering BBF products that match mineral fertilizers in both agronomic efficiency and physical attributes. To improve perception and acceptance of BBFs, it is imperative to work with stakeholders and end-users of BBFs and include them in research and development processes to address their logistical and agricultural needs (Nedelciu et al., 2019; Venegas et al., 2021). While agricultural efficiency is crucial, it is also important for BBFs to provide additional benefits to the soil, like addition of organic matter (Case et al., 2017). Practicality is another key element, along with customer and societal acceptance of products fertilized with BBFs. It is one of the most crucial factors to develop to unlock the BBF market. Literature has evidenced a demand for BBFs that offer the same convenience, meaning they are always available, can be applied without major logistical complications, and are designed for the needs of a specific crop, i.e., commercial products.

Based on the literature review, we propose that to enhance BBF acceptance among farmers and growers, it is important to demonstrate that: (1) BBFs have sufficient and reliable nutrient amounts, as well as crop-adequate nutrient ratios; (2) BBF can maintain or improve soil fertility by being a supply of organic matter to soils; (3) BBFs need to be priced competitively with mineral fertilizers, but not significantly cheaper than mineral fertilizers. The close pricing ensures that BBFs remain an attractive alternative while covering costs of production and innovation.; (4) Handling and application of BBF with existing machinery is possible; (5) There is willingness among consumers of the farmer’s products to buy products fertilized with BBFs.

Increasing refinement of bio-based fertilizing products will be associated with higher energy and material consumption, and environmental impacts. For instance, pollution swapping caused by some treatment techniques may result in trade-offs between the agronomic and

environmental benefits of BBFs. To overcome this, integrated measures that consider local conditions are crucial. Multiple LCAs have shown that the sustainability of BBFs is strongly influenced by local factors such as available biowastes, electricity sources, local legislation, soil and climatic conditions, which ultimately determine the agronomic value and environmental impacts (Jensen et al., 2020; Beyers et al., 2022). Thus, local, and small to middle-scale production of BBFs utilizing regional biowastes may be a more optimal solution than centralized, large-scale national facilities. Similarly, transforming waste management facilities like wastewater treatment plants into nutrient recovery centers, is an important aspect of locally sourcing BBFs.

Strategies of fertilization with BBFs should also consider adequate timing and placement to maximize nutrient efficiency and minimize nutrient losses to the environment (Lemming et al., 2016; Maris et al., 2021). Therefore, effective agricultural extension services are needed to assist farmers in transitioning to BBFs and ensure that they are used and placed appropriately to maximize their fertilizer value.

7 Conclusion

Maximizing the potential of BBFs in the European context and overcoming existing barriers requires a comprehensive assessment. We acknowledge that the agricultural use of BBFs at present could be demanding for farmers, given the regulatory framework, the scarcity of new, innovative BBFs, the planning required to ensure proper nutrient supply to crops, and the potential reluctance of consumers to buy products that have been fertilized with BBFs. It is crucial to carefully consider legislative, logistical, economic, soil fertility, and climatic requirements, with a primary focus on the crop's nutrient needs to develop BBFs that can be adopted by farmers. Therefore, substantial research and product development efforts are still required to overcome these barriers and provide viable alternatives. However, the goal is to achieve a circular nutrient economy and not only substitute mineral fertilizers with BBFs. Therefore, it is important for all stakeholders to recognize the value of producing and consuming products fertilized with recycled nutrients, understanding how their purchase contributes to a more circular economy and sustainable development in general.

Author contributions

MÁ: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. PS: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. TS: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. MR: Writing – review & editing, Writing – original

draft, Conceptualization. JN: Writing – review & editing, Writing – original draft, Methodology, Formal Analysis, Conceptualization. EM: Writing – review & editing, Writing – original draft, Visualization, Conceptualization. JM: Writing – review & editing, Writing – original draft, Validation. CK: Writing – review & editing, Writing – original draft. HC: Writing – review & editing, Writing – original draft. AU-J: Writing – review & editing, Writing – original draft. SC: Writing – review & editing, Writing – original draft. FL: Writing – review & editing, Writing – original draft. KW: Writing – review & editing, Writing – original draft. YZ: Writing – review & editing, Writing – original draft. SH: Writing – review & editing, Writing – original draft. DC: Writing – review & editing, Writing – original draft, Supervision. LJ: Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement no. 860127. Open access funding by ETH Zürich.

Acknowledgments

We would like to thank Astrid Oberson from ETH Zürich for her feedback on the later version of this manuscript and valuable discussions.

Conflict of interest

The authors declare that this review was conducted in the absence of any commercial or financial relationships that could be regarded as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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OPEN ACCESS

EDITED BY

Tom Misselbrook,
Rothamsted Research, United Kingdom

REVIEWED BY

Arturo F. Chica,
University of Cordoba, Spain
Elio Dinuccio,
University of Turin, Italy

*CORRESPONDENCE

Cristiane Romio
✉ ane.romio@bce.au.dk

RECEIVED 10 April 2024

ACCEPTED 24 June 2024

PUBLISHED 12 July 2024

CITATION

Romio C, Ward AJ and Møller HB (2024)
Characterization and valorization of biogas
digestate and derived organic fertilizer
products from separation processes.
Front. Sustain. Food Syst. 8:1415508.
doi: 10.3389/fsufs.2024.1415508

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Characterization and valorization of biogas digestate and derived organic fertilizer products from separation processes

Cristiane Romio^{1*}, Alastair James Ward² and
Henrik Bjarne Møller²

¹Department of Biological and Chemical Engineering, Aarhus University, Aarhus, Denmark,

²Department of Biological and Chemical Engineering, Aarhus University, Tjele, Denmark

Introduction: Anaerobic digestion of manure, together with other biowastes, produces biogas that can substitute fossil energy and thereby reduce CO₂ emissions and post-digestion greenhouse gas emissions. The final digestate of the process is an organic fertilizer rich in plant nutrients and recalcitrant organic constituents. The digestate characteristics and quality depend on several parameters, such as input feedstocks and operational conditions of the biogas plants. In Denmark, the rapid expansion of the biogas sector in recent years has resulted in a great variety of feedstocks used in the plants. The first generation of biogas plants mainly treated manure, industrial wastes, and energy crops with short retention times, while the new generation of biogas plants are co-digesting manure with higher amounts of lignocellulosic feedstocks and operating with longer retention times. This study evaluated whether this shift in feedstock composition could impact the fertilizer quality and post-digestion greenhouse gases and ammonia emissions during storage and application of digestate.

Methods: Digestate samples from 2015 to 2023 were collected and analyzed for composition and residual methane yields. The efficiencies of solid-liquid separation applied to several digestate samples from the new generation of biogas plants were investigated and the nutrients contents of the liquid and solid fractions of digestate were evaluated.

Results and discussion: The most evident change caused by the feedstock transition was an average increase of 52% in the total solids content of digestate, which can negatively impact ammonia emissions during digestate application. In contrast, similar average residual methane yields on a fresh matter basis of approximately 5 L/kg indicated comparable risks of methane emissions during storage. The liquid fraction of industrially separated digestate presented, on average, nutrient concentrations similar to those of unseparated digestate, while the solid fraction presented similar K, lower total ammoniacal nitrogen, and higher organic N and P contents than unseparated digestate on a fresh matter basis. The average residual methane yield of the industrially separated solid fraction of digestate was 101 L/kg volatile solids, while the average calorific value was 21 MJ/kg volatile solids, indicating its potential for additional energy generation.

KEYWORDS

residual methane potential, calorific value, screw press, decanter centrifuge, straw

1 Introduction

Anaerobic digestion (AD) is a common method of treating organic matter to produce energy in the form of methane rich biogas. However, the de-gassed digestate can be considered as a valuable secondary resource that is commonly used as a fertilizer (Teglia et al., 2011). The fertilizer value of digestates is usually associated with the NPK concentrations (Teglia et al., 2011), and sometimes more specifically to the ammonium nitrogen content (Alburquerque et al., 2012b). However, high concentrations of heavy metals may restrict their use (Alburquerque et al., 2012b), and concerns associated to ammonia and greenhouse gases emissions exist.

Digestate can be utilized in its raw form or separated into solid and liquid fractions for easier transport and application and for partitioning of the nutrients (Teglia et al., 2011). Normally, the liquid fraction of digestate (LFD) is valued by its ammonium and K concentrations, while the solid fraction (SFD) is attractive due to its organic matter and P contents (Drosg et al., 2015). The characterization of other nutrients, heavy metals, and plant-derived polymers and their partitioning into SFD and LFD are seldom reported in the literature. The LFD can be used for direct fertilization or fertilization combined with irrigation (Alburquerque et al., 2012b). The SFD is often considered as a soil amendment (Kovačić et al., 2022) or it can be used for additional energy generation through, for instance, prolonged AD by SFD recirculation to the digester, combustion, hydrothermal liquefaction, or pyrolysis (Monlau et al., 2015). The produced biochar from pyrolysis is suitable for use as a soil amendment (Kovačić et al., 2022) and can be a method for carbon capture and sequestration (Wu et al., 2023).

AD of agricultural wastes such as manures results in relatively low biogas yields (Orlando and Velazquez-Marti, 2020), so the use of energy crops such as maize silage to boost gas production became popular in many countries, with maize silage constituting 70% of the fresh matter input to German biogas plants (Daniel-Gromke et al., 2018). This has led to concerns regarding competition with food production and overall sustainability of the process (Bartoli et al., 2019). As a result of these concerns, the Danish government has progressively reduced the proportion of energy crops added to manure-based biogas plants in recent years, to a maximum of 4% of the fresh mass added from August 2024 to a complete restriction of maize silage from 2025 (KEFM, 2023). To replace energy crops and increasingly expensive and limited industrial wastes, Danish biogas plants have focused on the utilization of agricultural byproducts such as cereal straws (Larsen et al., 2023), grasses, and deep litter manures (Møller et al., 2022). Due to the poorer degradability of these substrates compared to energy crops, higher loads have been applied, along with longer hydraulic retention times (HRTs). This transition in AD feedstock may lead to changes in the digestate quality, affecting fertilizing and amendment properties, and associated ammonia and greenhouse gases emissions during digestate storage and after field application.

This study examines digestates from several biogas plants in Denmark from 2015 to 2016 and from 2020 to 2023. The two periods compare the digestate compositional changes with a reduced portion of energy crops and increased portion of straws and deep litter in the latter period, when compared to the earlier period. Samples of LFD and SFD from the latter period were also examined, and the partition of digestate components into these fractions were evaluated. The work

examines physical and chemical parameters including nutrients and heavy metals contents and fiber composition. Residual methane potentials were determined to assess the risks of methane emissions during storage and the potential of methane recovery by prolonged AD. Calorific values were estimated to evaluate the utilization of digestate for pyrolysis or combustion.

2 Materials and methods

2.1 Experimental set-up

Digestate samples were collected from the final digester or post-storage tank of a selection of Danish biogas plants. Samples were divided into Group 1 and Group 2. Group 1 consisted of samples collected between 2015 and 2016 and comprised samples from biogas plants treating mainly cattle and pig manure, industrial waste, energy crops, and some portions of deep litter. Group 2 contained samples collected between 2020 and 2023 from biogas plants treating cattle and pig manure, industrial waste, smaller amounts of energy crops, and increased portions of deep litter and straw. Within Group 2, ten of the collected digestate samples were separated into solid and liquid fractions in the lab on the same day as they were collected. Another 12 samples of solid and liquid digestate separated at the respective biogas plants were obtained. The main feedstock employed, the digestion temperature, and the HRTs are presented in Table 1. Digestate samples and their fractions were analyzed on the same day of collection or after storage at -18°C . They were characterized in terms of pH, conductivity, total solids (TS), volatile solids (VS), volatile fatty acids (VFA), total ammoniacal nitrogen (TAN), total Kjeldahl nitrogen (TKN), crude protein and lipids contents, fiber (cellulose, hemicellulose, and lignin) and elemental compositions (C, N, H, and S), calorific values, contents of macro-nutrients (Ca, Mg, K, and P), and micro-nutrients, and heavy metals (Na, Fe, Mn, Zn, Cu, Cr, Al, Ni, and Mo). Mass balances determined the efficiency of the separation methods. AD batch tests were employed to determine the residual methane potentials of the samples and started on the same day of sample collection, except for industrially separated SFD samples, which were previously frozen. Part of the unseparated digestate samples from Group 2 (samples A1-A10 and B1-B19) were characterized in terms of pH, TS, VS, VFA, TAN, fiber and elemental compositions, and methane yields in a previous study (Romio et al., 2023).

2.2 Solid–liquid separation

In the lab-scale separation, 3 kg of each digestate sample was separated with a 1 mm mesh sieve of 20 cm diameter, initially by manual screening, followed by applying a pressure of 5.3 kPa for 5 min. The weights of the solid and liquid fractions were recorded. The industrial separation was conducted with screw presses for eight samples and decanter centrifuges for four samples (Table 1).

2.3 Anaerobic digestion tests

The AD batch tests were conducted in triplicates to determine the residual methane yields of digestate and its fractions and lasted

TABLE 1 Operational conditions from the selected digesters.

Samples	Temperature	HRT	Feedstock	Separation method
	(°C)	(days)		
Group 1				
01 ^a	38.0	70.8	S, M, IW, PM	
02 ^b	52.0	27.7	M, IW, CM, PM	
03 ^c	39.0	29.5	M, IW, CM, PM	
04 ^d	52.0	17.5	M, IW, CM, PM	
05 ^e	52.0	38.0	M, G, IW, CM, PM	
06	53.0	29.5	M, IW, CM, PM	
07	50.0	30.3	S, G, DL, IW, CM, PM	
08	38.0	99.2	M, G, DL, IW, CM, PM	
09 ^f	52.0	43.1	M, G, IW, CM, PM	
10	38.0	26.6	IW, CM, PM	
11	38.0	23.2	M, IW, CM, PM	
12	52.0	27.0	IW, CM, PM	
13	51.0	69.0	S, M, G, DL, IW, CM, PM	
14	51.0	44.7	M, DL, IW, CM, PM	
15	52.0	55.0	M, DL, IW, CM, PM	
Average	47.20 (6.62)	42.07 (22.51)		
Group 2*				
A1 ^g , A1S, A1L	52.0	13.0	S, G, CM, PM	LS
A2 ^b , A2S, A2L	51.0	35.0	M, G, IW, CM, PM	LS
A3 ^h , A3S, A3L	51.0	70.0	S, G, B, IW, CM, PM	LS
A4 ⁱ , A4S, A4L	46.8	100.0	S, IW, CM, PM	LS
A5 ^j , A5S, A5L	51.0	75.0	G, IW, CM, PM	LS
A6, A6S, A6L	39.0	68.4	M, G, IW, CM, PM	LS
A7, A7S, A7L	42.5	60.4	M, G, IW, CM, PM	LS
A8, A8S, A8L	42.5	70.8	M, G, IW, CM, PM	LS
A9, A9S, A9L	35.0	48.0	M, G, IW, CM, PM	LS
A10 ^a , A10S, A10L	42.0	96.0	S, IW, PM	LS
B1 ^g	52.0	50.0	S, G, CM, PM	

(Continued)

TABLE 1 (Continued)

Samples	Temperature	HRT	Feedstock	Separation method
	(°C)	(days)		
B2 ^b	51.0	35.0	M, IW, CM, PM	
B3 ^b , B3S ^b , B3L ^h	51.0	70.0	S, G, B, IW, CM, PM	SP
B4 ⁱ	46.8	100.0	S, IW, CM, PM	
B5 ^j	51.0	75.0	G, IW, CM, PM	
B6 ^k	38.0	38.0	IW, CM, PM	
B7 ^k	43.0	38.0	IW, CM, PM	
B8 ^l , B8L ^l	49.0	60.0	S, DL, IW, CM, PM	DC
B9 ^l , B9S ^l , B9L ^l	49.0	60.0	S, DL, IW, CM, PM	DC
B10 ^f	52.0	40.0	M, IW, CM, PM	
B11	50.0	100.0	S, DL, IW, CM, PM	
B12 ^m	46.0	80.0	S, DL, IW, CM, PM	
B13 ⁿ	52.0	60.0	S, DL, IW, CM, PM	
B14 ^o , B14S ^o , B14L ^o	52.0	60.0	S, DL, IW, CM, PM	SP
B15, B15S, B15L	50.0	130.0	S, DL, IW, CM, PM	SP
B16	52.0	30.0	IW, CM, PM	
B17 ^e	30.0	25.0	IW, CM, PM	
B18 ^c , B18S ^c , B18L ^c	39.0	28.0	IW, CM, PM	DC
B19	50.0	70.0	S, G, CM	
B20	48.0	35.0	IW, CM, PM	
B21 ^p	52.0	43.0	S, DL, IW, CM, PM, CHM	
B22 ^p	38.0	43.0	S, DL, IW, CM, PM, CHM	
B23	51.0	60.0	S, DL, IW, CM, PM	
B24 ^d	51.0	45.0	S, DL, IW, CM, PM	
B25 ^d	51.0	45.0	S, DL, IW, CM, PM	
B26	51.0	32.0	S, DL, IW, CM, PM	
B27, B27S, B27L	40.0	90.0	S, DL, IW, CM, PM	SP
B28 ^o , B28S ^o , B28L ^o	52.0	60.0	S, DL, IW, CM, PM	SP
B29 ^m , B29S ^m , B29L ^m	46.0	150	S, DL, IW, CM, PM	SP
B30S ^g	52.0	50.0	S, G, CM, PM	DC
B31 ^q	38.0	55.0	S, DL, IW, CM, PM	

(Continued)

TABLE 1 (Continued)

Samples	Temperature		HRT (days)	Feedstock	Separation method
	(°C)				
B32 ^a	38.0		55.0	S, DL, IW, CM, PM	
B33 ^a	38.0		55.0	S, DL, IW, CM, PM	
B34 ^b , B34S ^b , B34L ^b	51.0		70.0	S, G, B, IW, CM, PM	SP
B35 ^c , B35S ^c , B35L ^a	52.0		60.0	S, DL, IW, CM, PM	SP
B36 ^c	52.0		60.0	S, DL, IW, CM, PM	
B37 ^c	52.0		60.0	S, DL, IW, CM, PM	
B38S ^c , B38L ^c	52.0		60.0	S, DL, IW, CM, PM	
Average**	46.90 (5.94)		60.95 (26.72)		

*Data for samples A1-A10 and B1-B19 had been presented by [Romio et al. \(2023\)](#). Values in parenthesis indicate the standard deviation.
**Considering only the unseparated samples. Samples named with the same number indicate collection in the same day from the same biogas plant (from the final digester or post-storage tank). S and L stand for the solid and liquid fractions of digestate, respectively. Same letter superscript indices indicate collection from the same biogas plant but at different days or from different parallel streams. Some plants changed number of digesters, HRT, digester temperature or feedstock composition within sample collections periods. HRT, hydraulic retention time; S, straw; M, maize; G, grass; B, beet; DL, deep litter; IW, industrial waste; CM, cattle manure; PM, pig manure; CHM, chicken manure; LS, lab-scale separation through sieving; SP, screw press; DC, decanter centrifuge.

between 84 and 148 days. The procedures of the AD tests and methods of determination of volumes of biogas and methane have been described elsewhere ([Romio et al., 2023](#)). An amount of 300 g digestate was employed for samples A1-A10 and lab-separated LFD samples A1L-A10L, while 200 g of digestate was employed for the remaining unseparated and industrially separated LFD samples. The samples were digested at the same temperatures as the digesters/storage tanks from which they were collected ([Table 1](#)).

Lab-separated SFD samples A1S-A10S were not included in the AD tests, and their methane productions were estimated through the methane productions of the respective unseparated and LFD samples (Section 2.5). Industrially separated SFD samples were digested with an inoculum, collected from the biogas plant at Aarhus University (Viborg, Denmark) 14 days before the start of the tests, filtered through a 1 mm mesh sieve, and incubated at 51°C for the partial deployment of its residual organic matter. 200 g of inoculum was employed in the AD tests, containing a VS content of 1.30%. The mass of the SFD samples added corresponded to the mass yielding an inoculum to substrate VS ratio of 2:1. AD tests were performed at 51°C.

2.4 Analytical methods

The determination of pH, TS, VS, TAN, and VFA contents, the elemental analysis, and the fiber characterization were performed as described by [Romio et al. \(2023\)](#). The fiber analysis fractionated the sample into neutral detergent soluble fraction (NDS), neutral detergent fiber (NDF), acid detergent fraction (ADF), and acid detergent lignin (ADL), as proposed by [Van Soest et al. \(1991\)](#). The hemicellulose content was considered to be the difference between NDF and ADF, the cellulose content as the difference between ADF and ADL, and the lignin content corresponded to the ADL fraction.

The conductivity was determined with an EcoSense EC300 conductivity meter (YSI, USA). The TKN was determined following the procedure described by [APHA \(2017\)](#), utilizing the digestion apparatus Tecator Kjeltac 2,400 (Foss Analytics, Denmark), the distillation system Vapodest 45 s (Gerhardt Analytical Systems, Germany), and the titration unit TitroLine easy (SI Analytics, Germany). The protein content was assumed to correspond to the difference between TKN and TAN multiplied by 6.25 ([Hattingh et al., 1967](#)). The lipids content was determined as described by [Jensen \(2008\)](#). The contents of nutrients and heavy metals were determined using optical emission spectrometry with inductively coupled plasma (ICP-OES, 4300 Optima, Perkin-Elmer, United States).

2.5 Calculations and data analysis

2.5.1 Calorific value

The theoretical calorific value was calculated through [Eqs. 1, 2](#) ([Annamalai et al., 1987](#)):

$$x_O = 100 - x_C - x_H - x_S - x_N - x_{ash}$$

(1)

$$\text{Calorific value} = 35160x_C + 116225x_H + 10465x_S - 11090x_O + 6280x_N \quad (2)$$

where the calorific value is given in kJ/kg, and x_C , x_H , x_S , x_N , x_O , and x_{ash} represent the contents of C, H, S, N (from elemental analysis), O, and ash (from VS determination) in % on a TS basis, respectively.

2.5.2 Separation efficiency

The enrichment of any analyzed component x into the solid fraction by the solid–liquid separation was evaluated by its relative separation index (RSI), calculated through Eqs. 3, 4 (Hjorth et al., 2011):

$$SI_x = \frac{m_{SFD}C_{x,SFD}}{m_{UD}C_{x,UD}} \quad (3)$$

$$RSI_x = \frac{SI_x - \frac{m_{SFD}}{m_{UD}}}{1 - \frac{m_{SFD}}{m_{UD}}} \quad (4)$$

where SI_x is the separation index of component x , RSI_x is the relative separation index of species x , m_{UD} represents the initial mass of unseparated digestate, m_{SFD} represents the mass of digestate recovered as SFD, and $C_{x,UD}$ and $C_{x,SFD}$ represent the contents of x in the unseparated digestate and SFD (on a fresh matter basis).

The RSI considers the amount of mass recovered in the SFD; therefore, it can determine if a component was selectively transferred to the solid fraction. An RSI value equal to 1 represents a complete recovery of a component in the SFD (occurring when $m_{SFD}C_{x,SFD} = m_{UD}C_{x,UD}$), while an RSI value close to 0 means that the given component was not selectively recovered and simply followed the overall mass fractionation into SFD and LFD (occurring when $C_{x,SFD} = C_{x,UD}$). Negative values of the RSI indicate the removal of a component from the SFD to the LFD (occurring when $C_{x,SFD} < C_{x,UD}$).

In the case of lab-scale separation, m_{UD} and m_{SFD} were determined experimentally. However, when considering the industrial separation, mass balances were required to estimate $\frac{m_{SFD}}{m_{UD}}$ to determine the

RSI. A general mass balance was combined with a mass balance of the TS, resulting in Eq. 5:

$$\frac{m_{SFD}}{m_{UD}} = \frac{C_{TS,UD} - C_{TS,LFD}}{C_{TS,SFD} - C_{TS,LFD}} \quad (5)$$

where $C_{TS,UD}$, $C_{TS,LFD}$, and $C_{TS,SFD}$ represent the contents of TS in the unseparated digestate, LFD, and SFD.

The TS content was chosen to be included in the mass balance due to the reliability of its determination method, allowing the use of greater amounts of sample compared to other analyses and, therefore, minimizing the impacts of sample heterogeneity. The calculations were performed for the subsets of ten samples with the unseparated digestate, SFD, and LFD that were collected on the same day (Table 1). Two of these samples underwent separation with decanter centrifuges while the remaining were separated with screw presses. Notice that the subsets of samples B9, B9L and B30S, which underwent separation

with decanter centrifuges, were not included in the calculations, since samples B9S, B30, and B30L were missing.

The RSI of any component x can, however, be severely impacted by inconsistencies in mass balances caused by sampling or analysis issues. Therefore, an error threshold of $\pm 20\%$ was assumed, and the RSI was only calculated when mass balances errors (E) did not surpass this limit (Eq. 6).

$$E = \frac{m_{SFD}C_{x,SFD} + m_{LFD}C_{x,LFD} - m_{UD}C_{x,UD}}{m_{UD}C_{x,UD}} 100\% \quad (6)$$

where m_{LFD} corresponds to the mass of digestate recovered as LFD and $C_{x,LFD}$ represents the content of any component x in the LFD (on a fresh matter basis).

2.5.3 Methane production

The methane production during the batch AD tests was modeled with the first-order kinetic model (Eq. 7):

$$B(t) = B_0 [1 - \exp(-kt)] \quad (7)$$

where $B(t)$ is the methane yield at time t (on a fresh matter or VS basis), B_0 is the ultimate methane yield (on a fresh matter or VS basis), and k is the apparent hydrolysis rate.

The first-order kinetic model was used for all samples except for the industrially separated SFD samples, for which the Modified-Gompertz model was employed due to a lag-phase in gas production (Eq. 8):

$$B(t) = B_0 \exp \left\{ -\exp \left[\frac{\mu_{\max} e}{B_0} (\lambda - t) + 1 \right] \right\} \quad (8)$$

where μ_{\max} is the maximum methane production rate, and λ is the lag-phase duration.

The kinetic parameters of both models were determined through the least squares method using the Solver function of Microsoft Excel.

For the lab-separated SFD samples, the methane production was estimated based on Eq. 9:

$$B_{SFD} = \frac{B_{UD} - B_{LFD} \frac{m_{LFD}}{m_{UD}}}{\frac{m_{SFD}}{m_{UD}}} \quad (9)$$

where B_{UD} , B_{LFD} , and B_{SFD} are the methane yields of the unseparated digestate, LFD, and SFD, respectively. In this equation, the methane yields are given on a fresh matter basis, and the conversion of B_{SFD} to a VS basis can be obtained by multiplying B_{SFD} by the VS content of the SFD sample.

2.5.4 Statistical analysis

Statistical analysis was performed with the software JMP Pro 16.0.0. The Tukey's HSD test was used to determine whether sample characteristics significantly differed among groups of samples. The Pearson correlation factor (r) was used to describe the linear relationships among the averaged ultimate methane yields, calorific values, digestate characteristics, and operational conditions of origin

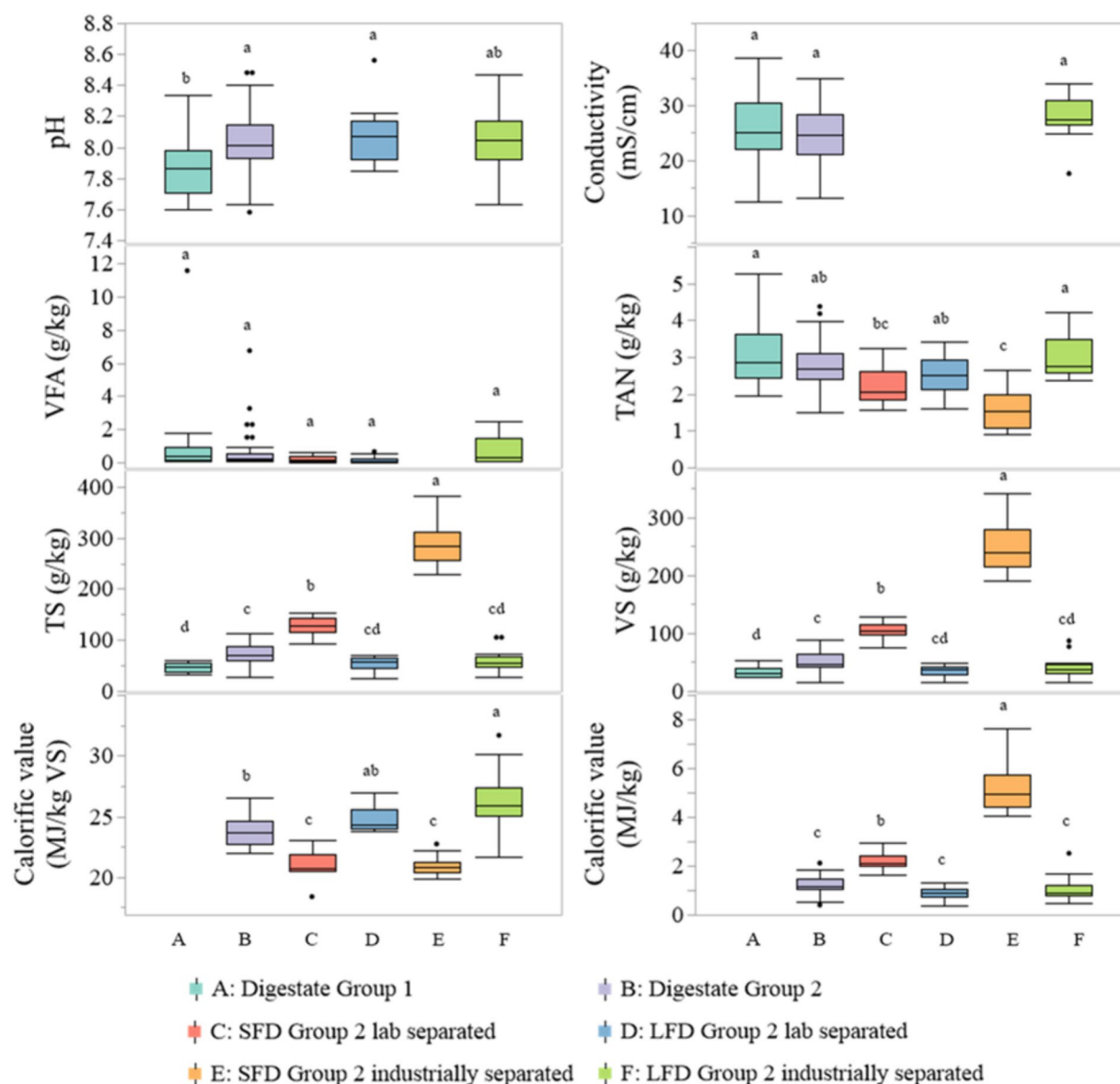


FIGURE 1

Digestate characterization: distribution of pH, conductivity, VFA, TAN, TS, VS, and calorific values. Letter indices above the boxplots originate from the Tukey's HSD test, comparing means at a significance level of differences set at $p < 0.05$, where different letters represent significant differences among the groups of samples. VFA, volatile fatty acids; TAN, total ammoniacal nitrogen; TS, total solids; VS, volatile solids; SFD, solid fraction of digestate; LFD, liquid fraction of digestate.

digesters. The significance of r was determined through a t -test. Tukey's HSD and t -tests considered a significance level of 0.05. Significant differences and correlations were represented by the probability value (p) < 0.05 .

3 Results

3.1 Digestate characterization

The distributions of digestate characteristics on a fresh matter basis are displayed in Figures 1–4. Table 2 shows the averages of digestate characteristics on fresh matter and TS basis. Results for the parameters below the quantification limits were not included. For the

characterization of every analyzed sample and for specific averages of samples that underwent separation and the resulting fractions, see [Supplementary material](#).

The pH of digestate samples belonging to Group 2 was, on average, 8.04, significantly ($p < 0.05$) higher than the average pH of digestate samples from Group 1, which was 7.87 (Figure 1). The same applies to the TS content, which was, on average, 71.03 g/kg for samples from Group 2 against 46.85 g/kg for digestates from Group 1 (Figure 1). The VS content followed the same trend on a fresh matter basis (Figure 1). However, no significant differences ($p > 0.05$) were found for the VS content on a TS basis, resulting in average VS contents of 705.50 and 709.42 g/kg TS for digestate samples from Group 1 and Group 2, respectively (Table 2). No significant differences ($p > 0.05$) were observed for the

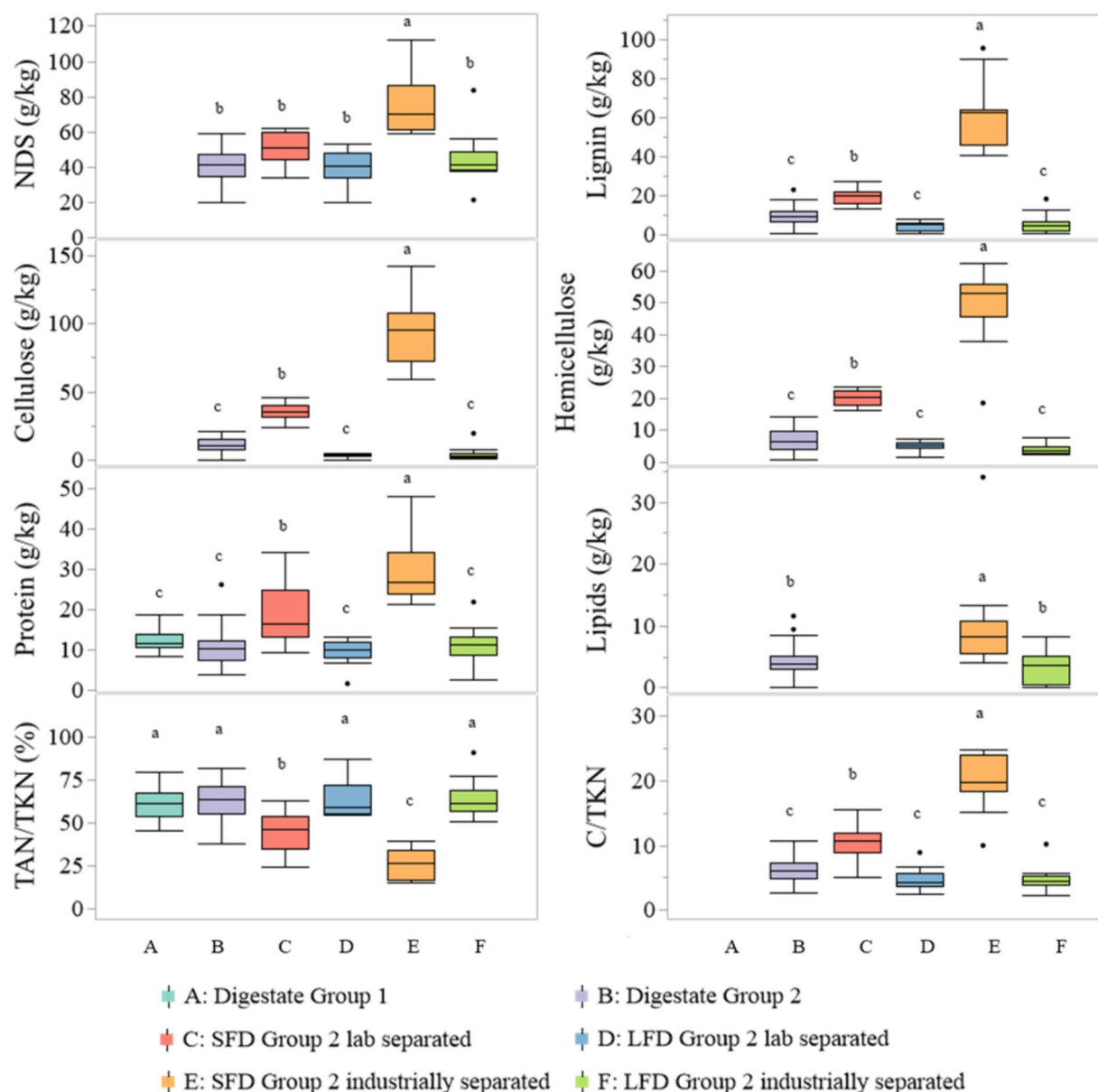


FIGURE 2

Digestate characterization: distribution of NDS, lignin, cellulose, hemicellulose, protein, lipids, and TAN/TKN and C/TKN ratios. Letter indices above the boxplots originate from the Tukey's HSD test, comparing means at a significance level of differences set at $p < 0.05$, where different letters represent significant differences among the groups of samples. VFA, volatile fatty acids; TAN, total ammoniacal nitrogen; TS, total solids; VS, volatile solids; NDS, neutral detergent soluble fraction; TKN, total Kjeldahl nitrogen; SFD, solid fraction of digestate; LFD, liquid fraction of digestate.

conductivities, VFA and TAN concentrations on a fresh matter basis, and TAN/TKN ratios between samples from Group 1 and Group 2 (Figures 1, 2). VFA contents were, on average, 1.25 and 0.62 g/kg for digestates from Group 1 and Group 2, respectively. However, a few outliers were present with high VFA concentrations. TAN contents were, on average, 3.01 and 2.80 g/kg for digestates in Group 1 and Group 2, and the average TAN/TKN ratios were 60.08 and 63.05%. The TAN (Figure 1), protein (Figure 2), TKN, K, P (Figure 3), Fe, Zn, and Cu (Figure 4) contents did not differ ($p > 0.05$) in samples from Group 1 and Group 2 when considered on a fresh matter basis. However, when their contents on a TS basis were evaluated, significantly ($p < 0.05$) higher contents in samples from Group 1 were encountered (Table 2). No significant differences

($p > 0.05$) were observed between the two groups when considering the contents of Na, Mg, S, Ca (Figure 3), Mn, Cr, Ni, Al, and Mo (Figure 4), neither on a fresh matter nor on a TS basis.

Within Group 2, the LFD samples did not differentiate ($p > 0.05$) from the unseparated digestate when characteristics on a fresh matter basis were compared (Figures 1–4). The same occurred for the ratios of TAN/TKN and C/TKN. When characteristics were evaluated on a TS basis, lower ($p < 0.05$) VS, lignin, cellulose, and C contents were obtained for lab or industrially separated LFD. In contrast, the contents of NDS, S, TKN, Ca, K, and Mo were higher ($p < 0.05$) (Table 2). On a fresh matter basis, the VFA (Figure 1), Na, K (Figure 3), and Cu (Figure 4) contents of SFD and unseparated digestate samples were similar ($p > 0.05$), while TAN contents were lower ($p < 0.05$) in

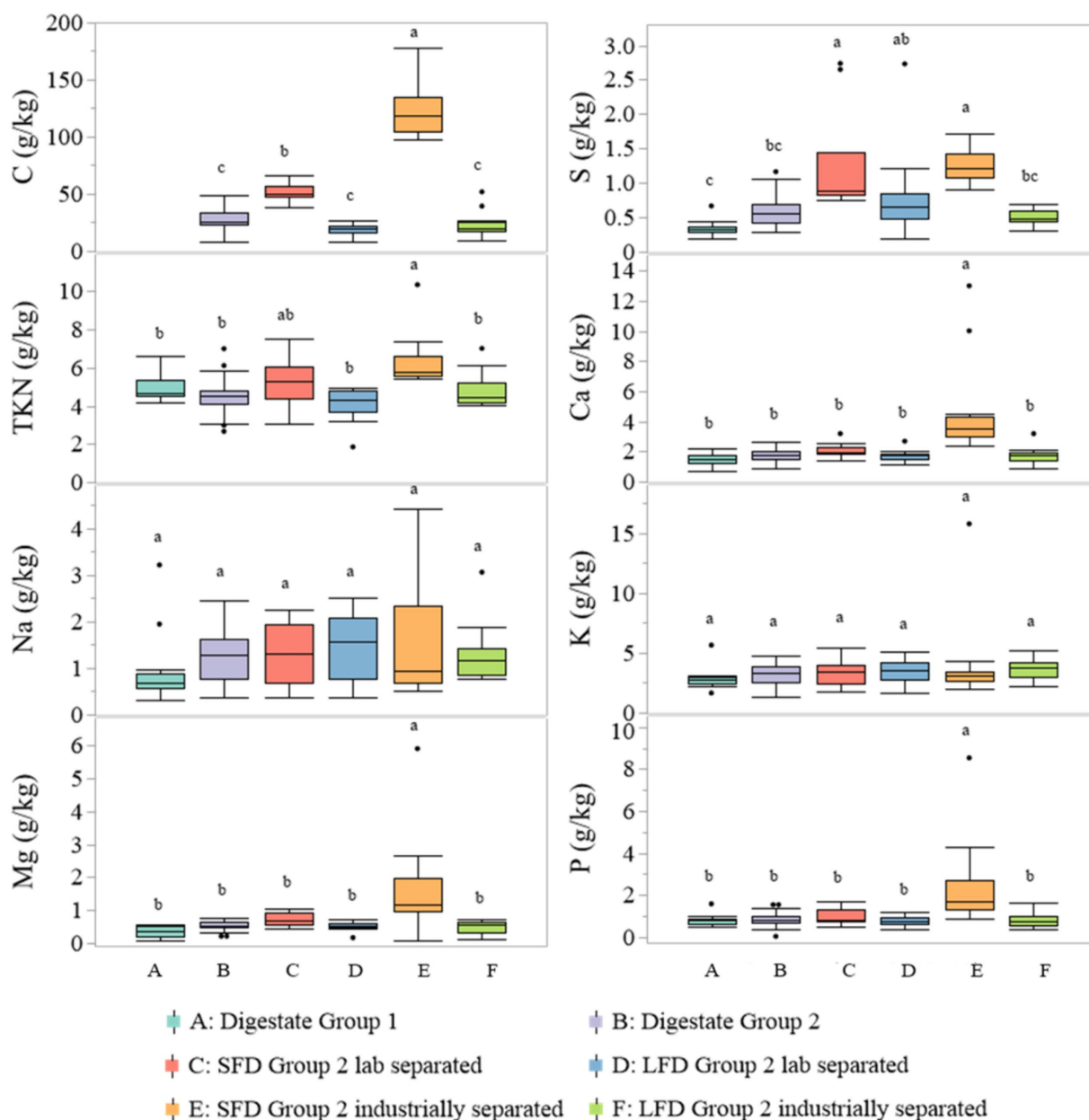


FIGURE 3

Digestate characterization: distribution of nutrients. Letter indices above the boxplots originate from the Tukey's HSD test, comparing means at a significance level of differences set at $p < 0.05$, where different letters represent significant differences among the groups of samples. TKN, total Kjeldahl nitrogen; SFD, solid fraction of digestate; LFD, liquid fraction of digestate.

industrially separated digestate (Figure 1). TAN/TKN ratios were lower, while C/TKN ratios were higher in SFD samples ($p < 0.05$) compared to unseparated digestate (Figure 2). C/TKN were, on average, 10.38 and 19.99 for lab and industrially separated SFD. The remaining components were higher in lab or industrially separated SFD samples than in unseparated digestates ($p < 0.05$) (Figures 1–4). When considering the concentrations on a TS basis, lab or industrially separated SFD samples displayed higher ($p < 0.05$) VS, cellulose, hemicellulose, lignin, C, and calorific values contents and lower ($p < 0.05$) TAN, NDS, TKN, S, K, Ca, Na, Mo, Al, Cu, Mn, and Zn concentrations than unseparated digestate samples (Table 2).

The average calorific value of unseparated digestates from Group 2 was 1.21 MJ/kg. The calorific values of lab and industrially separated SFD were higher ($p < 0.05$), on average, 2.22 and 5.28 MJ/kg, respectively (Table 2). On a TS basis, the average calorific value of the unseparated digestate samples was 16.88 MJ/kg TS. Only the industrially separated SFD achieved a significantly higher ($p < 0.05$) average calorific value, resulting in 18.33 MJ/kg TS (Table 2). When calculated on a VS basis, the average calorific value of unseparated digestate achieved a content of 23.83 MJ/kg VS. In this case, the calorific values of both SFD groups were lower while the calorific value of the industrially separated LFD was higher (Figure 1).

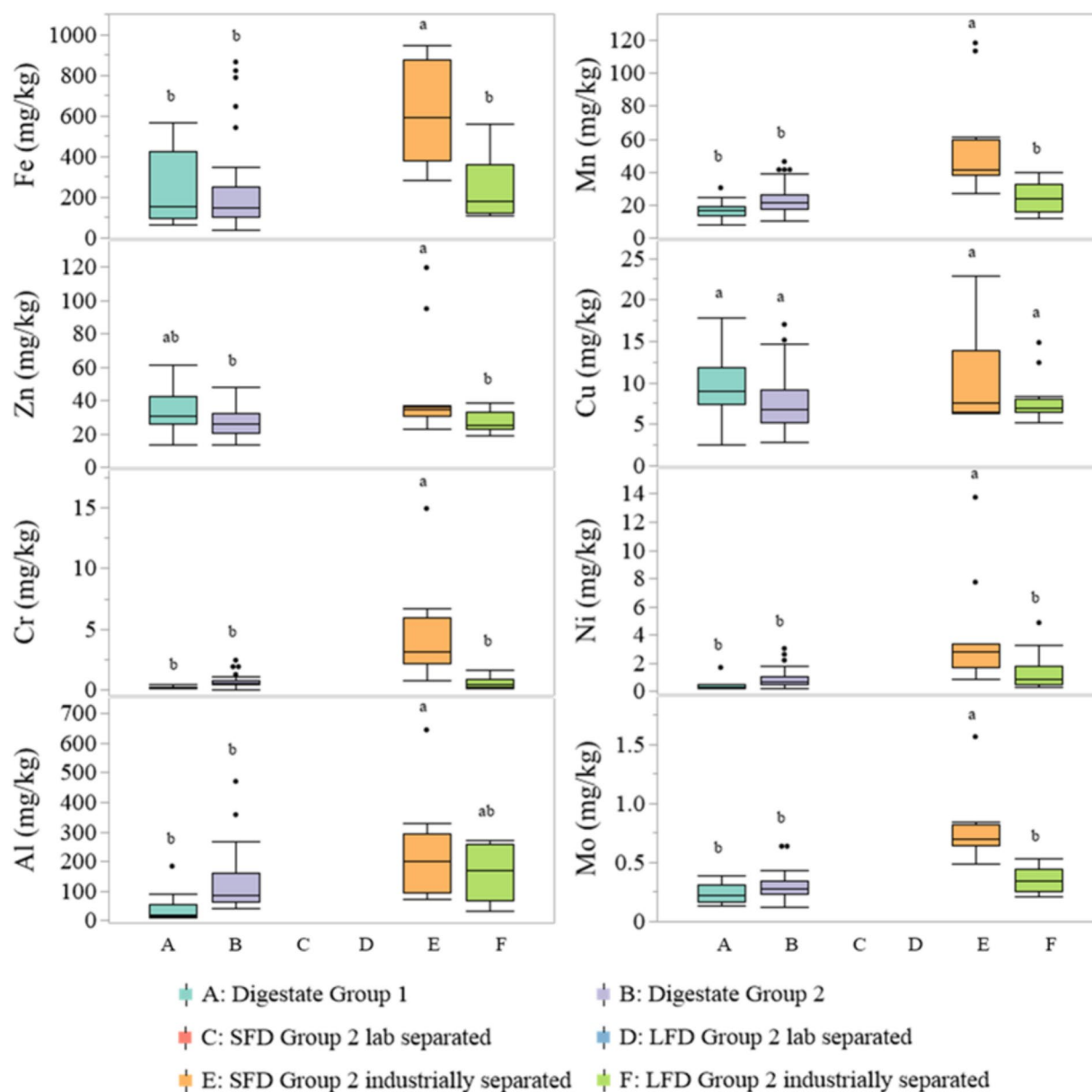


FIGURE 4

Digestate characterization: distribution of micro-nutrients and heavy metals. Letter indices above the boxplots originate from the Tukey's HSD test, comparing means at a significance level of differences set at $p < 0.05$, where different letters represent significant differences among the groups of samples. SFD, solid fraction of digestate; LFD, liquid fraction of digestate.

3.2 Separation efficiency

Figure 5 presents the TS contents of the unseparated digestate and the resulting LFD after separation by different methods, i.e., lab-scale and industrial separation by screw press and decanter centrifuge. Linear curves represented well the relationships between the TS contents of the samples. According to the curve slopes, the decanter centrifuges were the most efficient at reducing the TS contents of the LFD, followed by the lab-scale separation. Average reductions in TS contents from the unseparated digestate were 32.35, 26.82, and 18.69% for decanter centrifuge, lab-scale separation, and screw press, with ranges of 28–39, 19–39, and 2–32%, respectively. It should be noticed, however, that only three samples (B8, B9, and B18) were separated

with decanter centrifuges, and their initial TS contents were some of the lowest. Contrarily, screw presses were employed for the separation of some of the samples with the highest TS contents.

The distributions of digestate components fractionated into solid and liquid fractions and the RSI of lab-scale and industrially separated samples are presented in Figure 6. The averages of every component mass fraction in the SFD (corresponding to the SIs) and in the LFD and the RSIs are displayed in Table 3. Values presented in Figure 6 and Table 3 only considered groups of samples in which mass balance errors were not greater than 20%. The mass balances for all groups of samples, including the excluded ones, can be found in the Supplementary material, along with the absolute values of digestate fractionation into SFD and LFD, the SIs, and RSIs for all samples.

TABLE 2 Average composition of digestate and solid and liquid fractions.

Component	Digestate Group 1	Digestate Group 2	SFD Group 2 Lab-scale separation	LFD Group 2 Lab-scale separation	SFD Group 2 Industrial separation	LFD Group 2 Industrial separation
pH	7.87 (0.20)	8.04 (0.19)		8.09 (0.20)		8.05 (0.21)
Conductivity (mS/cm)	25.39 (6.10)	24.53 (4.82)				27.64 (4.17)
TS (g/kg)	46.85 (9.44)	71.03 (18.01)	128.02 (18.00)	54.65 (14.01)	286.97 (45.29)	61.27 (23.05)
VS (g/kg)	33.17 (8.86)	50.98 (15.19)	105.45 (15.62)	35.13 (9.54)	251.32 (45.67)	41.43 (18.93)
VS (g/kg TS)	705.50 ^{bc} (88.45)	709.42 ^b (48.27)	823.63 ^a (31.36)	642.07 ^c (44.99)	874.05 ^a (48.83)	660.40 ^{bc} (50.66)
VFA (g/kg)	1.25 (2.90)	0.62 (1.14)	0.25 (0.19)	0.20 (0.23)		0.70 (0.83)
VFA (g/kg TS)	31.97 ^a (80.17)	9.04 ^a (1.42)	1.94 ^a (1.42)	3.54 ^a (3.71)		15.81 ^a (22.07)
TAN (g/kg)	3.01 (0.81)	2.80 (0.63)	2.23 (0.51)	2.54 (0.59)	1.63 (0.58)	3.03 (0.59)
TAN (g/kg TS)	69.35 ^a (31.25)	42.18 ^b (14.89)	17.69 ^c (4.25)	48.78 ^{ab} (13.98)	5.88 ^c (2.36)	57.40 ^{ab} (30.98)
TKN (g/kg)	4.97 (0.76)	4.46 (0.80)	5.28 (1.35)	4.05 (0.93)	6.39 (1.38)	4.84 (0.92)
TKN (g/kg TS)	111.63 ^a (34.85)	66.06 ^c (17.15)	42.11 ^d (14.92)	77.43 ^{bc} (23.12)	22.57 ^d (5.25)	86.49 ^b (28.67)
TAN/TKN (%)	60.08 (8.63)	63.05 (10.46)	44.37 (12.25)	63.84 (10.78)	25.84 (8.57)	63.41 (11.34)
Protein (g/kg)	12.21 (2.59)	10.41 (4.04)	19.02 (8.67)	9.46 (3.49)	29.73 (7.81)	11.30 (4.77)
Protein (g/kg TS)	264.24 ^a (49.93)	149.28 ^{bc} (49.03)	152.63 ^{bc} (86.23)	179.07 ^b (78.01)	104.32 ^c (26.34)	181.82 ^b (43.61)
Lipids (g/kg)		3.96 (2.71)			10.06 (7.98)	3.51 (2.70)
Lipids (g/kg TS)		53.45 ^a (32.81)			33.18 ^a (19.19)	53.35 ^a (38.46)
NDS (g/kg)		41.21 (8.62)	50.32 (9.65)	39.95 (9.62)	76.12 (17.45)	44.90 (14.70)
NDS (g/kg TS)		592.99 ^b (88.82)	391.50 ^c (40.04)	738.54 ^a (56.48)	266.51 ^d (52.12)	749.74 ^a (86.42)
Cellulose (g/kg)		11.74 (5.16)	35.85 (6.45)	3.77 (1.43)	95.46 (24.39)	5.05 (4.95)
Cellulose (g/kg TS)		158.58 ^c (44.65)	280.09 ^b (32.79)	66.44 ^d (16.88)	330.03 ^a (48.47)	72.70 ^d (40.83)
Hemicellulose (g/kg)		6.66 (3.77)	19.98 (2.70)	4.96 (1.59)	49.48 (11.67)	4.17 (1.77)
Hemicellulose (g/kg TS)		91.10 ^b (42.48)	157.39 ^a (20.01)	89.34 ^b (14.42)	174.53 ^a (44.82)	71.76 ^b (22.59)
Lignin (g/kg)		9.70 (4.31)	20.08 (4.17)	4.69 (2.34)	60.98 (17.37)	5.95 (5.09)
Lignin (g/kg TS)		132.89 ^b (41.59)	156.68 ^b (22.32)	82.11 ^c (30.21)	210.51 ^a (37.33)	85.65 ^c (41.95)
C/TKN		6.12 (1.76)	10.38 (2.74)	4.86 (1.81)	19.99 (4.32)	4.75 (1.94)
C (g/kg)		27.07 (8.04)	51.84 (8.41)	18.84 (5.28)	124.19 (24.64)	23.29 (11.47)
C (g/kg TS)		376.97 ^b (27.61)	404.62 ^{ab} (21.83)	343.64 ^c (29.23)	431.10 ^a (27.22)	370.97 ^{bc} (39.44)
S (g/kg)	0.36 (0.11)	0.58 (0.19)	1.24 (0.76)	0.84 (0.72)	1.26 (0.24)	0.51 (0.11)
S (g/kg TS)	7.74 ^{bc} (2.42)	8.33 ^b (2.59)	9.63 ^{ab} (5.57)	14.40 ^a (9.30)	4.43 ^c (0.79)	8.83 ^{bc} (1.96)
Ca (g/kg)	1.49 (0.39)	1.77 (0.39)	2.09 (0.48)	1.74 (0.42)	4.72 (3.28)	1.76 (0.58)
Ca (g/kg TS)	31.41 ^{ab} (5.12)	25.89 ^b (6.44)	16.53 ^c (3.84)	33.22 ^a (8.34)	16.10 ^c (9.20)	29.56 ^{ab} (4.93)
Na (g/kg)	0.94 (0.79)	1.27 (0.52)	1.32 (0.66)	1.48 (0.72)	1.47 (1.18)	1.32 (0.64)
Na (g/kg TS)	19.76 ^{ab} (14.40)	18.68 ^{ab} (8.54)	10.00 ^{bc} (4.52)	26.47 ^a (11.57)	4.77 ^c (2.96)	22.24 ^a (7.36)
K (g/kg)	2.84 (0.93)	3.25 (0.88)	3.39 (1.11)	3.57 (1.04)	4.09 (3.74)	3.69 (0.82)
K (g/kg TS)	60.62 ^a (15.80)	46.69 ^b (11.12)	26.30 ^c (7.59)	65.59 ^a (9.36)	13.42 ^c (9.05)	64.37 ^a (15.56)
Mg (g/kg)	0.36 (0.17)	0.56 (0.12)	0.73 (0.22)	0.52 (0.14)	1.68 (1.48)	0.50 (0.18)
Mg (g/kg TS)	7.64 ^{ab} (3.16)	8.12 ^{ab} (1.76)	5.70 ^b (1.50)	9.53 ^a (1.98)	5.77 ^b (5.09)	8.27 ^{ab} (2.45)
P (g/kg)	0.81 (0.27)	0.84 (0.29)	0.95 (0.38)	0.75 (0.24)	2.46 (2.12)	0.83 (0.41)
P (g/kg TS)	17.52 ^a (5.92)	11.93 ^{bc} (3.86)	7.45 ^c (2.75)	13.75 ^{ab} (2.91)	8.44 ^{bc} (7.22)	13.32 ^{ab} (3.27)
Fe (mg/kg)	250.92 (183.43)	221.71 (200.72)			619.42 (239.70)	238.90 (149.91)
Fe (mg/kg TS)	5627.69 ^a (4398.88)	3095.08 ^b (2713.83)			2236.21 ^b (1001.42)	4226.07 ^{ab} (2536.45)
Mn (mg/kg)	17.09 (5.94)	23.83 (8.53)			54.45 (29.98)	24.38 (9.25)
Mn (mg/kg TS)	363.46 ^a (100.84)	340.67 ^a (93.88)			189.35 ^b (94.13)	415.32 ^a (143.15)
Zn (mg/kg)	33.12 (13.82)	27.08 (8.72)			44.66 (29.89)	27.74 (6.52)
Zn (mg/kg TS)	772.62 ^a (465.92)	411.92 ^b (198.92)			151.31 ^c (82.31)	498.86 ^b (193.81)
Cu (mg/kg)	9.45 (4.08)	7.65 (3.36)			10.67 (6.05)	7.89 (2.83)
Cu (mg/kg TS)	220.05 ^a (129.92)	117.04 ^b (64.72)			37.09 ^c (19.13)	145.02 ^{ab} (69.78)

(Continued)

TABLE 2 (Continued)

Component	Digestate Group 1	Digestate Group 2	SFD Group 2 Lab-scale separation	LFD Group 2 Lab-scale separation	SFD Group 2 Industrial separation	LFD Group 2 Industrial separation
Cr (mg/kg)	0.24 (0.09)	0.73 (0.44)			4.68 (3.92)	0.63 (0.43)
Cr (mg/kg TS)	5.44 ^b (2.98)	10.29 ^{ab} (6.22)			16.58 ^a (14.48)	10.51 ^{ab} (8.24)
Ni (mg/kg)	0.46 (0.38)	0.92 (0.59)			3.84 (3.76)	1.41 (1.38)
Ni (mg/kg TS)	10.12 ^b (8.49)	12.75 ^{ab} (7.16)			12.97 ^{ab} (11.09)	19.87 ^a (12.13)
Al (mg/kg)	42.02 (48.88)	123.75 (93.53)			217.19 (160.89)	152.52 (92.95)
Al (g/kg TS)	858.00 ^{bc} (946.61)	1807.93 ^{ab} (1234.89)			745.82 ^c (450.11)	2739.46 ^a (1817.69)
Mo (mg/kg)	0.23 (0.08)	0.30 (0.11)			0.79 (0.33)	0.35 (0.11)
Mo (mg/kg TS)	5.15 ^{ab} (2.20)	4.29 ^b (1.33)			2.64 ^c (0.76)	5.90 ^a (1.68)
Calorific value (MJ/kg VS)		23.83 (1.21)	21.00 (1.27)	24.78 (1.05)	20.96 (0.83)	26.24 (2.67)
Calorific value (MJ/kg TS)		16.88 ^b (1.13)	17.30 ^{ab} (1.30)	15.92 ^b (1.53)	18.33 ^a (1.28)	17.30 ^{ab} (2.09)
Calorific value (MJ/kg)		1.21 (0.35)	2.22 (0.41)	0.87 (0.25)	5.28 (1.04)	1.08 (0.54)

Values in parenthesis represent the standard deviations. Letter indices originate from the Tukey's HSD test, comparing means at a significance level of differences set at $p < 0.05$, where different letters represent significant differences among the groups of samples.

SFD, solid fraction of digestate; LFD, liquid fraction of digestate; TS, total solids; VS, volatile solids; VFA, volatile fatty acids; TAN, total ammoniacal nitrogen; TKN, total Kjeldahl nitrogen; NDS, neutral detergent soluble fraction.

On average, 31.31% of the digestate mass was retained in the SFD when separated in lab-scale (Table 3), resulting in an average TS content in the SFD of 128.02 g/kg and approximately equal fractions of TS in the LFD and SFD. The average TS of the SFD obtained through industrial separation was 286.97 g/kg and was significantly higher ($p < 0.05$) than that obtained with lab-scale separation (Table 2). In this case, the estimated mass retained in the SFD was, on average, only 7.24%, resulting in most of the TS (73.16%), remaining in the LFD (Table 3). The resulting RSIs of the TS were 31.59 and 21.65% for lab-scale and industrial separation, respectively (Table 3).

Except for VS, the RSIs of all components separated by both lab-scale and industrial methods did not differ significantly ($p > 0.05$) by the scale of separation (Figure 6). In the case of VS, the highest RSI was obtained when employing the lab-scale separation, with an average of 41.80% (Table 3). The separation methods also selectively highly retained lignin, cellulose, hemicellulose, and Cr in the SFD (Figure 6), with average RSIs between 36.07 and 68.60% (Table 3). Some moderate RSIs were obtained for protein, C, S, Mg, and Ni (10.67–37.56%) (Table 3). Lower RSIs were achieved by lipids, NDS, Ca, P, Fe, Mn, Zn, Cu, Al, and Mo (2.49–13.90%) (Table 3). The average RSIs of TAN, Na, and K were negative (Table 3), indicating that they were preferably transferred to the LFD.

The results of industrial separation in Figure 6 do not discriminate between separation by screw presses or decanter centrifuges. However, the outliers in the RSIs of NDS, Ca, Mg, P, Mn, and Cu correspond to sample B18, which was separated with a decanter centrifuge.

3.3 Residual methane yields

The experimental methane yields obtained in the batch tests and by mass balance for lab-scale separated SFD and the kinetic parameters calculated with models are presented in Table 4. The first-order kinetic model represented well the methane production of all groups of samples with a minimum r^2 of 0.939, except for the SFD

samples separated industrially. For the latter group, the Modified-Gompertz model was utilized, resulting in a minimum r^2 of 0.962. The digestion of these samples was accompanied by long lag-phases, averaging 15.47 days.

The average ultimate methane yields of digestate samples belonging to Group 1 were significantly higher ($p < 0.05$) than those achieved by samples from Group 2 (152.04 mL/g VS against 99.40 mL/g VS) (Table 4). On a fresh matter basis, the difference was not significant ($p > 0.05$), with average ultimate methane yields of 4.91 and 4.95 mL/g obtained for Group 1 and Group 2. Within Group 2, the methane yields of the digestate fractions (LFD and SFD) were not significantly different from those of unseparated digestate on a VS basis (Table 4). On a fresh matter basis, significant differences ($p < 0.05$) were observed only for the SFD, where average ultimate methane yields were 8.94 and 24.88 mL/g in samples separated in the lab and industrially.

When considering only the samples utilized in the lab-scale separation experiments, the average ultimate methane yields of the unseparated digestate, SFD, and LFD were 86.46, 86.86, and 80.95 mL/g VS (or 4.62, 8.94, and 2.73 mL/g). When samples separated industrially were evaluated, the average methane yields were 112.79, 104.49, and 98.40 mL/g VS (or 5.98, 26.87, and 3.81 mL/g) for unseparated digestate, SFD, and LFD. In the first case, approximately 60% of the methane potential would be captured in the SFD against 40% in the LFD. However, the industrial separation of digestate would result in most of the methane potential held in the LFD (59%).

3.4 Correlations among digestate characteristics, operational conditions, methane yields, and calorific values

In Table 5, the linear correlations factors are presented. Equations describing significant linear relationships are shown in the

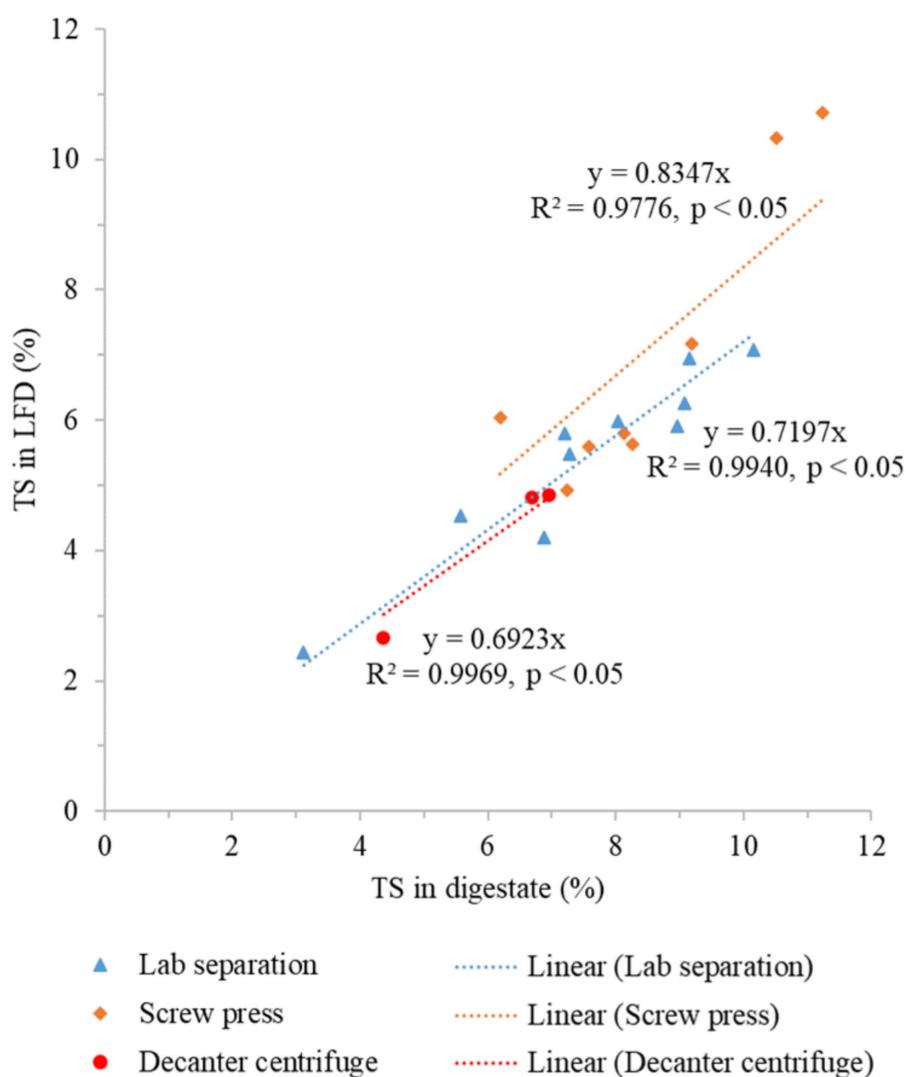


FIGURE 5

Total solids reduction in digestate separation. TS, total solids; LFD, liquid fraction of digestate.

Supplementary material. Correlation factors were not consistent within all groups of samples. Significant ($p < 0.05$) correlations included negative relationships between the ultimate methane yield in Group 2 and the temperature ($r = -0.31$) and the natural logarithm of the HRT ($r = -0.59$). When evaluating the digestate composition, the ultimate methane yield correlated positively with the protein content ($r = 0.54$) in Group 1, positively with the hemicellulose content in the SFD ($r = 0.71$ for both lab and industrially separated SFD), and negatively with the lignin content in industrially separated SFD and LFD ($r = -0.87$ and $r = -0.69$, respectively). The calorific value did not correlate with the operational conditions of the digesters. It presented a negative relationship with the protein content in industrially separated SFD ($r = -0.67$). Negative correlations were also observed for the NDS content in Group 2 and industrially separated SFD ($r = -0.50$ and $r = -0.82$). Positive correlation factors were found for the cellulose content in Group 2 and industrially separated SFD ($r = 0.43$ and $r = 0.64$) and for lignin and lipids contents in Group 2 ($r = 0.57$ and $r = 0.44$).

4 Discussion

4.1 Digestate characterization and its nutrient contents

4.1.1 Nutrient contents of unseparated digestate

Digestate characteristics of samples from Group 1 and Group 2 were, in general, in agreement with values previously reported for agricultural digestate (Schievano et al., 2008; Möller and Müller, 2012; Albuquerque et al., 2012a,b; Monlau et al., 2015; Sambusiti et al., 2015; Tambone et al., 2017; Akyol et al., 2019; Guilayn et al., 2019; Finzi et al., 2021; Ekstrand et al., 2022). Digestate characteristics vary widely in literature, depending on feedstock composition and operational parameters of digesters.

In Denmark all agricultural digestate is currently applied as a fertilizer without solid–liquid separation. Digestate has generally been considered as a good alternative to manure since digestate often presents higher ratios of TAN/TKN (Crolla et al., 2013) and other

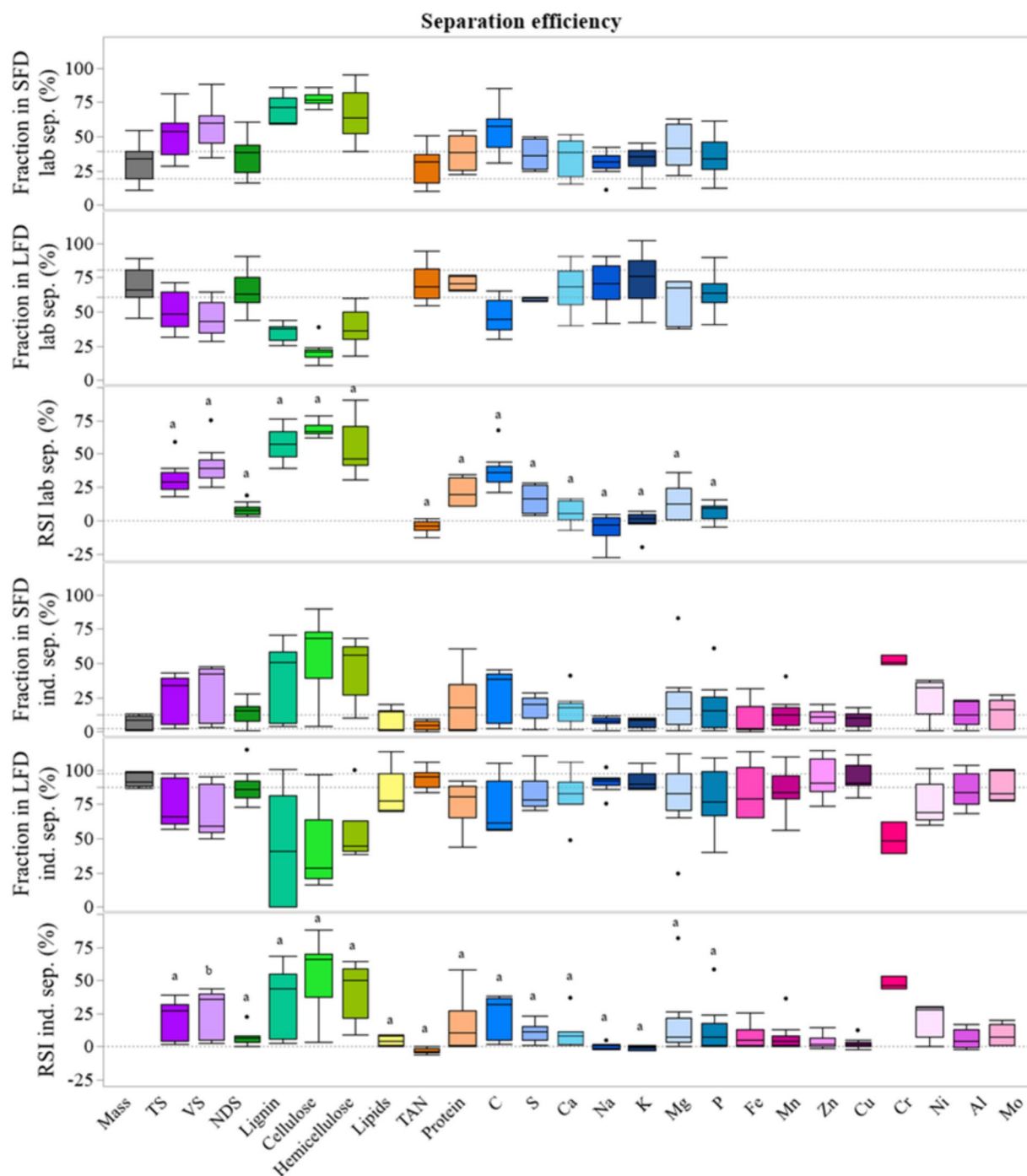


FIGURE 6

Digestate characterization: separation efficiency. Letter indices above the boxplots originate from the Tukey's HSD test, comparing means at a significance level of differences set at $p < 0.05$, where different letters represent significant differences (comparisons are made between the RSI of each component obtained through lab sep. and ind. sep.). SFD, solid fraction of digestate; LFD, liquid fraction of digestate; RSI, relative separation index; lab sep., lab-scale separation; ind. sep., industrial separation; TS, total solids; VS, volatile solids; NDS, neutral detergent soluble fraction; TAN, total ammoniacal nitrogen.

nutrients in more available forms for plant uptake (Möller and Müller, 2012). Furthermore, digestate contains less pathogens, less odorous compounds, and less easily degradable C than manure (Crolla et al., 2013). The reduction in the contents of readily degradable C during AD results in less availability of this component for denitrification, potentially reducing N_2O emissions from digestate when compared to

manure (Crolla et al., 2013). Additionally, the loss of N through soil microbial immobilization, stimulated by high C contents and C/TKN ratios, is reduced (Albuquerque et al., 2012b). Despite the benefits, the increase in pH and TAN content during AD and the associated potential increase in ammonia emissions due to a shift in the NH_4^+ / NH_3 equilibrium towards ammonia formation are well known (Crolla

TABLE 3 Average components distribution after solid–liquid separation of digestate.

Component	Lab-scale separation			Industrial separation		
	Fraction in the SFD* (%)	Fraction in the LFD (%)	RSI (%)	Fraction in the SFD* (%)	Fraction in the LFD (%)	RSI (%)
Mass	31.31 (13.32)	68.70 (13.32)		7.24 (4.63)	92.76 (4.63)	
TS	51.80 (15.71)	50.77 (13.34)	31.59 (11.54)	26.84 (16.29)	73.16 (16.29)	21.65 (13.66)
VS	58.57 (15.67)	45.21 (12.10)	41.80 (13.74)	31.97 (19.22)	67.70 (18.09)	27.34 (16.83)
TAN	28.71 (12.58)	70.94 (13.04)	−4.17 (4.41)	4.82 (3.03)	93.70 (7.11)	−3.49 (1.94)
Protein	38.15 (13.43)	70.85 (4.93)	21.20 (11.54)	20.72 (22.01)	76.13 (17.20)	15.97 (21.54)
Lipids				11.52 (7.34)	85.83 (16.99)	4.24 (3.06)
NDS	36.68 (14.00)	65.17 (13.89)	8.26 (4.92)	14.31 (8.49)	87.80 (12.41)	7.11 (6.40)
Cellulose	77.64 (4.69)	20.97 (7.58)	68.60 (4.92)	58.09 (28.11)	40.62 (30.65)	55.54 (27.30)
Hemicellulose	66.39 (17.83)	38.56 (13.45)	54.17 (19.18)	47.17 (22.00)	53.22 (23.32)	42.89 (20.98)
Lignin	70.23 (9.93)	35.96 (6.49)	58.14 (12.25)	39.50 (27.38)	42.74 (39.75)	36.07 (25.84)
C	55.80 (15.48)	46.63 (11.97)	37.56 (12.43)	29.58 (17.76)	71.49 (18.79)	24.71 (15.13)
S	36.96 (11.37)	59.82 (1.43)	16.16 (11.35)	17.66 (9.17)	83.30 (13.32)	10.67 (6.91)
Ca	35.20 (13.54)	67.84 (15.90)	6.54 (7.99)	16.78 (11.77)	81.91 (15.96)	9.79 (10.88)
Na	30.56 (8.90)	70.82 (16.08)	−5.57 (10.25)	7.74 (3.32)	91.11 (7.14)	−0.31 (2.13)
K	33.74 (10.07)	74.25 (18.80)	−0.68 (8.38)	6.79 (3.68)	92.92 (6.83)	−1.18 (1.32)
Mg	43.46 (16.07)	57.98 (17.49)	12.77 (14.49)	22.78 (24.80)	80.31 (25.14)	17.37 (25.48)
P	35.70 (14.32)	63.84 (14.50)	6.84 (6.32)	18.50 (18.61)	79.19 (21.23)	12.53 (18.65)
Fe				10.30 (12.09)	83.65 (19.52)	7.45 (9.59)
Mn				13.90 (11.23)	85.74 (14.21)	7.20 (10.81)
Zn				10.28 (6.01)	94.64 (13.35)	3.25 (4.84)
Cu				9.29 (5.81)	94.90 (10.04)	2.49 (4.13)
Cr				52.04 (3.91)	49.93 (11.72)	47.65 (5.13)
Ni				26.11 (14.88)	75.63 (15.82)	20.26 (13.08)
Al				13.39 (9.02)	85.94 (12.99)	5.66 (7.38)
Mo				13.01 (11.27)	88.07 (10.94)	8.42 (8.39)

*Equivalent to the separation index. Values in parenthesis represent the standard deviations.

SFD: solid fraction of digestate, LFD, liquid fraction of digestate, RSI, relative separation index, TS, total solids, VS, volatile solids, TAN, total ammoniacal nitrogen; NDS, neutral detergent soluble fraction.

et al., 2013). Nonetheless, digestate is still widely applied as a soil fertilizer. In the past, when the use of a higher share of manure with minimal amounts of co-substrates in biogas plants was predominant, it was expected that AD would reduce the viscosity of manure due to TS reduction during the process, allowing it to infiltrate faster into the soil, therefore balancing off the effects of higher pH and TAN content on ammonia emissions. However, substantial changes in the substrates fed to anaerobic digesters in Denmark may challenge the fertilizer value of digestate and its acceptance by farmers, as it is discussed in Section 4.1.2.

Besides N, digestate offers appreciable amounts of the other primary nutrients, P and K. However, it is known that the excessive application of nutrients can lead to pollution, for instance, due to the leaching of N and P to water bodies and the consequent stimulation of eutrophication (Teglia et al., 2011). Therefore, in Denmark, the amount of N supplied in digestate must currently not exceed 170 kg/ha/year, in agreement with the EU Nitrates Directive, while P addition is limited to 30 kg/ha/year from 2025 (Sommer and Knudsen, 2021). Depending on the N and P concentrations in each digestate and crop

requirements, either of these nutrients should be supplemented by commercial fertilizers. In Group 1 and Group 2, four out of 13 and 26 out of 46 samples would have their application limited by P. Secondary essential nutrients, such as S, Ca, and Mg, are also present in digestate, along with other elements required by plants in smaller amounts, such as Fe, Na, Cu, Zn, Mo, and Mn (Crolla et al., 2013). The source of nutrients in the sampled digestates was restricted to the feedstock material treated by biogas plants since no supplementation is performed in Danish digesters to boost microbial activity in AD. However, Cu and Zn are commonly added to animal diets to prevent pig diseases. Results in this study showed some high variability in the concentrations of all nutrients, especially Fe, Na, Zn, and Cu, evidenced by the high standard deviations compared to the concentrations averages in Table 2. Differences in nutrient composition in digestate can lead to different supplementation requirements to accommodate the needs of different plants (Coelho et al., 2018). Solid–liquid separation of digestate can result in the concentration of different nutrients in the different streams, easing the adjustment of nutrients during land application to the needs of the

TABLE 4 Kinetic parameters.

Sample	Experimental methane yield (ml/g/VS)	B ₀ (ml/g VS)	B ₀ (ml/g)	First order kinetic model		Modified-Gompertz model		
				10 ³ k (1/day)	r ²	μ _{max} (ml/g VS/day)	λ (day)	r ²
Digestate – Group 1								
01	138.97 (7.73)	127.74	3.04	44.47	0.977			
02	110.05 (2.88)	102.87	3.28	42.19	0.987			
03	143.27 (0.73)	216.21	5.08	10.23	0.980			
04	152.17 (7.62)	147.04	5.59	41.59	0.996			
05	119.50 (5.33)	113.69	4.77	42.91	0.988			
06	172.87 (5.03)	164.26	5.03	45.83	0.991			
07	208.98 (3.22)	209.51	5.64	36.20	0.988			
08	107.82 (7.98)	112.58	5.05	26.01	0.996			
09	180.13 (11.43)	166.82	6.55	50.08	0.980			
10	221.58 (6.53)	218.05	7.96	33.31	0.971			
11	112.16 (17.55)	108.07	5.72	35.34	0.993			
12	127.37 (11.81)	139.08	3.59	16.05	0.998			
13	110.75 (2.09)	120.97	3.06	19.92	0.995			
14	192.11 (1.46)	172.64	4.25	34.53	0.928			
15	102.92 (11.03)	161.05	5.08	9.61	0.999			
Average		152.04 ^a (39.44)	4.91 ^{b,c} (1.35)	32.55 ^a (13.18)				
Digestate – Group 2*								
A1	147.59 (3.33)	138.71	7.52	68.38	0.988			
A2	96.26 (2.05)	92.09	3.57	37.05	0.991			
A3	73.50 (1.24)	68.72	4.67	39.59	0.986			
A4	60.42 (1.24)	59.10	3.50	31.19	0.992			
A5	82.67 (1.77)	77.19	6.07	38.23	0.981			
A6	77.00 (2.10)	75.15	4.85	40.88	0.995			
A7	64.26 (2.25)	74.64	3.36	17.50	0.998			
A8	77.19 (6.45)	75.32	4.07	42.53	0.996			
A9	97.17 (2.45)	98.05	6.37	34.04	0.999			
A10	101.49 (2.15)	105.62	2.22	27.42	1.000			
B1	95.05 (0.99)	95.96	4.38	41.11	0.999			
B2	59.20 (4.28)	91.01	3.79	11.47	0.997			
B3	107.14 (7.87)	108.77	6.08	35.98	0.995			
B4	41.85 (1.30)	58.80	2.80	13.12	0.987			
B5	132.60 (4.46)	143.89	9.47	33.62	0.990			
B6	120.70 (1.99)	112.46	4.93	48.32	0.939			
B7	149.07 (0.81)	139.63	6.26	54.49	0.971			
B8	117.60 (4.68)	112.93	5.33	45.22	0.992			
B9	108.98 (3.03)	105.34	4.80	42.35	0.986			
B10	123.30 (4.44)	120.92	4.36	45.19	0.995			
B11	63.87 (5.77)	65.95	4.18	29.40	0.993			
B12	53.39 (2.46)	54.87	2.36	28.48	0.989			
B13	69.70 (2.42)	73.66	5.00	29.58	0.999			

(Continued)

TABLE 4 (Continued)

Sample	Experimental methane yield (ml/g/VS)	B ₀ (ml/g VS)	B ₀ (ml/g)	First order kinetic model		Modified-Gompertz model		
				10 ³ k (1/day)	r ²	μ _{max} (ml/g VS/day)	λ (day)	r ²
B14	88.31 (3.63)	86.42	6.94	40.33	0.991			
B15	84.19 (7.76)	83.88	7.40	34.22	0.989			
B16	116.85 (2.49)	111.49	4.15	38.52	0.973			
B17	96.26 (4.61)	207.94	8.64	6.92	0.989			
B18	154.04 (7.52)	235.87	6.88	12.18	0.995			
B19	94.81 (1.08)	90.44	6.08	51.52	0.991			
B20	128.31 (1.89)	120.97	4.31	79.13	0.977			
B21	68.33 (0.98)	64.13	3.55	54.66	0.983			
B22	83.32 (1.18)	87.91	4.39	29.29	0.996			
B23	79.90 (1.46)	78.88	5.20	33.85	0.986			
B24	114.89 (1.88)	118.47	5.89	30.49	0.996			
B25	85.57 (1.11)	89.15	4.24	27.07	0.995			
B26	94.19 (2.13)	102.50	4.89	25.70	0.997			
B27	68.91 (0.57)	68.52	2.86	34.98	0.984			
B28	108.12 (1.93)	100.70	6.92	45.85	0.982			
B29	79.34 (1.07)	83.00	4.95	24.72	0.996			
B31	83.66 (2.12)	95.46	3.73	21.58	0.993			
B36	77.06 (2.69)	79.92	1.25	28.81	0.983			
B37	117.91 (0.93)	120.44	5.76	36.87	0.998			
Average		99.40 ^b (35.73)	4.95 ^c (1.71)	35.52 ^a (14.15)				
SFD – Group 2								
Lab-scale separation								
A1S	157.51 (5.43)	148.19	14.39	69.97	0.985			
A2S	108.23 (6.79)	103.44	11.09	42.19	0.990			
A3S	66.60 (2.82)	62.83	8.16	36.04	0.983			
A4S	55.63 (2.19)	54.98	5.81	28.84	0.993			
A5S	67.30 (1.90)	61.97	7.92	40.58	0.977			
A6S	71.57 (3.53)	70.25	7.22	38.91	0.995			
A7S	61.74 (4.02)	73.80	7.23	16.46	0.998			
A8S	74.73 (10.26)	73.04	7.17	48.80	0.997			
A9S	98.40 (3.06)	102.88	11.52	30.07	0.998			
A10S	116.46 (6.83)	117.25	8.91	41.52	0.998			
Average		86.86 ^b (29.94)	8.94 ^b (2.61)	39.34 ^a (14.03)				
Industrial separation								
B3S	93.21 (7.95)	91.73	23.74			3.78	13.19	0.995
B9S	152.85 (35.60)	152.31	34.95			4.27	13.59	0.996
B14S	74.28 (1.23)	76.38	24.28			4.21	13.83	0.997
B15S	43.58 (10.76)	43.49	14.91			1.34	24.21	0.962
B18S	155.51 (12.43)	151.67	31.86			6.12	11.47	0.996
B27S	81.83 (4.09)	81.16	20.27			4.14	15.81	0.995
B28S	137.24 (50.27)	134.69	38.11			3.61	14.24	0.986

(Continued)

TABLE 4 (Continued)

Sample	Experimental methane yield (ml/g/VS)	B ₀ (ml/g VS)	B ₀ (ml/g)	First order kinetic model		Modified-Gompertz model		
				10 ³ k (1/day)	r ²	μ _{max} (ml/g VS/day)	λ (day)	r ²
B29S	93.39 (7.95)	92.63	21.15			5.01	17.04	0.993
B30S	39.02 (4.47)	36.66	7.00			5.23	20.30	0.967
B34S	95.33 (7.07)	91.09	18.86			4.21	14.36	0.994
B35S	165.89 (9.13)	164.11	37.64			5.80	14.41	0.997
B38S	101.64 (28.00)	95.94	25.72			3.37	13.16	0.987
Average		100.99 ^b (41.61)	24.88 ^a (9.44)			4.26 (1.26)	15.47 (3.55)	
LFD – Group 2								
Lab-scale separation								
A1L	134.90 (3.38)	126.48	3.74	65.27	0.989			
A2L	94.51 (1.95)	90.91	2.51	33.77	0.990			
A3L	82.85 (2.32)	77.11	3.15	43.52	0.987			
A4L	61.90 (1.08)	60.04	2.39	33.99	0.990			
A5L	81.14 (2.21)	61.97	3.04	40.58	0.977			
A6L	79.30 (1.52)	76.87	3.34	43.73	0.994			
A7L	63.47 (3.09)	71.91	2.29	18.50	0.995			
A8L	68.41 (0.82)	67.71	2.49	33.30	0.995			
A9L	80.24 (1.37)	74.04	2.76	50.17	0.979			
A10L	86.69 (2.95)	102.44	1.56	17.08	1.000			
Average		80.95 ^b (20.46)	2.73 ^c (0.62)	37.99 ^a (14.28)				
Industrial separation								
B3L	101.15 (5.46)	98.10	3.35	43.19	0.973			
B8L	129.68 (5.77)	138.91	4.08	32.02	0.994			
B9L	114.59 (4.15)	111.66	3.32	43.03	0.986			
B14L	59.38 (6.95)	61.58	4.74	29.95	0.992			
B15L	55.17 (1.45)	55.80	4.43	33.85	0.991			
B18L	151.01 (8.37)	191.30	3.00	19.03	0.983			
B27L	69.25 (0.98)	68.92	2.77	35.09	0.983			
B28L	105.05 (1.02)	101.45	5.05	35.39	0.989			
B38L	112.53 (3.40)	117.74	3.64	31.74	0.997			
Average		105.05 ^b (42.53)	3.82 ^c (0.80)	33.70 ^a (7.23)				

*Data for samples A1–A10 and B1–B19 had been presented by [Romio et al. \(2023\)](#). Values in parenthesis represent the standard deviations; the batch tests were performed in triplicates. Letter indices originate from the Tukey’s HSD test, comparing means at a significance level of differences set at $p < 0.05$, where different letters represent significant differences among the groups of samples.

VS, volatile solids; LFD, liquid fraction of digestate; SFD, solid fraction of digestate.

plants. Further processing, for instance, ammonia stripping to recover TAN as (NH₄)₂SO₄ and struvite precipitation to recover N and P, may be relevant ([Barampouti et al., 2020](#)).

Among the mentioned nutrients, Zn and Cu can also have detrimental effects on plants and the environment, as they are heavy metals ([Teglia et al., 2011](#)). Zn and Cu, along with the other analyzed heavy metals Ni and Cr, were below the limits set for waste in Danish legislation on a TS or P basis ([FLFM, 2018](#)), except for one sample, which surpassed the limit for Ni on a TS basis. In general, digestate does not need to comply with the waste legislation in Denmark since

digestate is regarded as manure with no heavy metals restrictions. However, biogas plants cannot treat any waste products that exceed the limits. When the biomass is anaerobically digested, the TS based heavy metal contents are almost doubled since approximately 50% of the TS are converted into biogas. Therefore, when waste is treated, it can be more appropriate to use the heavy metals contents in relation to P, which is conserved in the AD process. Therefore, it can be inferred that the materials fed to the digester were within the limits set for waste.

High concentrations of Na⁺ and other free ions may also negatively affect crop growth by impacting the biological activity in soil ([Teglia](#)

TABLE 5 Relationships among ultimate methane yields, calorific values, solids composition, and operational conditions.

Dependent variable	Independent variable	Linear correlation factor					
		Digestate Group 1	Digestate Group 2	SFD Group 2 Lab-scale separation	LFD Group 2 Lab-scale separation	SFD Group 2 Industrial separation	LFD Group 2 Industrial separation
Ultimate methane yield (ml/g VS)	Temperature (K)	−0.09	−0.31*				
	HRT (days)	−0.34	−0.50*				
	ln HRT (ln days)	−0.28	−0.59*				
	Calorific value (MJ/kg VS)		0.30	−0.30	0.13	−0.55	0.37
Ultimate methane yield (ml/g TS)	Protein (g/kg TS)	0.54*	−0.04	0.09	−0.32	−0.14	−0.53
	Lipids (g/kg TS)		0.19			−0.22	−0.44
	NDS (g/kg TS)		−0.13	−0.49	0.26	0.31	0.43
	Cellulose (g/kg TS)		0.08	0.37	−0.27	−0.23	−0.52
	Hemicellulose (g/kg TS)		0.22	0.71*	0.14	0.71*	0.61
	Lignin (g/kg TS)		−0.04	−0.31	−0.32	−0.87*	−0.69*
Calorific value (MJ/kg VS)	Temperature (K)		−0.17				
	HRT (days)		−0.19				
Calorific value (MJ/kg TS)	Protein (g/kg TS)		0.11	0.34	−0.37	−0.67*	−0.30
	Lipids (g/kg TS)		0.44*			0.26	−0.19
	NDS (g/kg TS)		−0.50*	−0.48	−0.30	−0.82*	0.15
	Cellulose (g/kg TS)		0.43*	0.35	0.13	0.64*	−0.08
	Hemicellulose (g/kg TS)		0.01	0.48	0.13	−0.08	−0.51
	Lignin (g/kg TS)		0.57*	−0.19	0.39	0.45	0.17

*Significant correlations set at $p < 0.05$.

SFD, solid fraction of digestate; LFD, liquid fraction of digestate; HRT, hydraulic retention time; VS, volatile solids; TS, total solids; NDS, neutral detergent soluble fraction.

et al., 2011) and causing osmotic stress, affecting the uptake of essential nutrients (Machado and Serralheiro, 2017). Conductivity can be an indicator of salinity since it indicates the concentration of free ions (Coelho et al., 2018). While Na concentrations differed notably within each group of samples, the conductivities presented low variability, indicating overall similar salinities of the samples and, possibly, similar risks of harmful effects.

Despite part of the C fed to digesters being recovered as biogas, substantial contents of C remained in digestate. A fraction of it was present as VFA, which are easily degradable and can lead to odorous (Crolla et al., 2013) and greenhouse gas emissions during digestate storage and application. Most digestates presented low concentrations of VFA, but some samples contained high VFA levels, achieving values up to 11.55 g/kg. Soluble organic compounds present in NDS, hemicellulose, lipids, and proteins may also be degraded during storage and after incorporation into the soil. At the same time, cellulose and, especially, lignin are supposed to be more stable forms of C, partially contributing to C sequestration into the soil.

4.1.2 Effects of feedstock shift on characteristics of unseparated digestate

The shift in feedstock in Danish biogas plants seen in recent years was accompanied by important changes in digestate quality. Although

Group 2 focused on biogas plants that employed large shares of lignocellulosic substrates, such as straw and deep litter, and do not represent a national average, it still is a good representative of future digestates (Pedersen and Hafner, 2023). The most evident change in digestate quality was the increase of average TS content (approximately 52%) seen in samples from Group 2 despite the longer HRTs. The higher TS can make digestate handling and field application difficult and potentially increase ammonia emissions following broadcast or band application by reducing digestate infiltration into the soil and prolonging its exposure to the atmosphere (Sommer and Hutchings, 2001). Additionally, samples in Group 2 presented an increase ($p < 0.05$) in average pH of 0.17 units while the average TAN contents remained unaffected ($p > 0.05$). This may further increase ammonia emissions by the shift in $\text{NH}_4^+/\text{NH}_3$ equilibrium towards ammonia formation (Sommer and Hutchings, 2001). Modeling predictions showed that field application by trailing hose of samples from Group 2 would cause higher ammonia emissions than undigested cattle and pig slurry (Pedersen and Hafner, 2023). For some samples, losses of 60% of the applied TAN were estimated (Pedersen and Hafner, 2023). Evidently, experimental trials are required to endorse these predictions. The potential loss of TAN reduces the attractiveness of digestate for the farmers, requiring the implementation of ammonia emissions mitigation strategies. Digestate soil injection can reduce

ammonia emissions by lowering the emitting surface area (Pedersen and Hafner, 2023); however, the method is energy demanding and can damage crops (Hansen et al., 2003). Additionally, injection may stimulate N_2O emissions by lowering the O_2 supply due to a more concentrated placement of the slurry (Thomsen et al., 2010; Petersen and Sommer, 2011). Therefore, alternative treatments to reduce digestate TS and pH may become crucial. Feedstock physical, chemical, and biological pre-treatments (Carrere et al., 2016) may result in a reduced TS content in digestate due to enhanced organic matter degradation during AD but can be limited by high costs. Solid–liquid separation of digestate appears as an obvious option to reduce TS contents; however, as seen in Section 4.1.5, enhancements of the currently applied technologies are required. Acidification with H_2SO_4 , commonly applied to animal slurry (Fangueiro et al., 2015), can be challenging on digestate due to its high buffer capacity, requiring large amounts of acid (Møller et al., 2022), which increases costs and risks of overfertilization with S (Pedersen and Hafner, 2023). A recently explored technology to reduce digestate pH is plasma treatment, which fixes N from the atmosphere in the digestate as NO_3^- and NO_2^- , forming the acids HNO_3 and HNO_2 (Nyangau et al., 2024). However, the treatment is costly and the risks of increased N_2O emissions should be assessed.

Regarding the TS composition of digestate, significant differences ($p < 0.05$) were observed between Group 1 and Group 2. Samples from Group 2 showed average lower TAN, TKN, protein, K, P, Fe, Zn, and Cu contents, indicating the lower contents of these compounds in the substrates substituting energy crops and other materials. Compositional differences between straws and energy crops can be considerable. For example, Garcia-Ruiz et al. (2019) found average wheat straw C/N ratios between 82.4–152.1 depending on variety, soil and management techniques, which were much higher than typical values for cropped silages of 30.8 for maize silage and 19.4 for grass silage (Bahar et al., 2005). These differences can directly affect the digestate quality.

4.1.3 Nutrient contents of the liquid fraction of digestate

In general, pH, conductivities, TS contents, TS/VS, TAN/TKN, and C/TKN ratios, and concentrations of some nutrients and heavy metals in the LFD obtained with screw presses were in line with those in the literature (Tambone et al., 2017; Guilayn et al., 2019; Mazzini et al., 2020; Akhiar et al., 2021; Finzi et al., 2021; Cathcart et al., 2023), except for Cu and Zn, whose concentrations were higher than those reported. TS contents of the LFD originating from the separation with decanter centrifuges were higher than those reported by Akhiar et al. (2021) and Cathcart et al. (2023), resulting in also higher concentrations of other components on a fresh matter basis. Data regarding the lignocellulosic composition and lipids contents in LFD are still scarce in the literature.

Within Group 2, LFD samples presented lower TS contents than unseparated digestate, which were brought to levels closer to those presented by samples from Group 1. However, differences in TS contents were not statistically significant ($p > 0.05$). Some samples of LFD still presented very high TS contents, achieving values of up to 107.12%. Considering the similar levels of pH and TAN in the LFD as those of unseparated digestate ($p > 0.05$), the application of LFD with a more efficient solid–liquid separation, achieving higher reduction in TS, could contribute to counterbalance the potential increase in

ammonia emissions during digestate soil application in unseparated digestate from Group 2 due to its increased TS content compared to Group 1. Lower TS contents in the LFD could also ease digestate handling and soil application since the material may become more pumpable, and blockages of application equipment may be avoided. Pedersen et al. (2022) estimated the ammonia emissions in unseparated digestate and LFD based on literature data concerning emission factors and digestate separation efficiency. The authors showed that a clear reduction in emissions could be obtained by utilizing LFD as a soil fertilizer instead of the unseparated digestate when trailing hoses and trailing shoes were employed, even when including the emissions from the SFD during storage and application. However, estimates were based on few data available in the literature, with even fewer being representative of Danish digestates containing high TS contents. Additionally, the authors reviewed that, in some cases, emissions factors were higher for the liquid fraction of cattle and pig slurry compared to those of raw slurry. The reasons were not clear, but a reduction in TS may reduce the mass transfer resistance, which impedes ammonia volatilization (Pedersen et al., 2022), or increase the exposed surface area of the material due to more spreading out after application (Pedersen and Hafner, 2023). Therefore, field experiments are crucial to determine whether attainable TS reductions with the currently utilized separation methods are sufficient to deliver significant reductions in ammonia emissions from the new Danish digestates or if such a technique could have undesirable consequences as those observed for some samples of separated animal slurry. The assessment of emissions of other environmentally harmful gases, such as CH_4 and N_2O , during storage of LFD and SFD and their comparison to the emissions associated with the unseparated digestate, is also important.

Regarding other characteristics, results showed comparable concentrations to unseparated digestate on a fresh matter basis ($p > 0.05$), even though the concentrations of some components in the SFD were significantly higher ($p < 0.05$). This may be explained by the low mass of digestate retained as SFD when industrial separators were employed and the low differences in concentrations between LFD and SFD obtained in lab-scale separation. Comparable concentrations in LFD and unseparated digestate have also been reported in literature, for instance for TKN, P (Finzi et al., 2021; Cathcart et al., 2023), and TAN (Finzi et al., 2021) when separations were performed with screw presses and for K when separations were conducted with either screw presses or decanter centrifuges (Finzi et al., 2021; Cathcart et al., 2023). However, when decanter centrifuges were utilized, Cathcart et al. (2023) observed clear reductions in concentrations of TKN and P in the LFD. The reduction in contents of VS, lignin, cellulose, and C contents and the increase in NDS, S, TKN, Ca, K, and Mo on a TS basis ($p < 0.05$) in the industrially separated LFD compared to unseparated digestate samples indicate that the first group of components may be associated with larger particles, while the former may be associated with smaller ones. Contents of measured heavy metals were within legislated recommendations for waste, except for Ni, which surpassed the limit for two LFD samples, but only on a TS basis.

4.1.4 Nutrient contents of the solid fraction of digestate

In most cases, TS contents, VS/TS and C/TKN ratios, and concentrations of some nutrients and heavy metals in industrially

separated SFD were comparable to those reported for SFD obtained by screw presses (Thygesen et al., 2014; Sambusiti et al., 2015; Tambone et al., 2017; Guilayn et al., 2019; Brémont et al., 2020; Mazzini et al., 2020; Akhiar et al., 2021; Brémont et al., 2021; Finzi et al., 2021; Cathcart et al., 2023) and decanter centrifuges (Kratzeisen et al., 2010; Thygesen et al., 2014; Akhiar et al., 2021; Cathcart et al., 2023), except for C contents, which were higher than those presented in the literature. TAN contents and TAN/TKN ratios in the SFD samples obtained after separation with decanter centrifuges were lower than those in the literature, while data from SFD originated from screw presses were similar to reported values. As in the case of LFD, data about lignocellulosic composition and lipids contents are still limited.

In comparison to the unseparated digestate from Group 2, the concentrations of VFA, Na, K, and Cu remained constant in industrially separated SFD on a fresh matter basis ($p > 0.05$), while TAN contents and TAN/TKN contents decreased ($p < 0.05$). C/TKN ratio and the contents of other parameters increased ($p < 0.05$). Finzi et al. (2021) and Cathcart et al. (2023) also reported higher TKN and P and similar K contents in the SFD compared to the unseparated digestate. Finzi et al. (2021) additionally observed similar TAN contents in SFD, while Cathcart et al. (2023) described higher C contents in this fraction. On a TS basis, lower contents of NDS, TKN (likely driven by TAN), S, Ca, K, and Mo and higher contents of VS, cellulose, lignin, and C were found in the industrially separated SFD compared to unseparated digestate ($p < 0.05$). The opposite trends were noticed for LFD. Hence, these observations corroborate the idea that the first group of compounds may be associated with smaller particles, while the second should be part of larger ones.

The differences in nutrients concentrations in SFD compared to unseparated digestate, especially the higher P contents, make SFD an attractive fertilizer to be applied to soils lacking P. Considering the legislation limits for application of N and P, 11 out of 12 of the industrially separated SFD would have their soil application limited by P concentrations. Soils lacking P, however, are usually located in areas far from agricultural biogas plants, where intensive farming is present. The higher TS contents of SFD could reduce the transportation costs of digestate to these locations. The contents of heavy metals were lower than the national limits for waste, except for Ni, where the concentration on a TS basis was higher than allowed for one SFD sample.

Although the SFD has fertilizer properties, it has mainly been proposed as a soil amendment due to its high organic matter content, which is more stable than the organic matter of undigested organic wastes. Soil amendments improve soil properties, such as water retention, permeability, water infiltration, aeration, or structure, providing a better environment for roots (Teglia et al., 2011). Surface-applied SFD should be incorporated into the soil to avoid odor emissions and loss of nutrients through runoff or volatilization (Crolla et al., 2013). Given the higher C/TKN ratios in SFD than in unseparated digestate, the risks of N immobilization may be increased. Most industrially separated SFD samples presented C/TKN ratios higher than 18, often assumed as a threshold for immobilization stimulation (Teglia et al., 2011). However, immobilization risks do not rely solely on C/TKN ratios but also depend on the stability of the organic carbon in soil amendments (Teglia et al., 2011). Although the concentrations of

recalcitrant fractions, such as cellulose and lignin, increased in the SFD, the attained methane yields demonstrate that the organic matter in SFD was not completely stabilized.

A process capable of enhancing organic matter stability before soil application is composting, in which part of the organic matter is degraded under controlled aerobic conditions (Akyol et al., 2019). A fraction of the organic substances is transformed into humic compounds, ensuring stability after soil application. Additionally, the increase in temperature during the process can eliminate pathogens that may have remained after AD (Lu et al., 2021). However, composting of SFD faces some challenges. For instance, the C/TKN ratios of the SFD obtained in the present study were lower than those reported as optimal for initiating composting (25–35) (Akyol et al., 2019). The high cellulose and lignin contents may also prolong the duration of composting of SFD due to their recalcitrance (Lu et al., 2021). Finally, the TS contents of SFD were, with few exceptions, also lower than the recommended range of 35–60%, which is optimal for microbial activity and allows proper air transport through the solids pile during the composting process (Akyol et al., 2019). A potential solution for these issues includes adding dry materials with high C contents to the SFD to adjust C/TKN ratios and TS contents for more favorable composting conditions (Akyol et al., 2019). This strategy may also reduce methane and N₂O emissions by minimizing the formation of anaerobic zones due to facilitated aeration (Li et al., 2020). However, ammonia emissions may increase as aeration boosts microbial activity, leading to increased temperature in the SFD pile (Li et al., 2020).

4.1.5 Efficiency of solid–liquid separation

Decanter centrifuges were employed to separate digestate in the lowest range of TS contents containing fewer fibers and coarse particles, while screw presses were applied to digestates with more TS and fibrous materials. Decanter centrifuges are generally more efficient at retaining smaller particles than screw presses (Finzi et al., 2021), which explains the higher reductions in TS contents observed in LFD originating from these separators. However, the TS reductions in the LFD obtained with decanter centrifuges were lower than those reported in the literature, which is, on average, approximately 50%, as reviewed by Pedersen et al. (2022). Screw presses were very inefficient for three samples, with TS reductions of less than 5%. The TS reductions in the remaining samples separated by screw presses were in line with those in the literature (Pedersen et al., 2022). In general, both separator types resulted in low amounts of digestate mass recovered as SFD, on average, only 7.24%. It is unclear why the separation efficiencies were, in many cases, lower than those in previous studies. The differences might have been caused by changes in substrate composition and operation of anaerobic digesters. The increasing amounts of lignocellulosic substrates fed to anaerobic digesters have forced biogas plants to implement maceration as a pre-treatment of certain recalcitrant substrates, such as straw. The process reduces the sizes of particles, which, to some extent, are recovered in the LFD. At the same time, HRTs have been prolonged, also leading to smaller particles in digestate. The separation with screw presses may be improved by utilizing smaller screen sizes with 0.5 mm, since 1 mm screen size is currently used. Furthermore, the separation process could be optimized by the utilization of precipitating agents, polymers, and flocculants and by integrating

sequential separation steps, such as screw presses and decanter centrifuges followed by microfiltration (Barampouti et al., 2020).

The major part of the digestate mass and its components remained mainly within the industrially separated LFD, except cellulose, hemicellulose, lignin, and Cr, which were retained mainly in the SFD. Accordingly, Guilayn et al. (2019) have reported that most of the TS, VS, organic N, TAN, P, K, S, Ca, and Mg remained in the LFD, while Tambone et al. (2017) observed the same trend for TS, TKN (likely driven by TAN), and P. Excluding the three mentioned samples with very inefficient separation, the SIs of industrially separated digestate were comparable to those reported in the literature for fresh mass, TS, VS, organic N (or protein), TAN, P, K, and C (Tambone et al., 2017; Guilayn et al., 2019; Finzi et al., 2021; Cathcart et al., 2023). The RSIs of TS, K, and TAN of digestate separated by screw press were similar to those reported by Finzi et al. (2021). Data on the RSIs of components resulting from the separation of agricultural digestate with a decanter centrifuge are limited in the literature. However, Cathcart et al. (2023) showed that the SIs of TS, C, TKN, P, and K were higher for decanter centrifuges than screw presses. Likewise, in the present study, the separation with decanter centrifuges resulted in higher RSIs for P (see Supplementary material). Literature data concerning the RSIs of other components in agricultural digestate have not been found for comparison. The high RSIs obtained for cellulose, hemicellulose, and lignin are linked to their presence mainly in large fibrous particles. Likewise, Cr presented high RSIs. While the reason for this is unclear, Zhu and Guo (2014) showed that Cr had high stability in digestate, being present mainly in residual form and being unlikely to be released to the digestate solution. The separation of the remaining analyzed components resulted, in general, in lower RSIs, suggesting that they were, to a larger degree than cellulose, hemicellulose, lignin, and Cr, also associated with smaller particles (as an original part of them or adsorbed) or partially solubilized (Möller and Müller, 2012; Zhu and Guo, 2014). The negative RSIs obtained for TAN, K, and Na can be explained by their high solubility in water (Baryga et al., 2021; Finzi et al., 2021).

Industrially separated SFD was obtained with different equipment types likely operating with different capacities and at different conditions, such as different applied pressures in screw presses and bowl speeds in decanter centrifuges (Cathcart et al., 2023). Therefore, the lab-scale separation was performed to evaluate RSIs by applying the same separation method to several digestate samples. This type of separation was not efficient, with larger mass proportions retained in the sieve, but with lower TS contents in SFD and smaller differences in components concentrations between LFD and SFD when compared to industrial separation. Nevertheless, the lab-scale separation resulted in average RSIs comparable to those obtained with industrial separation.

4.2 Energetic potential of digestate

Significant amounts of digestible fractions (protein, lipids, cellulose, hemicellulose, part of NDS) were still present in digestate, representing a potential for additional methane recovery in biogas plants. Methane yields of unseparated digestate on a VS basis were within the range of those presented in a previous publication, including some samples belonging to Group 2 (Romio et al., 2023). The average methane yield on a VS basis of samples from Group 1 was

approximately 50% higher than that from Group 2 ($p < 0.05$). The average longer HRT and the more recalcitrant nature of substrates applied to digesters in Group 2 explain this difference. However, the higher TS contents ($p < 0.05$) of digestates from Group 2 resulted in similar ($p > 0.05$) average methane yields on a fresh matter basis for both groups. This observation indicates comparable risks of methane emissions during digestate storage, unless the different TS contents affect crust formation, which may also influence gaseous emissions (Wood et al., 2012). The negative relationships ($p < 0.05$) between the residual methane yield on a VS basis and the temperature and logarithm of HRT found by Romio et al. (2023) were maintained but slightly weakened by the addition of data from new samples in Group 2. Higher temperatures should enhance organic matter degradation, resulting in lower residual methane yields in the digestate. Extending the HRT by expanding digestion volume capacity can be economically feasible for several biogas plants, as previously demonstrated (Romio et al., 2023). Besides enhancing the revenues of biogas plants, this strategy also has the potential to reduce methane emissions during digestate storage prior to field application.

Digestate separation resulted in similar ($p > 0.05$) methane yields on a VS basis in both SFD and LFD, indicating comparable overall recalcitrance of these fractions despite the different compositions. Protein and hemicellulose contents positively affected the methane yields in some groups of samples, while lignin contents had a negative impact. Protein and hemicellulose are digestible fractions, while lignin is hardly degradable under anaerobic conditions and contributes to increasing the resistance of holocellulose to degradation (Schievano et al., 2008).

Methane yields of the SFD agreed with values found in the literature (Thygesen et al., 2014; Maynaud et al., 2017; Romio et al., 2021). The methane production of LFD started rapidly, while long lag-phases were observed in the digestion of industrially separated SFD. The reasons behind this behavior are unclear, but the larger particles and the reduction in soluble organic compounds in SFD may have had an influence. Moreover, it should be noted that SFD samples had been frozen before digestion, while LFD samples were utilized immediately after collection. The inactivation of AD microbes during the freezing period may have contributed to the longer start-up phase. The same batch assay run included samples of raw cattle manure and wheat straw (both from an unrelated study), and these all showed good biogas production with no significant lag-phase (data not shown). Therefore, low inoculum activity should not have been a reason for the occurrence of the long lag-phases.

On a fresh matter basis, the average methane yield of the industrially separated SFD was approximately seven times higher than that of LFD. The average methane yield of the SFD on a fresh matter basis achieved 46–100% and 126–222% of the yields of samples of maize and grass analyzed by Triolo et al. (2011). However, only 8% of the methane yield of straw was achieved by the SFD (Triolo et al., 2011). The results indicate that SFD has the potential to be reutilized in AD and be a valuable substitute for some expensive, low-quality, or scarce feedstock or simply be included as additional material to the substrate mix in a system where it is recirculated to the digester (Brémond et al., 2021). Likely, only a portion of the SFD should be used to avoid the accumulation of inorganic matter and inhibitors in the digester, such as heavy metals (Brémond et al., 2021), and to avoid an excessive increase in the TS content in the digester, which could difficult mixing and

mass and heat transfer. As shown by [Dinuuccio et al. \(2013\)](#), the recirculation of the totality of the SFD in continuous reactors operating with a HRT of 40 days resulted in an increase of 16% in the TS content and maintained the volumetric methane production 4% higher than that of control reactors without recirculation for 120 days. After this period, the methane production of the two sets of reactors leveled out, possibly due to the accumulation of recalcitrant compounds in the reactors to which SFD was recirculated. The economic viability of the SFD recirculation would depend on the resulting methane yields, prices of substituted substrates, and costs of digestate solid–liquid separation. Methane production associated with SFD recirculation could be enhanced by applying post-treatment methods to the SFD to open up its structure, increasing its methane yield or accelerating the hydrolysis rate. Several studies evaluated the effects of post-treatments, such as thermal, mechanical, chemical, and biological methods, in the SFD. Varying results were obtained in batch tests, from reductions in methane yields to increases of up to 300%, as reviewed by [Romio et al. \(2021\)](#). In a continuous test, [Jagadabhi et al. \(2008\)](#) showed that recirculating untreated or alkali treated SFD to the digester was unjustified in a system operating with a HRT of 20 days and with a ratio between the mass load of SFD and fresh feedstock of 10%. Nevertheless, studies evaluating continuous tests mimicking the recirculation of the SFD to the digester at different rates with or without SFD post-treatment are still lacking in the literature.

Although industrially separated SFD presents higher methane yields on a fresh matter basis than LFD, most of the methane potential, about 2/3, is held in the LFD due to its higher recovered mass in separation. This means that, although reduced, risks of methane emissions during LFD storage remain, as observed by [Gioelli et al. \(2011\)](#); hence, storage of LFD in covered tanks with methane capture is recommended. The emissions from the SFD should also be considered for an overall assessment of the effects of separation in methane emissions. Emissions from the SFD will depend on storage type (in covered or uncovered tanks or piles) and utilization, i.e., recirculated to the digester, as a soil amendment, composting, or for another purpose.

Alternative uses of SFD include bioethanol production, hydrothermal carbonization with the production of hydrochar, pyrolysis with the production of biochar, syngas, and bio-oil, and combustion, as reviewed by [Monlau et al. \(2015\)](#) and [Peng et al. \(2020\)](#). While bioethanol, hydrochar, syngas, and bio-oil can be further processed for energy generation, biochar has been considered a soil amendment, with a significant role in sequestering carbon in soil ([Monlau et al., 2015](#)). Regarding combustion, calorific values were similar to the experimental ones obtained by [Czekala \(2021\)](#) and [Kratzeisen et al. \(2010\)](#) for SFD and comparable to those of wood. These authors suggested that the waste heat in plants owning combined heat and power units could be utilized for SFD drying to a moisture content of about 10–20% and pelletizing could be applied to produce a homogeneous and transportable material. [Cathcart et al. \(2021\)](#) and [Kratzeisen et al. \(2010\)](#) estimated positive energy balances for the combustion of SFD, considering the energy demand for separation, drying, and pelletizing. According to [Cathcart et al. \(2021\)](#), the highest costs of the process would derive from the investment in a belt dryer (corresponding to 61 and 78% of the total costs when separating digestate with a decanting centrifuge and a screw press), as mechanical aid is likely required in the drying process. The profitability

of the process would highly depend on the amount of SFD being produced. [Kratzeisen et al. \(2010\)](#) showed that the emissions of flue gas during combustion of SFD pellets were in agreement with German limits set for biofuels and suggested that the ash residue could be a valuable fertilizer since nutrients such as P, K, and minerals are conserved. However, the contents of the heavy metals Ni and Cr were above the safe limits for land application.

5 Conclusion

The transition to higher shares of lignocellulosic feedstock seen in Danish biogas plants in recent years has caused an average increase of 52% in the TS content of digestate and an average pH increase of 0.17 units. These changes may impact ammonia emissions during digestate soil application. Solid–liquid separation and the utilization of the LFD as a fertilizer may alleviate the increased risks of ammonia emissions by reducing TS contents and accelerating digestate soil infiltration rate. However, currently employed separation technologies have not proved highly efficient at reducing TS contents in the LFD, indicating that new approaches are necessary. The SFD was shown to be an attractive fertilizer to be applied to P deprived areas or a valuable source for additional energy recovery through its recirculation to the digester (with average methane yield of 101 mL/g VS) or combustion (with average calorific value of 21 MJ/kg VS).

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

Author contributions

CR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. AW: Writing – review & editing. HM: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was funded by the Ministry of Food, Agriculture, and Fisheries of Denmark through a project with the title Methods for reducing ammonia loss and increased methane yield from biogas slurry (MAG) (journal nr.: 34009-21-1829) and the FertiHood project (journal nr.: 34009-20-1707) as part of Organic RDD6, coordinated by the International Centre for Research in Organic Food Systems (ICROFS).

Acknowledgments

The authors acknowledge the biogas plants that provided samples for the conduction of this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

EDITED BY

Rachel E. Thorman,
Agricultural Development Advisory Service,
United Kingdom

REVIEWED BY

Maria Pilar Bernal,
Spanish National Research Council
(CSIC), Spain
Diogenes Antille,
Commonwealth Scientific and Industrial
Research Organisation (CSIRO), Australia

*CORRESPONDENCE

Laura Zavattaro
✉ laura.zavattaro@unito.it

RECEIVED 13 February 2024

ACCEPTED 19 June 2024

PUBLISHED 15 July 2024

CITATION

Sitzmann TJ, Alpignano A, Lerda C, Moretti B,
Zavattaro L and Grignani C (2024) Response
of tomato to innovative organo-mineral
fertilizers.

Front. Sustain. Food Syst. 8:1385828.
doi: 10.3389/fsufs.2024.1385828

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Response of tomato to innovative organo-mineral fertilizers

Tomas Javier Sitzmann¹, Andrea Alpignano¹, Cristina Lerda¹,
Barbara Moretti¹, Laura Zavattaro^{2*} and Carlo Grignani¹

¹Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Turin, Italy,

²Department of Veterinary Sciences (DSV), University of Turin, Turin, Italy

Organo-mineral fertilizers (OMFs) with low organic carbon (C_{org}) content have been associated with higher mineral fraction nutrient use efficiency. However, the extraction of peat, which is typically used in these OMFs, from endangered ecosystems causes long-time stored C_{org} to mineralize and to be released back into the atmosphere as carbon dioxide (CO_2). This study analyzes the replacement of peat in OMFs with biowaste materials. These materials, considered organic byproducts that microorganisms and other living things can decompose through composting and aerobic or anaerobic digestion, offer a viable opportunity. This study investigated three stabilized biowastes—green compost (GC) from pruning residues, municipal solid waste compost (MSWC), and manure-based vermicompost (VC)—as the organic matrices for granular OMFs. These matrices were impregnated with dissolved ammonium sulfate and urea and used to coat diammonium phosphate granules. Each biowaste OMF contained 7.5% C_{org} , 20% mineral N, and 10% mineral P_2O_5 (OMF_{20–10}). Fertilizers with high nutrient concentrations have the advantage of requiring low application volumes, facilitating their application in the field. Biowaste OMFs were compared with peat OMFs with the same C_{org} -N- P_2O_5 concentration. Peat and MSWC were also used to create OMFs containing 7.5% C_{org} , 10% mineral N, and 5% mineral P_2O_5 (OMF_{10–5}). A 75-day tunnel trial was conducted under semi-controlled conditions using tomato plants (*Solanum lycopersicum* L.) fertilized to an equivalent of 81 mg N kg⁻¹ soil and 18 mg P kg⁻¹ soil. Controls included no fertilization (N₀P₀) and mineral N and P fertilization (MF_{NP}). The Soil Plant Analysis Development (SPAD) chlorophyll meter and the BBCH (from German Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) scale as well as the number of shoots were measured over time, as berry and total aboveground yield, N and P uptakes, and N and P use efficiencies (NUE and PUE, respectively) were calculated at harvest. All treatments outperformed the control N₀P₀ in most indicators. Peat_{20–10} did not have more berry yield than other OMF_{20–10}; however, the higher number of shoots indicated a higher potential yield in the event of prolonging the experiment. At the end of 75 days, VC_{20–10} and MSWC_{20–10} showed similar PUE to peat, suggesting that those materials can be used as replacements. In the case of OMF_{10–5}, MSWC_{10–5} had yield and N and P uptakes like peat OMFs, confirming the potential use of MSWC as peat replacement even at different nutrient concentrations. This research provides reassuring evidence of the effectiveness of biowaste OMFs, offering a positive outlook for sustainable agriculture. However, their use is not recommendable for short growing seasons.

KEYWORDS

biowaste, municipal solid waste, peat replacement, pruning compost, vermicompost

1 Introduction

Organo-mineral fertilizers (OMFs) refers to a combination of an organic material with one or more mineral fertilizers (Smith et al., 2020). The mixture is made to correct the nutrient ratio of an organic material (Rady, 2012; Antille et al., 2013b; Anetor and Omuetti, 2014) or to use the organic material as a matrix for the mineral nutrients (Florio et al., 2016). Generally, this second type of OMF adopts low quantities of organic carbon (C_{org}), which in Europe can be as low as 3 or 7.5% for liquid or solid OMFs, respectively (EC, 2019), and their use has been reported to reduce N losses, improve P availability, and enhance N and P use efficiency (Richards et al., 1993; Antille et al., 2013b, 2014; Florio et al., 2016).

The proportion of the organic matrix in the OMF chemically and physically influences the mineral fraction in close contact with it. In OMFs with different organic material fractions, the lower organic material fractions in the OMF decrease the fertilizer density and increase the abrasion fragility and crushing strength (Pare et al., 2010); physical differences in fertilizer granules can create discrepancies in P release as they interact differently with the soil. Moreover, it has been shown that a higher C_{org} content in close contact with mineral nutrients increases the immobilization of P (Sitzmann et al., 2024b), probably due to Ca-P bonds and a pH effect.

In low- C_{org} OMFs, the organic matrix proportion can be considered fixed, but the proportion of the mineral fertilizer can be adjusted to adopt a desirable nutrient concentration (Sitzmann et al., 2023). However, reaching a specific nutrient concentration in OMFs will require using a mixture of mineral fertilizers that can cause one type of mineral fertilizer preference over another, e.g., urea for a high N concentration over ammonium sulfate. Differences in concentration in OMFs—and therefore in mineral fertilizers—will not only cause different interactions in soil and plant nutrient uptake but could also interact differently based on the organic fraction of the OMF.

Low- C_{org} OMFs can be produced from organic geogenic materials such as peat, leonardite, or lignite (EC, 2019). Traditionally, peat has been preferred over other materials because of its high humic carbon (C) content (Alianiello et al., 1999; Florio et al., 2016). This fraction has been linked to promoting crop growth by influencing microbial and physicochemical soil properties (Li et al., 2019), stimulating plant metabolism (Asli and Neumann, 2010; Vujinović et al., 2020), and helping the remediation of soil contaminants due to an increase in sorption capacity (Piccolo, 2002; Conte et al., 2005). However, peatlands—which account for 55,000 Mt C, corresponding to 27% of the soil C stock in the world (Parish et al., 2008)—should be protected from mining to avoid the volatilization of long-term stored C as carbon dioxide (CO_2) and the emissions of other greenhouse gases (Saarikoski et al., 2019; Humpenöder et al., 2020). Therefore, replacing organic geogenic materials with degraded and stabilized renewable biowaste materials that are produced locally has been proposed for improved circularity of carbon and other nutrients (Schmilewski, 2008; Pare et al., 2010; Taparia et al., 2021).

Replacing a raw material with another in an industrial process involves a series of challenges. The search process can be long and expensive, and companies may face reluctance to change from the

market and the established industry (Alexander et al., 2008), as well as establish a new production chain of quality-certified products. For OMF manufacturing, any potential raw organic material needs to be stable over time, homogeneous in larger volumes, and interact foreseeably with mineral fertilizers (Sakurada et al., 2016; Bouhia et al., 2022). Materials with high and easy availability on the market are desirable for OMF production to provide them with circularity through reutilization (Taparia et al., 2021). Low cost and certified technological quality are also fundamental when selecting a suitable organic material.

In a previous study from this research group, composted and digested dry materials similar to peat with a particle size below 5 mm, C_{org} content between 17 and 25%, and a humification degree >50% were proposed as peat replacements for preparing granular and pelletized low- C_{org} OMFs (Sitzmann et al., 2023). The results identified three potential candidates: green compost (GC) from pruning residues, municipal solid waste compost (MSWC), and manure-based vermicompost (VC). GC and VC can be used as organic fertilizers or soil amendments; on the other hand, municipal solid waste is not recognized as a fertilizer by the European Union (EU) regulation unless the organic fraction is separated from any other residue (EC, 2019), which can pose technical and economic challenges. However, when other potential organic fractions, such as manure, are unavailable, MSWC can serve as an alternative to traditional fertilizers in the future (Ros et al., 2006). MSWC used in the research by Sitzmann et al. (2023) is a physically separated organic source of municipal waste, which is on the threshold of being considered an organic fertilizer. The use of MSWC has been considered because its contribution to heavy metals can be almost negligible in OMFs with 7.5% C_{org} . The inclusion of MSWC among potential peat replacements can be a reference for future revisions of the current fertilizer regulations.

In a previous small pot trial with OMFs using GC, MSWC, VC, and peat, with C_{org} , N, and P_2O_5 concentrations of 7.5, 10, and 5%, respectively, peat and mineral control led to higher maize plant P uptake and growth yield after 4 weeks compared to biowaste OMFs (Sitzmann et al., 2024a). The reduced uptake in biowaste treatments was attributed to a slow release of nutrients, bonds between metals in the organic material and the phosphates from the mineral fertilizer, or the shift of phosphates to unavailable forms caused by the high pH of the organic material, which reduced the nutrient use efficiency of GC, MSWC, and VC OMFs. Microbial immobilization in soil caused by the organic matrix of OMFs was discarded in low- C_{org} OMFs due to the limited C_{org} addition compared to mineral controls (Sitzmann et al., 2024a). Considering the 1st days of plant growth, an initial lower nutrient use efficiency based on the ratio of absorbed nutrients to total applied nutrients was frequently observed in OMFs due to slow chemical release (Bouhia et al., 2023; de Moraes et al., 2023) or initial nutrient immobilization (Mandal et al., 2007; Mazeika et al., 2016; Carneiro et al., 2021; Llovet et al., 2023), and both processes could increase nutrient availability in more prolonged periods. While a previous study on GC, MSWC, and VC granular OMFs focused on their performance over a few weeks, further study is needed to determine if the nutrient immobilization is temporary and if these OMFs will increase their efficiency after 1 month; alternatively, it must be determined if the

immobilization is permanent and if the addition of these organic materials to mineral fertilizers is counterproductive.

This study aimed to evaluate the N and P use efficiencies (NUE and PUE, respectively) of three new granulated biowaste-based OMFs characterized by low- C_{org} content. They were compared with peat OMFs and a mineral control in a pot experiment where tomato plants (*Solanum lycopersicum*), as a medium-term growing crop, were managed for 75 days. Additionally, it sought to evaluate if the ratio between organic fraction and mineral nutrient can affect the nutrient use efficiency and physiological development of the plants; therefore, the MSWC and peat materials were used with two different concentrations of mineral nutrients but with the same C_{org} concentration.

We hypothesized the following:

- 1) differences in growing parameters would emerge in the initial and medium growth phases of tomato plants due to differences in N and P release of OMFs compared to the mineral control;
- 2) granular OMFs made with GC, MSWC, and VC would have a similar nutrient use efficiency compared to peat OMF and the mineral control after 75 days; and
- 3) a lower concentration of minerals N and P in the OMF, which results in higher C_{org}/N and C_{org}/P , would result in a higher immobilization effect.

Compared to the study conducted by Sitzmann et al. (2024a), this study contributes to a deeper understanding of the nutritional dynamics derived from biowastes and peat-based OMFs over a mid-term period that is more appropriate for a broader range of commercial crops such as tomatoes. Additionally, this study evaluates the significance of mineral nutrient concentration within the OMF granules, serving as an indicator for fertilizer application per unit soil area.

2 Materials and methods

This study was conducted through a plant growth trial with tomato plants (*Solanum lycopersicum* L.). It involved two sets of comparisons: (i) four OMFs made from three different biowastes or peat and (ii) four OMFs made from one biowaste or peat with two different concentrations of the mineral nutrient. In the first trial, four OMFs with a C_{org} -N- P_2O_5 content of 7.5-20-10 (4.2% of total P) were created using peat (hereafter mentioned Peat₂₀₋₁₀), green compost (hereafter mentioned GC₂₀₋₁₀), municipal solid waste compost (hereafter mentioned MSWC₂₀₋₁₀), and vermicompost (hereafter mentioned VC₂₀₋₁₀). Two control treatments were also included: (1) no fertilization (N_0P_0) and (2) mineral fertilization (MF_{NP}), consisting of a blend of ammonium sulfate, urea, and triple superphosphate (TSP) at an N:P ratio of 10-2.1. For the second comparison, two additional OMFs were created using a C_{org} -N- P_2O_5 content of 7.5-10-5 (2.1% of total P) using peat (Peat₁₀₋₅) and municipal solid waste compost (MSWC₁₀₋₅). These treatments were compared to Peat₂₀₋₁₀ and MSWC₂₀₋₁₀.

Therefore, for the given amount of N and P fertilization per kg of soil, the 7.5-10-5 OMFs will provide twice the amount of C_{org} of the 7.5-20-10 OMFs. Treatment details and amounts of fertilization are described in Table 1.

2.1 Fertilizer production

Biowaste materials and peat for OMFs were collected from industries across Italy. Biowaste materials were selected based on their similarities to peat C_{org} content, C_{org} stability based on the alkyl C/O alkyl C ratio and the humification degree, low heavy metal contents, and processing facilities. The GC was produced by combining vegetable waste with pruning residues composted for 6 months and was manufactured by Roffino S.R.L. The VC was produced from bovine manure that was composted for 6 months, which then undergoes a vermicomposting process, and was manufactured by Conitalo S.p.A. ACEA Pinorelese Industriale S.p.A. manufactured the MSWC by mechanically separating the organic fraction of municipal household waste and stabilizing it through integrated anaerobic digestion and aerobic composting (Mainero, 2008). Details regarding MSWC production can be found in the study conducted by Moretti et al. (2020). Organic materials were fully characterized in the study of Sitzmann et al. (2023).

The OMFs were manufactured by SCAM S.p.A. (Modena, Italy). The OMF manufacturing process involved mixing the organic material with ammonium sulfate, urea, diammonium phosphate, and water in a granulator. The water dissolves the mineral N, whereby impregnating the organic material with the dissolved fertilizer, forming a cohesive dough. This cohesive mass acts as a coating for the diammonium phosphate that acts as the granule nucleus. After creating the OMF granules, they are dried at 60°C until they reach ~4% moisture content. The mineral N fertilizer is added slightly over the target concentration of OMF N, accounting for volatilization of a partial N concentration during the OMF drying. Finally, the granules are sieved, resulting in OMFs with a diameter of 2–5 mm (60% <3 mm). For more details, see Sitzmann et al. (2024a,b).

2.2 Experimental setup

A pot experiment was conducted in the summer of 2022 on the campus of the Department of Agricultural, Forest, and Food Sciences of the University of Turin in Grugliasco (NW, Italy) under semi-controlled conditions in an open tunnel greenhouse with a North-South orientation. The greenhouse was covered with a transparent cloth to guarantee proper illumination for plant development while preventing rainwater from entering. During the growing period, the minimum and maximum average temperatures recorded were 18 and 33°C, respectively. A tomato (*Solanum lycopersicum* L.) cultivar H1301, suitable for industrial processing, was used as a test plant. Twenty-day-old tomato plants grown in a nursery were transplanted, one per pot, into pre-fertilized pots on 25th May.

The experiment was a randomized block design separated by benches with four replicates for each treatment (the total number of pots was 36). However, one block was discarded due to pest development during the 2nd month of the tomato growth cycle. Each pot was 20 cm in diameter at the top and slightly thinner at the bottom, and it was placed on a plate that was used to provide sub-irrigation. Each pot was filled with 12 kg of air-dried soil sieved

TABLE 1 Treatment description and contribution of nutrients from organic and mineral fractions.

Treatments	OMF details			Organic matrix C _{org}	Organic matrix N	Organic matrix P	Mineral N	Mineral P
	Organic material	Mineral fertilizers	C _{org} -N-P ₂ O ₅ concentration	mg pot ⁻¹	mg pot ⁻¹	mg pot ⁻¹	mg pot ⁻¹	mg pot ⁻¹
N ₀ P ₀	No organic material	No mineral fertilizers		0.00	0.00	0.00	0.00	0.00
MF _{NP}	No organic material	Ammonium sulfate; urea; and triple superphosphate	0-20-10	0.00	0.00	0.00	971	212
Peat ₂₀₋₁₀	Peat	Ammonium sulfate; urea; and diammonium phosphate	7.5-20-10	364	22	5	971	212
GC ₂₀₋₁₀	Green compost from pruning residues	Ammonium sulfate; urea; and diammonium phosphate	7.5-20-10	364	21	8	971	212
MSWC ₂₀₋₁₀	Municipal solid waste compost	Ammonium sulfate; urea; and diammonium phosphate	7.5-20-10	364	40	14	971	212
VC ₂₀₋₁₀	Manure-based vermicompost	Ammonium sulfate; urea; and diammonium phosphate	7.5-20-10	364	27	16	971	212
Peat ₁₀₋₅	Peat	Ammonium sulfate; urea; and diammonium phosphate	7.5-10-5	728	44	10	971	212
MSWC ₁₀₋₅	Municipal solid waste compost	Ammonium sulfate; urea; and diammonium phosphate	7.5-10-5	728	79	29	971	212

The OMF treatments had two different concentrations of C_{org}, mineral N, and mineral P₂O₅: (i) a concentration of 7.5% C_{org}, 20% N, and 10% P₂O₅ (OMF₂₀₋₁₀); and (ii) a concentration of 7.5% C_{org}, 10% N, and 5% P₂O₅ (OMF₁₀₋₅). Organic materials used to create OMF₂₀₋₁₀ were green compost from pruning residues (GC₂₀₋₁₀), municipal solid waste compost (MSWC₂₀₋₁₀), manure-based vermicompost (VC₂₀₋₁₀), and peat (Peat₂₀₋₁₀). For OMF₁₀₋₅, municipal solid waste compost (MSWC₁₀₋₅) and peat (Peat₁₀₋₅) served as the organic matrices. Each OMF was mixed with ammonium sulfate, urea, and diammonium phosphate to obtain the ideal C_{org}-N-P₂O₅ concentration. A mineral control (MF_{NP}) was used to replicate the mineral composition of OMF₂₀₋₁₀. Additionally, a control without fertilization was used (N₀P₀).

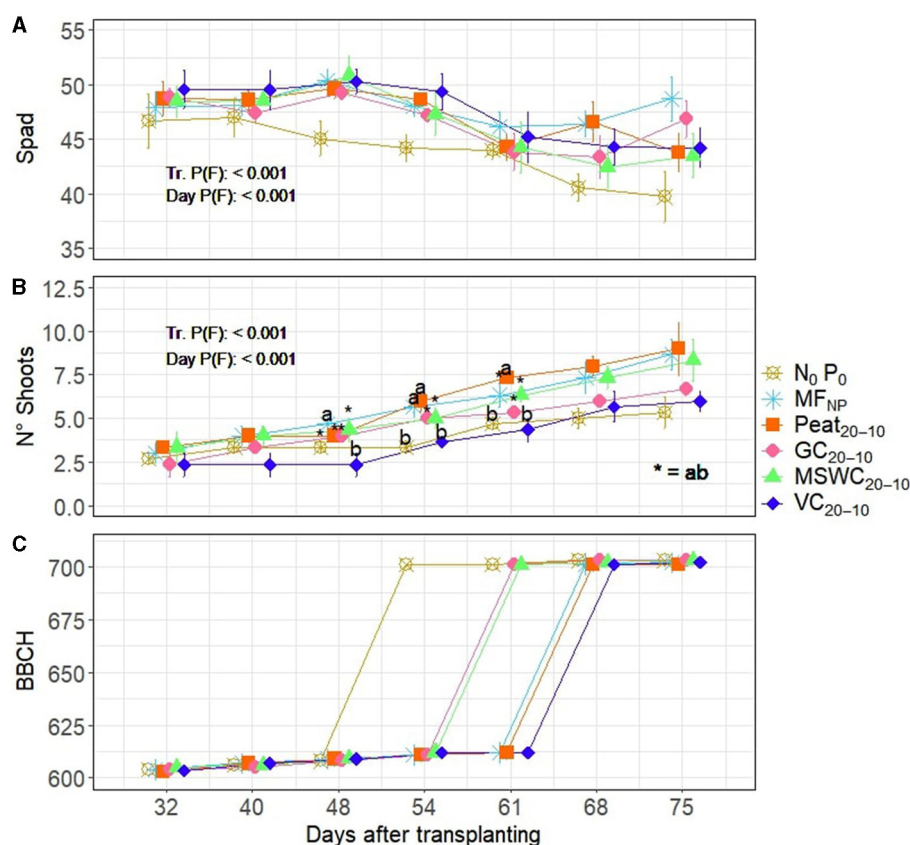


FIGURE 1

Growing parameters of tomatoes between weeks 5 and 11 for OMF_{20-10} , mineral control (MF_{NP}), and unfertilized control (N_0P_0). The parameters are average and standard error SPAD index (A), number of shoots (B), and median BBCH (C). Differences between treatment (Tr) and day are calculated as the average values of the multiple sampling events. The letters indicate differences between treatments during each specific sampling day. No differences were calculated for BBCH because the values are expressed on a scale with disjoint intervals.

to 5 mm at a density of 1.40 g cm^{-3} , resulting in a soil depth of 30 cm. The soil used in the experiment has a loam texture, a neutral pH in water (7.2), 8.4 g kg^{-1} organic C, and 1.1 g kg^{-1} total N. The total carbonate content was 4.4 g kg^{-1} . The exchangeable K was low (0.1 meq g^{-1}), while the exchangeable Ca and Mg were 7.1 and 0.9 meq g^{-1} , respectively, giving a low cation exchange capacity of 110. The Olsen P-value was 13.5 mg kg^{-1} .

To simulate fertilizer incorporation, fertilization was carried out by removing soil of $\sim 2 \text{ cm}$ depth, then dispersing the fertilizers homogeneously, and later covering again with the previously removed soil. Except for N_0P_0 , each treatment received 81 mg N kg^{-1} soil (equivalent to 170 kg N ha^{-1} for 15 cm soil depth) and 18 mg P kg^{-1} soil in pre-planting (Table 1). OMFs with a $C_{org}\text{-N-P}_2\text{O}_5$ concentration of 7.5-20-10 and 7.5-10-5 $\text{kg } 100 \text{ kg}^{-1}$ OMF received ~ 4.9 and $9.8 \text{ g OMF pot}^{-1}$, respectively. For mineral control, MF_{NP} , urea, and ammonium sulfate were combined so that 75% of the N was supplied in the form of ammonium and 25% in the form of urea to emulate the N forms of OMF. Supplemental fertilization was carried out using with potassium chloride (KCl 60%), adding 79 mg K kg^{-1} soil to all pots to avoid potential K deficiencies.

During the 1st month, pots were watered daily with 1 L of water and then with 2 L daily until harvesting. Water was added to the bottom plate to avoid nutrient leaching.

Tomato plants received lambda-cyhalothrin (KarateZeon[®], Seneffe, Belgium; 17th June), deltamethrin (Decis[®] Evo, Villefranche, France; 1st July), and copper oxide (Fenicrit[®], Bologna, Italy; 6th June and 4th July) as insecticides and fungicides.

2.3 Plant analyses on nutritional status and yield production

During the 2nd and 3rd months of tomato growth, phenology development, the number of stems, and leaf chlorophyll content were measured weekly. Phenological stage measurements were performed using the BBCH scale, focusing on the growth stage coding system for *Solanaceae*, provided by the Federal Biological Research Center for Agriculture and Forestry (Meier, 2001). The BBCH index was measured by considering the emergence of inflorescences, flowering, and fruit development, while the number of stems and fruits was counted manually. All shoots and branches with at least one visible node and a first genuine well-extended leaf were considered lateral stems. The measurements were taken on the same dates as the phenological stage survey. Leaf chlorophyll content was measured on a 6-mm^2 area using the SPAD-502 Portable Chlorophyll Meter (Konica Minolta Camera Co. Ltd.,

Tokyo, Japan). The tomato was harvested on 8th August, 75 days after the transplant. Tomato fruits were collected manually, and shoot mass was harvested at the soil level. The soil contained in the pot was divided into two sections (top and bottom) of 15 cm in height each, and from each section, roots were manually collected and hand-washed with the help of sieves. Dry matter (DM) biomass was measured separately for fruits, shoots, and top and bottom roots after drying samples at 60°C for 72 h. The total epigeal biomass (fruit and shoot mass) was ground to 0.5 mm. Total N concentration in tissue was measured in an elemental analyzer (Thermo Scientific FlashEA 1112, Thermoquest). Similarly, total P concentration in tissue samples was analyzed through digestion with sulfuric and perchloric acid, followed by a spectrophotometer analysis using green malachite (Ohno and Zibilske, 1991). The epigeal biomass N and P uptake was calculated as the product between the N and P concentration and the total epigeal biomass.

The N use efficiency (NUE) and the P use efficiency (PUE) were used as indicators for the fertilizer's efficiency. The nutrient use efficiency was calculated as follows:

$$NUE \text{ or } PUE \text{ (\%)} = \frac{N \text{ or } P \text{ uptake (g)} - N \text{ or } P \text{ uptake}_{N_0P_0} \text{ (g)}}{N \text{ or } P \text{ added with the fertilizer (g)}} * 100$$

2.4 Statistical analyses

Data analysis was performed using R statistical software (version 4.2.2). For each crop, two sets of comparisons were made: (a) treatments N₀P₀, MF_{NP}, GC₂₀₋₁₀, MSWC₂₀₋₁₀, Peat₂₀₋₁₀, and VC₂₀₋₁₀ were analyzed using treatments and block as independent values to determine differences between controls and organic materials in OMFs and (b) treatments MSWC₂₀₋₁₀, MSWC₁₀₋₅, Peat₂₀₋₁₀, and Peat₁₀₋₅ were analyzed using C_{org} to mineral fertilizer ratios and block as independent variables. Values were tested for normality and homogeneity and transformed into a log or square scale when needed. A general linear model (GLM) with two or three factors was performed to compare treatments. The SPAD index and the number of stems were analyzed, including the day after transplanting, as independent variables and also independently for each sampling date. In contrast, biomass production, nutrient uptake, and efficiency were analyzed only once.

3 Results and discussion

3.1 Plant growth parameters

The chlorophyll content in leaves was analyzed based on the SPAD index, which is associated with the N content in tomato leaves (Wood et al., 1993) and fruits (Jiang et al., 2017). The SPAD index decreased by an average of 8% between 23rd June and 4th August (Figure 1A), indicating a lower chlorophyll concentration over time. Fertilized treatments had a significantly higher SPAD index overall than the

TABLE 2 Dry matter (DM) production for different crop sections.

Measurement	Unit	N ₀ P ₀	MF _{NP}	Peat ₂₀₋₁₀	GC ₂₀₋₁₀	MSWC ₂₀₋₁₀	VC ₂₀₋₁₀	Peat ₁₀₋₅	MSWC ₁₀₋₅
Top root DM (0-15 cm)	g DM pot ⁻¹	3.5 ± 0.6 ^b	5.4 ± 0.6 ^{ab}	7.2 ± 0.9 ^a	5.7 ± 0.5 ^a	5.9 ± 0.5 ^a	5.7 ± 0.7 ^a	5.3 ± 0.1	5.8 ± 0.3
		Tr. P (F): <0.01.						Tr. P (F); ns; C _{org} P (F): ns.	
Bottom root DM (15-30 cm)	g DM pot ⁻¹	0.7 ± 0.2	0.9 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	1.1 ± 0.0
		Tr. P (F); ns.						Tr. P (F); ns; C _{org} P (F): ns.	
Shoot dry matter	g DM pot ⁻¹	30.6 ± 2.4 ^c	54.5 ± 3.9 ^{ab}	64.7 ± 3.6 ^a	48.7 ± 5.7 ^b	51.4 ± 2.0 ^{ab}	51.3 ± 0.8 ^{ab}	52.8 ± 4.3	53.6 ± 4.9
		Tr. P (F): <0.01.						Tr. P (F); ns; C _{org} P (F): ns.	
Fruit dry matter	g DM pot ⁻¹	22.2 ± 1.5	23.0 ± 1.3	20.2 ± 0.5	19.7 ± 0.6	19.1 ± 1.1	22.6 ± 2.0	17.9 ± 1.1	17.9 ± 0.4
		Tr. P (F); ns.						Tr. P (F); ns; C _{org} P (F): ns.	

Treatments with a C_{org}-N-P₂O₅ content of 7.5-20-10, mineral control (MF_{NP}), and unfertilized control (N₀P₀) are compared by treatment (Tr) and block, and their differences are shown in lowercase letters. The block effect was always non-significant. Treatments with two different C_{org}-N-P₂O₅ concentrations (7.5-20-10 vs. 7.5-10-5) are compared by treatment (Tr), block, and mineral content (Min). The treatment effect was always not significant, ns, not significant.

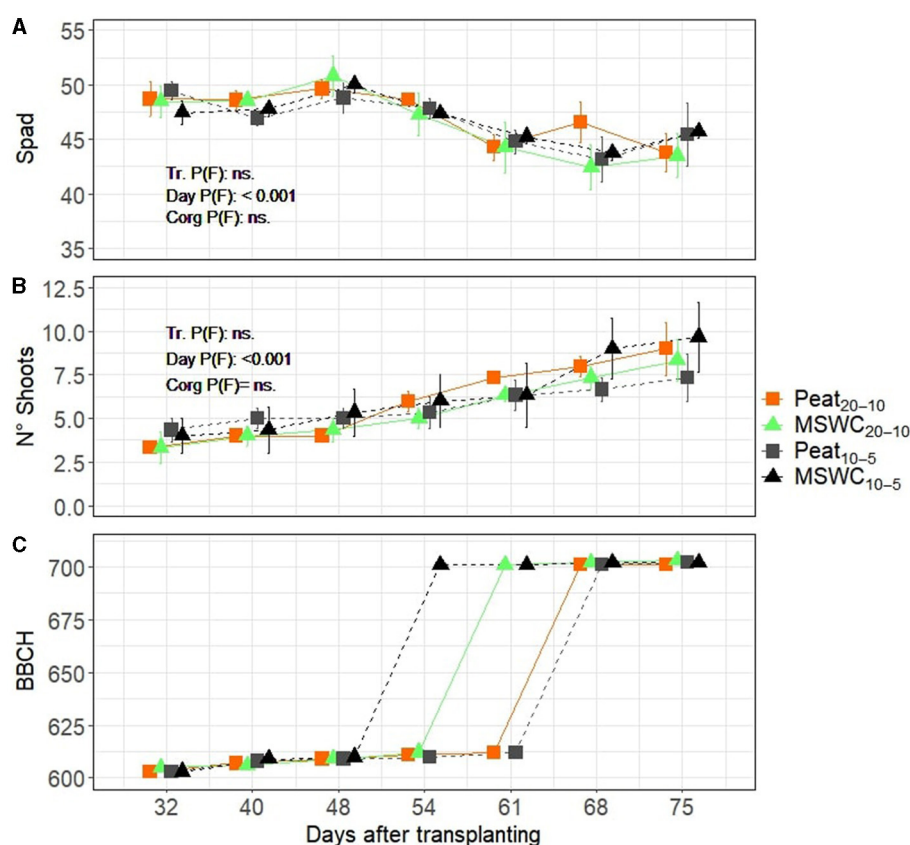


FIGURE 2

Growing parameters of tomatoes between weeks 5 and 11 for OMF₁₀₋₅ and OMF₂₀₋₁₀. Treatments Peat₂₀₋₁₀ and MSWC₂₀₋₁₀ are the same as those shown in Figure 1. Parameters are SPAD index (A), number of shoots (B), and BBCH (C). Differences between treatment (Tr) and day are calculated as the average values of the multiple sampling events. The letters above indicate differences between treatments during each specific sampling day. No differences were calculated for BBCH because the values are expressed on a scale with disjoint intervals.

control N₀P₀. However, when the data were analyzed for each day separately, there were no significant differences between treatments.

The chlorophyll content in leaves indicates no differences in N availability in soil between OMFs and mineral control, contradicting other reports that observed a slower N release into the N solution (Richards et al., 1993; Antille et al., 2013a, 2014; Florio et al., 2016). However, in previous reports from our study, we did not find significant differences in N release or NUE between OMFs and mineral controls in short-term ryegrass or maize pot experiments (Sitzmann et al., 2024a,b), where the interactions of the OMF organic fraction were mostly with P.

Morphological parameters such as the number of stems, or branch number, in tomatoes can be used as a reference for potential yield, as more stems can lead to increased branching and potentially more fruit-bearing sites, influencing overall yield and plant health (Ece and Darakci, 2009). In general, the number of shoots was significantly higher in Peat₂₀₋₁₀, the mineral control MF_{NP}, and MSWC₂₀₋₁₀ than in the N₀P₀ control and VC₂₀₋₁₀, with intermediate values for GC₂₀₋₁₀ (Figure 1B). Differences in the number of shoots occurred between the third,

fourth, and fifth sampling dates, and the trend followed was higher values for Peat₂₀₋₁₀ and MF_{NP} and lower values for N₀P₀ and VC.

The earlier entrance into the reproductive stage on week 7 for N₀P₀ was expected compared to the OMFs and the mineral control MF_{NP} (Figure 1C), as an earlier entrance to a reproductive phenological stage can indicate a nutritional deficiency in plants (Gaj et al., 2020). Peat₂₀₋₁₀, MSWC₂₀₋₁₀, and VC₂₀₋₁₀ had the latest entrance to the reproductive stage (week 9). The results for VC₂₀₋₁₀ are opposite to the number of shoots; however, as with Peat₂₀₋₁₀ and MSWC₂₀₋₁₀, those treatments are correlated to a higher P uptake. In the formerly mentioned study, Sitzmann et al. (2024a) found that peat had the same initial P availability as the mineral control. At the same time, a vermicompost OMF—as well as the other biowaste materials—immobilized a fraction of the mineral P. This initial lower P availability could explain why VC₂₀₋₁₀ initially had fewer stems than Peat₂₀₋₁₀, limiting their counting for the rest of the experiment.

The hypothesis of differences in growing parameters between OMFs and mineral control has not been confirmed. However, the results show that peat OMFs tend to outperform biowaste OMFs in

TABLE 3 Shoot plus fruit N and P plant concentration, uptake, and efficiencies (NUE and PUE, respectively).

Measurement	Unit	N ₀ P ₀	MF _{NP}	Peat _{20–10}	GC _{20–10}	MSWC _{20–10}	VC _{20–10}	Peat _{10–5}	MSWC _{10–5}
N concentration	mg N g ^{−1} plant	13.7 ± 0.8 ^b	16.5 ± 0.3 ^{ab}	14.6 ± 0.6 ^{ab}	17.3 ± 1.2 ^a	14.3 ± 0.5 ^{ab}	15.5 ± 0.1 ^{ab}	16.5 ± 0.6	15.6 ± 1.1
		Tr. P (F): <0.05.						Tr. P (F): ns; C _{org} P (F): ns.	
P concentration	mg P g ^{−1} plant	2.5 ± 0.0 ^a	2.0 ± 0.0 ^b	2.3 ± 0.2 ^{ab}	2.1 ± 0.1 ^{ab}	2.0 ± 0.1 ^b	2.3 ± 0.1 ^{ab}	2.1 ± 0.1	2.1 ± 0.0
		Tr. P (F): <0.05.						Tr. P (F): ns; C _{org} P (F): ns.	
N uptake	g N kg ^{−1} soil	34.7 ± 1.4 ^b	74.7 ± 5.0 ^a	79.3 ± 7.3 ^a	69.1 ± 4.4 ^a	61.2 ± 1.2 ^a	66.2 ± 0.8 ^a	72.8 ± 8.2	69.0 ± 5.4
		Tr. P (F): <0.001.						Tr. P (F): ns; C _{org} P (F): ns.	
P uptake	g P kg ^{−1} soil	6.4 ± 0.4 ^c	9.2 ± 0.6 ^{ab}	12.6 ± 1.1 ^a	8.5 ± 0.8 ^{bc}	8.7 ± 0.7 ^{abc}	9.7 ± 0.4 ^{ab}	9.2 ± 0.5	9.2 ± 0.9
		Tr. P (F): <0.01.						Tr. P (F): ns; C _{org} P (F): ns.	
NUE	%		52.6 ± 6.2	58.2 ± 9.0	45.7 ± 5.4	35.8 ± 1.5	42.1 ± 1.0	50.2 ± 10.1	45.6 ± 6.6
		Tr. P (F): ns.						Tr. P (F): ns; C _{org} P (F): ns.	
PUE	%		18.6 ± 3.4 ^{ab}	37.1 ± 5.8 ^a	14.4 ± 4.2 ^b	15.9 ± 4.0 ^{ab}	21.1 ± 1.9 ^{ab}	18.4 ± 2.9	18.6 ± 5.0
		Tr. P (F): <0.05.						Tr. P (F): ns; C _{org} P (F): ns.	

Treatments with a C_{org}-N-P₂O₅ content of 7.5-20-10, mineral control (MF_{NP}), and unfertilized control (N₀P₀) are compared by treatment (Tr) and block, and their differences are shown in lowercase letters. The block effect was always non-significant. Treatments with two different C_{org}-N-P₂O₅ concentrations (7.5-20-10 vs. 7.5-10-5) are compared by treatment (Tr), block, and mineral content (Min). The treatment effect was always not significant. ns, not significant.

terms of early growth, but no significant differences were observed after 75 days.

3.2 Nutrient use efficiency of OMFs

Adding mineral fertilizers alone or through OMFs increased the dry matter production in the shoots and top roots of the tomato plants compared to the control N₀P₀ (Table 2). Differences between treatments were observed only in the first 15 cm of the soil, which had, on average, six times higher root dry matter than the bottom 15 cm; the root DM was significantly higher in fertilized treatments than in the control N₀P₀. The shoot DM was significantly higher in fertilized treatments than in the N₀P₀ control; among the fertilized treatments, GC_{20–10} had a considerably lower DM than Peat_{20–10}, with other fertilized treatments being between these two. The highest total—berries, shoots, and roots—DM production was found in Peat_{20–10} and the mineral control, while GC_{20–10}, MSWC_{20–10}, and VC_{20–10} had intermediate values between the mineral control and N₀P₀.

Total plant N uptake at the end of the growing season was correlated to the SPAD index measured on the harvest day by a Pearson value of $r = 0.5$ (p -value < 0.01), as all fertilized treatments were significantly higher than the control N₀P₀ (Table 3). OMFs did not present significant differences in NUE compared to the mineral control MF_{NP}.

As mentioned above, biowaste OMFs made with the same materials as the one used in this experiment had a lower content of plant-available P in the soil after 10 days of incubation than a peat OMF and mineral control, which caused a lower P uptake in maize plants after 30 days (Sitzmann et al., 2024a). The lower P availability caused by the biowaste OMF partially remained after 75 days, especially for GC_{20–10}, which demonstrated a lower P uptake and PUE than Peat_{20–10} at harvest (Table 3). Other biowaste materials mitigated the immobilization effects, showing intermediate values for P uptake, and did not differ from Peat_{20–10}. The organic material influence on the P availability of low-C_{org} OMFs has been associated with chemical interactions between both fractions rather than microbial immobilization (Sitzmann et al., 2024a). The Ca-P bonds in the OMF and the organic matrix pH have a higher influence on reducing P availability. However, as OMFs contained large fractions of ammonium sulfate and urea, it was expected that the nitrification process would reduce the pH in the fertilizer hotspot (Bouman et al., 1995; Dal Molin et al., 2020), and therefore, it could increase the P availability. However, this effect was not observed. The OMF granules were not concentrated in one unique hotspot, and hence, the buffer capacity of the soil, one with a high pH, could have readily adsorbed the H ions released during the nitrification, thereby reducing the impact of potential soil acidification on P availability.

Our second hypothesis considered that, despite differences during plant growth, after 75 days, tomato plants would have the same yield and nutrient use efficiency across OMF treatments. The second hypothesis has been confirmed for MSWC_{20–10} and VC_{20–10} but not for GC_{20–10}.

3.3 Effects of C_{org} and mineral fertilizer ratios in OMFs

Differences in the mineral composition of the OMFs were expected to cause differences in N uptake between treatments. However, no differences were observed between OMF_{20–10} and OMF_{10–5} during the growth (Figure 2) and final yield (Table 2) of tomatoes. To fertilize at a ratio of 81 mg N kg^{−1}, OMF_{10–5} treatments used twice the amount of organic material. During the fertilization process, adding supplementary organic material will result in an increase of 4% N and 6% P for MSWC_{10–5} compared to MSWC_{20–10}. Furthermore, there will be an increase of 2% N and 2% P for Peat_{10–5} compared to Peat_{20–10} (Table 1). In addition, as urea is a richer source of N than ammonium sulfate, it will be in higher proportion in OMFs with 20% N (OMF_{20–10}). It can be considered that the OMF_{10–5} treatment, which contains a higher content of ammonium sulfate, received an additional S fertilization because the soil used in the experiment did not receive a supplementary S fertilization. A synergism between S and N was reported as S fertilization increases the NUE by promoting a higher N uptake (Jackson, 2000; Salvagiotti et al., 2009; Rietra et al., 2017).

However, a higher ratio of C_{org} to mineral nutrients could have promoted a higher degree of immobilization in soil, which would reduce the benefits of the additional organic N, humic C, and mineral S fertilization. Based on the characterization of the organic fraction discussed in the study conducted by Sitzmann et al. (2023), the immobilization would be more relevant for P than for N since pH and Ca values of MSWC (9.0, 47 mg g^{−1} DM) and peat (8.0, 41 mg g^{−1} DM) can enhance Ca-P bonds between the organic fraction and the mineral fertilizer and between the mineral fertilizer and the surrounding soil (Sica et al., 2023). The low soil *P*-values at the beginning of the experiment, associated with a lower release in OMF_{10–5} treatments, can be the reason for the lack of differences between the treatments, as P resulted in a limiting nutrient for a higher N and S uptake (Robertson and Vitousek, 2009; Vitousek et al., 2010).

Although the results showed no differences between using different concentrations of mineral fertilizers in OMFs, the results were inconclusive. Therefore, it is not possible to accept or reject the original hypothesis.

4 Conclusion

This study investigated the influence of organic materials in the impact of OMFs on nutrient availability and the performance of tomato plants. Materials such as vermicompost (VC_{20–10}) and municipal solid waste compost (MSWC_{20–10}) had a similar final PUE compared to that of a peat OMF (Peat_{20–10}) and a mineral control (MF_{NP}), suggesting that these materials could be used to replace peat in OMFs designed to be used for crops with medium to long growing cycles. Green compost (GC_{20–10}) had a lower PUE and overall growing parameters compared to Peat_{20–10}, indicating that not all organic materials are suitable to replace peat, even when the OMFs are used in crops that grow over several months.

The lack of differences between treatments with different mineral concentration implies that, in low- C_{org} OMFs, the organic fraction has a limited effect on performance, allowing flexibility

in adjusting the mineral fraction based on specific market requirements. Although the OMF used in this study, with its low- C_{org} content, supplies low amounts of organic C to the soil through fertilization, lower concentrations of the mineral component imply that more significant amounts of the organic materials are distributed for the same fertilization target. Therefore, this type of OMF could contribute to recycling biowaste materials.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

Author contributions

TS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. AA: Investigation, Methodology, Writing – review & editing. CL: Methodology, Writing – review & editing. BM: Conceptualization, Investigation, Methodology, Writing – review & editing. LZ: Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. CG: Funding acquisition, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This project has received funding from the European Union's Horizon 2020 Research and Innovation Program under the Marie Skłodowska-Curie Grant (Agreement No. 860127).

Acknowledgments

We appreciate the support of Stefano Tagliavini from SCAM S.p.A. for manufacturing the OMFs used in this experiment.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor RT declared a past co-authorship with the author LZ.

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OPEN ACCESS

EDITED BY

Fiona Nicholson,
Agricultural Development Advisory Service,
United Kingdom

REVIEWED BY

Rafael Clemente,
CEBAS-CSIC, Spain
Victor Riau,
Institute of Agrifood Research and
Technology (IRTA), Spain

*CORRESPONDENCE

Alberto Finzi
✉ alberto.finzi@unimi.it

RECEIVED 25 March 2024

ACCEPTED 03 July 2024

PUBLISHED 19 July 2024

CITATION

Finzi A, Ferrari O, Riva E and Provolo G (2024)
Nitrogen recovery from intensive livestock
farms using a simplified ammonia stripping
process.
Front. Sustain. Food Syst. 8:1406962.
doi: 10.3389/fsufs.2024.1406962

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Nitrogen recovery from intensive livestock farms using a simplified ammonia stripping process

Alberto Finzi *, Omar Ferrari, Elisabetta Riva and
Giorgio Provolo

Department of Agricultural and Environmental Sciences, University of Milan, Milan, Italy

The ammonia (NH₃) stripping process can recover nitrogen (N) from slurry and digestates as a mineral fertilizer, but it is currently expensive and difficult to manage at the farm level. Hence, a simple process is required. This study aimed to test a modular (based on farm N surplus) slow-release NH₃ stripping process at a pilot plant scale. NH₃ volatilization was promoted in a closed reactor, and then, the NH₃ was removed by an air stream through the reactor headspace. The NH₃-loaded air was purified in a scrubber, where NH₃ reacted with sulfuric acid to form ammonium sulfate (AS). In total, 11 trials were conducted using pig slurry, dairy cattle slurry, and digestates: 7 trials were carried out with the reactors heated to 40°C, 2 trials were carried out at an ambient temperature, and the other 2 trials were carried out with the addition of sodium hydroxide (NaOH). To assess the technical-economic sustainability of the pilot plant, the total ammoniacal nitrogen (TAN) removal rate, electricity consumption, acid requirements, and AS quality and costs were evaluated. The pilot plant yielded TAN recovery amounts of 45% in 2 weeks with the reactors heated to 40°C, 64% in 1 week with NaOH addition, and 25% in 2 weeks at an ambient temperature. The N concentration in the AS solution reached 85.9 g kg⁻¹, with an average value of 35.2 g kg⁻¹. The electricity consumption, acid requirement, and operational costs in an optimized system were approximately 0.52 kWh kgN⁻¹ recovered, 3.5 kg pure acid kgN⁻¹ recovered, and 0.86 € kgN⁻¹ recovered, respectively. Compared to other technologies, the simplified stripping process is slower but with similar removal efficiencies and lower energy consumption. Thus, this finding could be suitable for improving the N use in intensive livestock farms.

KEYWORDS

digestate, pig and cattle slurry, nitrogen recovery, ammonium sulfate, pilot plant

1 Introduction

In a well-balanced system where nutrient inputs and crop requirements offset each other, livestock manure and digestates are valuable sources of crop nutrients. However, in regions with highly intensive livestock farming, there are high nitrogen (N) loads compared with the available land for proper livestock manure application in accordance with the Nitrates Directive (91/676/EEC) (Velthof et al., 2014). The increased livestock density has resulted in an excess of plant nutrients, particularly N, in European livestock farms. These surpluses pose environmental risks, such as ammonia (NH₃) volatilization and nitrate (NO₃⁻) leaching, leading to the potential eutrophication of ecosystems (Sommer, 2013).

To reduce these pollution phenomena, it is necessary to adopt treatment systems that reduce the N load of the effluents (EC DGE, 2016). In particular, there is an increasing need to reduce excess N in critical areas without eliminating N but by recovering it in a more easily manageable form within the same farm or in a form that is transportable to other areas (Huygens et al., 2020).

Nutrient recovery from slurry and digestates has been deemed feasible, with membrane technologies, struvite precipitation, and NH_3 stripping as the most extensively studied processes (Rizzioli et al., 2023). Pressure-driven membrane technologies, including ultrafiltration and reverse osmosis, are gaining significance as crucial processes for nutrient recovery. Despite their non-selective nature, the primary limitation of these membrane processes is membrane fouling. This fouling hinders their widespread implementation on a large scale because of the challenges associated with effective management. Struvite precipitation is a method that enables the recovery of phosphorus and NH_3 in the form of a magnesium (Mg) ammonium (NH_4^+) phosphate (PO_4^{3-}) hexahydrate ($6\text{H}_2\text{O}$) mineral. This process is primarily used in the municipal sector, particularly for treating anaerobic sewage sludge digestates. However, its application in the agricultural sector is relatively infrequent, as outlined by Rizzioli et al. (2023). NH_3 stripping is the most developed technology in the European Union (EU) context, as noted by Rizzioli et al. (2023). It allows the removal of ammonia nitrogen (N-NH_3) from the slurry by retaining it in an acid solution, usually sulfuric acid (H_2SO_4), to form ammonium sulfate (AS) (Vaneekhaute et al., 2017; Pandey and Chen, 2021). Ammonia stripping is recognized as an effective technique, with N removal rates of up to 90–95% after treatment for a few hours (Flotats et al., 2011; Vaneekhaute et al., 2017). This process is usually performed in packed-bed stripping towers to increase the available area for mass transfer, requiring a high removal rate of the solids in the slurry to avoid stripping column clogging, as well as the high demand for heat or chemical additives (Provolo et al., 2017; Pandey and Chen, 2021; Rizzioli et al., 2023).

Despite its applications in waste treatment plants (Brienza et al., 2021; Pigoli et al., 2021), the diffusion of NH_3 stripping in livestock farms is still very limited (Rizzioli et al., 2023).

The driving forces of the NH_3 stripping process are those that favor the volatilization of NH_3 from the slurry, such as high temperature, pH, and airflow velocity, all of which are required to remove the released NH_3 from the slurry (Flotats et al., 2011).

However, the electricity required to produce 1 kg of N as AS by treating digestates and slurry with stripping processes (at ambient temperature or recovering heat surplus from other processes, i.e., combined heat and power (CHP) engine of a biogas plant) is approximately 3.8–12 kWh_{el} (Brienza et al., 2021, 2023). In comparison, the Haber–Bosch optimized process requires 9.5 kWh_{el} to produce 1 kg of N as chemical fertilizer (Smith et al., 2020). Therefore, an optimized and/or simplified NH_3 stripping process is required to produce renewable fertilizers more sustainably from digestates and slurry (Brienza et al., 2021; Vingerhoets et al., 2023).

The slow-release stripping system can be a valid solution to simplify the treatment, making it more easily applicable to livestock farms (Provolo et al., 2017; Heidarzadeh Vazifehkhora et al., 2022). This system requires that volatilized NH_3 is removed by an air stream through the headspace of the reactor without using a stripping tower. Thus, a reduced need for strong solid–liquid separation of the initial

slurry/digestates and reduced risks of clogging and foam formation is observed. In addition, slow-release stripping can work at a process temperature of approximately 40°C, which can be maintained by taking advantage of the heat produced by a CHP engine in anaerobic digestion plants that are often dissipated to the environment. Furthermore, slow-release stripping takes advantage of the natural increase in pH taking place due to the fast carbon dioxide (CO_2) volatilization (Hafner et al., 2012).

In the laboratory-scale trials conducted by Provolo et al. (2017) and Heidarzadeh Vazifehkhora et al. (2022), an NH_3 removal efficiency of 70–80% was obtained at 40°C after approximately 2 weeks of treatment. Ammonia removal efficiencies close to 90% were achieved by extending the treatment to 3 weeks (Heidarzadeh Vazifehkhora et al., 2022) or adjusting the pH to 9 using sodium hydroxide (Provolo et al., 2017).

The strength of the NH_3 stripping process is the production of AS, a mineral fertilizer regarded as a renewable substitute for synthetic fertilizers (Vaneekhaute et al., 2013; Sigurnjak et al., 2016). AS has a higher N content compared to slurry or digestates: 4–7.4% (Pigoli et al., 2021) vs. 0.13–0.93%, for pig slurry and digestate, respectively (Finzi et al., 2015). The same amount of N is therefore contained in a lower volume, reducing the transport costs for AS.

The implementation of the slow-release NH_3 stripping process on a farm scale will have important effects on the nutrient management of livestock slurries, which is in line with the objectives of the European Commission's Farm to Fork strategy (reduce nutrient losses by at least 50% by 2030), and specifically with the principle of recycling of organic waste into renewable fertilizers.

To test the performance of the slow-release NH_3 stripping process on a farm, the laboratory prototype tested by Provolo et al. (2017) and Heidarzadeh Vazifehkhora et al. (2022) was upscaled to a farm-scale pilot plant with a reactor capacity of 30 m^3 . In this pilot plant, trials were conducted using different slurries (pig, cattle, and digestates) to assess the process performance by evaluating the amount of N recovered as mineral fertilizer, i.e., AS; the quality of the AS obtained in terms of N content; and the energy and (H_2SO_4) requirements of the process. A technical–economic sustainability assessment was also included.

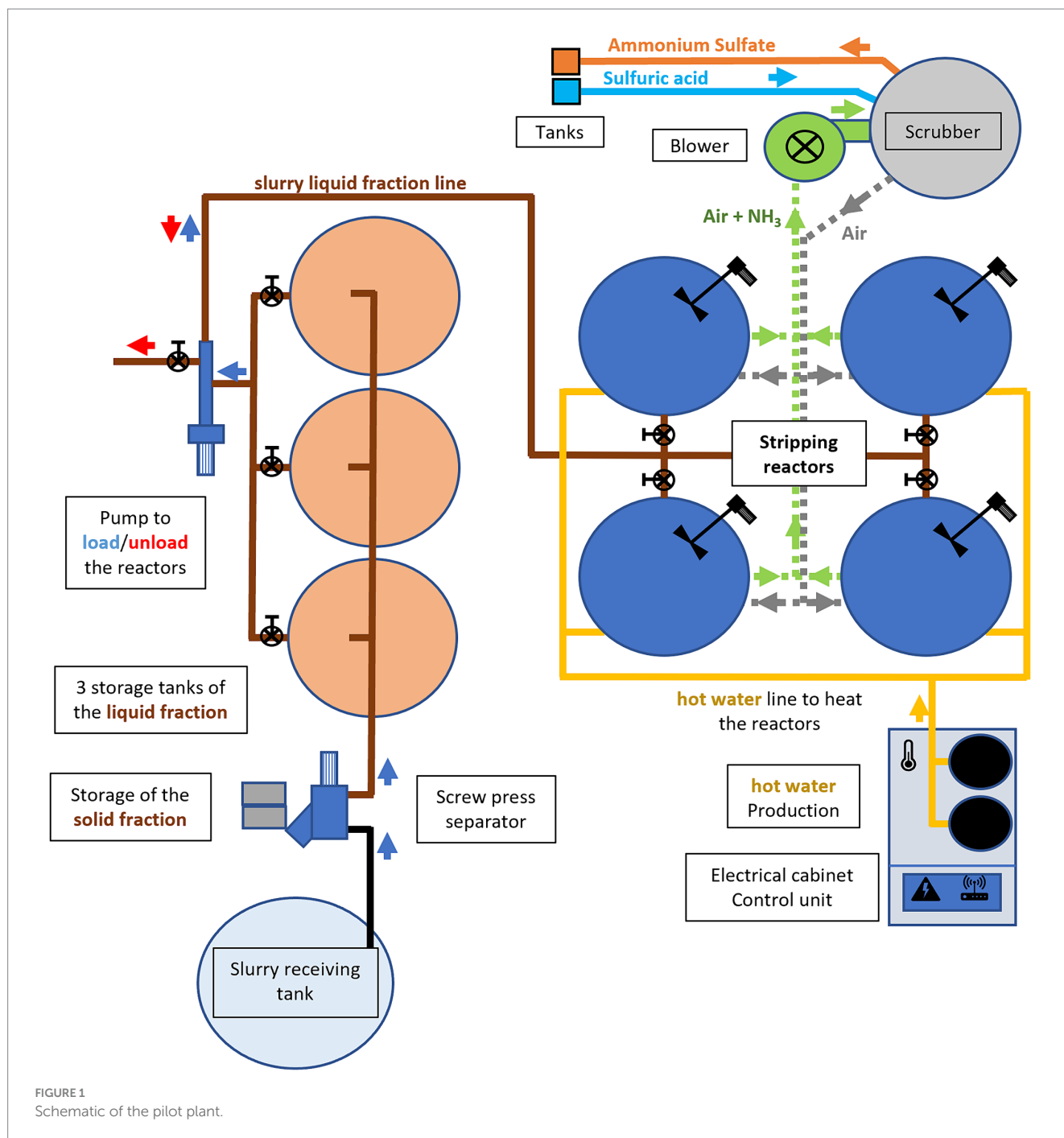
2 Materials and methods

2.1 Pilot plant description and experimental plan

2.1.1 Pilot plant description

Pilot plant functioning was based on the slow-release NH_3 stripping process conducted under batch conditions. In this process, the recovery of NH_3 from the slurry or digestates occurred in a reactor, where air was introduced into the headspace. The airflow served a dual purpose: to encourage the release of NH_3 from the surface of the slurry or digestates and to carry it away. The NH_3 -laden airflow was then directed into a wet scrubber, where NH_3 reacted with a (H_2SO_4) solution, resulting in the formation of AS.

The pilot plant (Figure 1) had a 30- m^3 steel tank to receive the input slurry and digestates to be treated, from which a centrifugal pump (7.5 kW) sent the input slurry/digestates to a screw-press solid–liquid separator (SEPCOM Biogas Vertical—WAM Italia



S.p.A, 4kW, screen 0.25 mm) to remove coarse solids. The liquid fraction was collected in three 7.5-m³ high-density polyethylene (HDPE) tanks equipped with a radar sensor for electronic level measurement (VEGAPULS 31, Vega). A progressive cavity pump (C.M.O. Pompe s.n.c., 1.8kW) loaded and emptied the liquid fraction into four 7.5-m³ HDPE stripping reactors. Each reactor was equipped with the following: a heating system using hot water circulated in a pipe exchanger inside the tank, a mechanical mixing system using an electric motor with a vertical paddle mixer (100 rpm), a temperature and pH sensor (B&C electronics PH 4326; probe B&C electronics SZ165), a slurry level sensor (VEGAPULS 31, Vega), and an effluent sampling valve. The hot water, which is

used to heat the reactors, was produced by an electric boiler (36 kW) that simulates the available heat from a biogas plant. The reactors were half-filled at a volume of 3.75 m³, and the airflow necessary for NH₃ recovery was ensured by a blower (Project Plast, Cremona, Italy, 2.2 kW, 2000 m³ h⁻¹) that operated in a closed circuit and connected the reactors and scrubbers. The scrubber had a tank containing an acid solution for cleaning NH₃-laden air from the reactors. As the acid was gradually neutralized to form AS, it became necessary to add more acid. This was done when the solution reached a pH of 1.5, as suggested by [Sigurnjak et al. \(2019\)](#). The pilot plant was monitored and controlled using a programmable logic controller (PLC), which could also be remotely accessed.

TABLE 1 Experimental conditions of the trials performed. sh = sodium hydroxide, NaOH 30% w/w; at = ambient temperature.

Trials	Reactors heated to 40°C							Ambient temperature		NaOH addition	
	Pig1	Pig2	Pig3	Dig1	Dig2	Cattle1	Cattle2	Pig4. at	Cattle3. at	Pig5. sh	Cattle4. sh
Starting day	May 30th	Sept. 30th	Oct. 28th	June 15th	Sept. 30th	Aug. 22nd	Oct. 25th	June 14th	July 7th	June 14th	July 7th
Ambient temperature (°C)	23.2	17.3	10.7	27.2	17	23.2	11.4	25.6	26.1	25.6	26.1
Reactor temperature (°C)	36.8	37.1	37.9	40.8	36.2	39.1	36.1	29.3	29.8	29.9	30.3
Treated volume (m ³)	15.01	7.67	7.67	15.12	7.75	7.64	6.38	7.64	7.64	7.90	7.90
Additive addition	-	-	-	-	-	-	-	-	-	NaOH	NaOH
Duration (d)	15	28	28	32	17	24	31	15	21	15	21

2.1.2 Experimental plan

The experimental trials were conducted in the pilot plant under batch conditions (Table 1). Seven trials (i.e., Pig1, Pig2, Pig3, Dig1, Dig2, Cattle1, and Cattle2) were conducted by heating the stripping reactors at 40°C. Considering that an electric boiler was used for heating the reactors, an upscaling of the pilot plant is feasible only with a surplus heat to be valorized (i.e., from a CHP of a biogas plant). Otherwise, it is advisable to conduct the treatment at ambient temperature or with the addition of additives. For this reason, trials Pig4.at and Cattle3.at were conducted at ambient temperature, and trials Pig5.sh and Cattle4.sh were conducted at ambient temperature (at) with the addition of sodium hydroxide (i.e., sh; NaOH 30% w/w). NaOH 30% w/w was added to the reactor before starting the trial to increase the pH. It was necessary to add 30.4 kg and 59.4 kg of a previously prepared solution of NaOH 30% w/w per cubic meter of slurry to raise the pH to 10.7 for cattle slurry (Cattle4.sh) and pig slurry (Pig5.sh), respectively.

The trials were monitored using the following procedure:

- During the trials, every 5 min, the PLC recorded the pH, temperature, and level values of the reactors and the pH of the scrubber.
- The sampling of the slurry/digestates of each reactor and the AS solution in the scrubber was conducted at the start of the trial, during the trial every 72–96 h, and at the end of the trial.

Furthermore, during each sampling, the level of the AS solution inside the scrubber tank was manually measured and recorded. When the safety level for AS was exceeded, the AS solution was unloaded by a dosing pump (model BT-MA/AD, Etatron D.S., Rome – Italy), and if necessary, more acid (H₂SO₄ at 50% w/w) was added to the scrubber, by a dosing pump (model Tekna Evo AKL 803, SEKO, Rieti – Italy). Each acid/AS load/unload was measured and recorded by the operator.

- At each sampling, electricity consumption was recorded from the plant's electricity meter.

A total of 196 samples were collected during the trials. The samples were stored at +4°C and analyzed within 24 h, to determine the content of total solids (TS), volatile solids (VS), ash, total Kjeldahl nitrogen (TKN), total ammoniacal nitrogen (TAN), pH, EC, phosphorus (P), and potassium (K). In particular, P and K were

evaluated using the rapid tests NANOCOLOR 0–55 (REF 985055) and NANOCOLOR 0–45 (REF 985045), respectively. All parameters were analyzed using standard methods (APHA/AWWA/WEF, 2012). Otherwise, organic N was derived by subtracting the TAN content from the TKN content.

The concentrations of the major elements (Na, Mg, Ca, K, and P) and trace elements (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, and Pb) in the AS solution were analyzed by inductively coupled plasma mass spectrometry (EPA, 2007; APHA/AWWA/WEF, 2012) using an ICP-MS (Bruker Aurora-M90).

2.2 Data analysis

The results obtained from the tests conducted in the pilot plant allowed us to evaluate its performance under different conditions, in terms of both the type of slurry treated (different animal species and farms; chemical and physical compositions) and the test conditions (warm and cold seasons, treatment duration). All the trials have been performed at least in duplicate, but a statistical analysis to compare the different trials has not been reported due to the mentioned different conditions. However, an in-depth evaluation of how such plants would work in practice has been performed.

2.2.1 Nitrogen removal efficiencies and mineral fertilizer production

Using both the results of the analyses on the samples and the data recorded during monitoring, the performance of the pilot plant was evaluated in terms of the TAN removal rate, TKN removal rate, and slurry volume reduction during the stripping treatment.

To determine the percentage of removal efficiencies achieved by the pilot plant, the difference between the effluent and influent masses of the above-mentioned parameters was considered.

The technical performances of the process were assessed by interpolating the TAN removal efficiency achieved in the pilot plant trials with heated reactors, to define a regression model over time considering the results of all trials performed. For the trials conducted at ambient temperature or with NaOH addition, the TAN removal efficiency was assessed by averaging the values obtained from the trials. The electricity consumption, acid requirement, and NaOH addition (when used) were then evaluated to determine the operational costs of the process and its economic sustainability.

Furthermore, the AS solution was evaluated in terms of quantity by assessing its N concentration and the mass of AS produced.

2.2.2 Technical–economic assessment

The electricity consumption recorded in each trial included the pretreatment of solid–liquid separation; reactor loading and unloading; reactor mixing, heating, and aeration; scrubber functioning; and PLC. Considering that electricity was used in the pilot plant to heat the reactors, the electricity consumption and thermal energy consumption have been analyzed separately. The H₂SO₄ requirement was the amount introduced into the scrubber during each trial, whereas the NaOH requirement was the amount added to the slurry or digestates in the reactors. The operational costs related to electricity consumption were 0.2297 € kWh^{−1} as determined from the annual report referred to in 2021 by the Italian Regulatory Authority for Energy, Networks, and Environment (ARERA). In the trials with reactors heated to 40°C, the costs do not include the electricity consumption resulting from heating, considering that an upscaling of the pilot plant is feasible only with a surplus heat to be valorized. The cost of pure H₂SO₄ and NaOH 30% w/w were, respectively, 0.21 and 0.21 € kg^{−1} (Vingerhoets et al., 2023). Consumption and costs were expressed per unit of N removed in each trial.

In addition to the treatment costs, it is worth noting that the AS solution produced has an economic value as a mineral fertilizer. Because there is no market for this product, the value of AS was considered a cost avoided in the purchase of mineral fertilizers based on the price of 1 kg of N as urea (46% N), with reference to the average market price in Europe in spring 2023 (March, April, and May) equal to 495 €/t, i.e., 1.08 € kgN^{−1} (WUR, 2024).

2.3 Pilot plant performance assessment

Based on the performance achieved in the pilot plant, a generalized mass and N balance was defined, considering the likely operating conditions of the upscaled process at full scale. The mass balance was based on the following 5 conditions: (1) setting a TAN removal rate that could be considered affordable; (2) the mass of the input slurry treated was equal to the treatment capacity of the pilot plant; (3) the mass of the output slurry was calculated considering a small volume reduction of the treated slurry, due to water evaporation; (4) the mass of the AS solution was obtained by calculating the amount of acid required to retain a given amount of TAN removed from the stripping reactors; (5) and the N in the output slurry was the difference between the N in the input slurry and that in the AS solution. The effect of input slurry dilution on the quality of the achievable AS solution was evaluated by comparing slurries with different initial TKN and TAN contents. Regarding upscaling the process, an assessment has been made based on the expected N removal performance and the associated electricity consumption, acid requirements, and operating costs.

3 Results

3.1 Nitrogen removal efficiencies and mineral fertilizer production

3.1.1 Characteristics of the treated slurries and digestates

The analyses reported in Table 2 refer to the mean values of the samples collected from each reactor at the beginning of each trial. The

TABLE 2 Characteristics of treated slurry in the pilot plant.

Trial	Reactors heated to 40°C							Ambient temperature		NaOH addition	
	Pig1	Pig2	Pig3	Dig1	Dig2	Cattle1	Cattle2	Pig4. at	Cattle3. at	Pig5. sh	Cattle4. sh
TS (%)	3.07 ±(0.20)	2.19 ±(0.11)	2.41 ±(0.04)	2.41 ±(0.19)	2.71 ±(0.03)	1.32 ±(0.04)	2.12 ±(0.05)	4.49 ±(0.22)	3.68 ±(0.12)	6.75 ±(0.60)	5.22 ±(0.14)
TAN (kg m ^{−3})	2.97 ±(0.10)	1.11 ±(0.02)	2.22 ±(0.08)	2.33 ±(0.04)	1.69 ±(0.12)	0.66 ±(0.01)	0.93 ±(0.02)	3.77 ±(0.11)	1.83 ±(0.01)	3.75 ±(0.11)	1.44 ±(0.04)
TKN (kg m ^{−3})	4.25 ±(0.18)	2.42 ±(0.01)	3.06 ±(0.02)	3.05 ±(0.01)	2.86 ±(0.07)	1.25 ±(0.09)	1.67 ±(0.09)	5.54 ±(0.13)	3.09 ±(0.06)	5.26 ±(0.21)	2.70 ±(0.00)
TAN/TKN (%)	70 ±(1)	46 ±(1)	73 ±(2)	76 ±(1)	59 ±(3)	53 ±(3)	56 ±(4)	68 ±(1)	59 ±(1)	71 ±(1)	53 ±(2)
P (kg m ^{−3})	0.56 ±(0.08)	0.20 ±(0.00)	0.40 ±(0.09)	0.26 ±(0.05)	0.16 ±(0.02)	0.25 ±(0.04)	0.12 ±(0.01)	2.08 ±(0.22)	0.74 ±(0.09)	2.26 ±(0.04)	0.65 ±(0.02)
K (kg m ^{−3})	3.94 ±(0.05)	1.23 ±(0.04)	2.45 ±(0.00)	2.86 ±(0.08)	3.18 ±(0.04)	1.53 ±(0.04)	1.98 ±(0.11)	3.93 ±(0.28)	2.98 ±(0.01)	3.97 ±(0.12)	3.08 ±(0.06)
pH	7.8 ±(0.1)	7.2 ±(0.3)	7.8 ±(0.1)	7.9 ±(0.1)	8.1 ±(0.1)	7.4 ±(0.2)	7.7 ±(0.0)	7.9 ±(0.1)	7.4 ±(0.1)	10.7 ±(0.1)	10.7 ±(0.0)
EC (mS cm ^{−1})	27.1 ±(0.4)	11.2 ±(0.1)	19.3 ±(0.1)	21.4 ±(0.07)	17.0 ±(0.5)	8.7 ±(0.1)	11.6 ±(0.5)	35.7 ±(0.9)	25.0 ±(0.2)	40.7 ±(2.1)	29.8 ±(2.1)

The mean and standard deviation (in brackets) of the parameter analyzed.
*Analysis referred after sodium hydroxide addition.

TS content of the treated slurries in trials Pig5.sh and Cattle4.sh also included the addition of NaOH.

The cattle slurry used in trials Cattle1 and Cattle2 had a high dilution with TS and lower nutrient content than that found in samples taken in the same area (Finzi et al., 2015). This was due to the consistent use of cooling systems based on the use of water (showers) during the period in which the slurry was treated in the pilot plant. The cattle slurry used in the trial in Cattle3.at was collected in a barn that did not have cooling systems with showers and therefore had a higher TS and nutrient content.

The digestates had a lower TS and nutrient content than that found in the same area (Finzi et al., 2015) probably because they were obtained from biogas plants fed mainly with livestock slurries (supplemented with small amounts of energy crop silage). In the Dig1 trial, the digestate used was from a biogas plant fed with pig slurry, while in the Dig2 trial, the digestate used was from a biogas plant fed with cattle slurry. Conversely, the pig slurry had a TS and nutrient content in line with (Pig1, Pig2, and Pig3) or higher (Pig4.at) than the values recorded in the same area (Finzi et al., 2015).

3.1.2 TKN and TAN removal and volume reduction

The results of the trials conducted (Table 3) showed TKN removal rates between 42 and 72% and TAN removal rates between 35 and 79% in the trial with reactors heated to 40°C; TKN removal rates between 3 and 26% and TAN removal rates between −5 and 25% in the trial with reactors kept at ambient temperature; and TKN removal rates between 50 and 67% and TAN removal rates between 76 and 82% in the trial with pH reactors adjusted to 10.7. The values depended on the treatment duration, which differed among trials. The TKN removal rate was lower than the TAN removal rate because the stripping treatment affected the amount of ammoniacal N. The TKN removal rate was higher than the TAN removal rate only in trials Pig2 and Cattle3.at. In trial Pig2, this removal effect could be due to a lower TAN/TKN ratio of the treated pig slurry compared with the expected values for this type of slurry (Finzi et al., 2015). The low TAN/TKN ratio could be due to the high content of organic N. A fraction of organic N had undergone a mineralization process (equal to 27% of the initial TKN content), producing TAN and leading to higher removal of TKN compared with TAN, the content of which was increased by the amount of organic N mineralized. In fact, organic N transformation to ammonium is temperature-dependent, as it is related to the transformation of organic matter. Sommer et al. (2007) observed the highest rates during the storage of pig slurry at 20°C in

the initial period of 10–15 days when mineralization of organic N of approximately 19% (relative to the initial TKN content). In trial Cattle3.at, the removal effect could be due to the very slow TAN removal because the mineralization rate of organic N was 6%, consistent with the mean value of 10% found in other trials.

During the treatment, together with NH_3 , a part of the water in the effluent also evaporated. A slurry/digestates volume reduction from 3 to 23% was observed in the trials with reactors heated to 40°C. The reduction in the volume of the treated effluent was very variable and depends on the duration of the treatment. It may seem like an additional advantage to be able to reduce the volume of the effluent in addition to N, but this would be true if water exits the process. However, these results were only due to the absence of insulation of pipes and scrubbers in the pilot plant. The water vapor in the exhaust air from the reactors condensed in the pipes and scrubber, especially when the external temperature was significantly lower than that of the reactor. The volume reduction can be limited in an insulated installation, or when the external temperature is close to the temperature of the reactor. The volume reduction in trial Pig1 was 3% after 15 days of treatment, with an average difference of 13.6°C between the temperatures in the reactors and the external temperature. The trials carried out at ambient temperature, with a temperature difference of 3.7–4.3°C, had a volume reduction of 1–3% in a process of 15–21 days. A higher volume reduction (23%) occurred in trial Pig3 with a temperature difference of 27.2°C and a duration of 28 days.

3.1.3 Tan removal trend

The TAN removal efficiencies achieved in the trials at 40°C were used to define a simple regression model over time that provides an achievable performance, regardless of the slurry characteristics and treatment duration (Figure 2). Each point on the graph corresponds to 1 sampling day and represents the removal of the TAN content from the start of the trial; 1 week after the start of treatment, a TAN removal rate of approximately 25% can be expected, 45% after 2 weeks, and 55% after 3 weeks. These average removal rates fit within a confidence interval, presenting an overall margin of variability of approximately $\pm 15\%$, linked to the different operating conditions. The advantage of this process is the possibility of modulating the intensity of the treatment. The treatment can be stopped at any time once the necessary N removal level has been reached, based, for example, on the N surplus that a livestock farm has to manage. By observing the levels of TAN removal as a function of treatment time, the process highlights higher removal rates in the early stages. This means that longer treatment times allow higher levels of overall removal, but in the advanced stage of the process,

TABLE 3 Performance achieved in the pilot plant.

Trial	Reactors heated to 40°C							Ambient temperature		NaOH addition	
	Pig1	Pig2	Pig3	Dig1	Dig2	Cattle1	Cattle2	Pig4.at	Cattle3.at	Pig5.sh	Cattle4.sh
Volume reduction	3%	17%	23%	9%	9%	17%	17%	2%	1%	2%	3%
TAN removal	50%	35%	67%	79%	67%	57%	63%	25%	−5%	82%	76%
TKN removal	42%	44%	57%	72%	47%	53%	46%	26%	3%	67%	50%

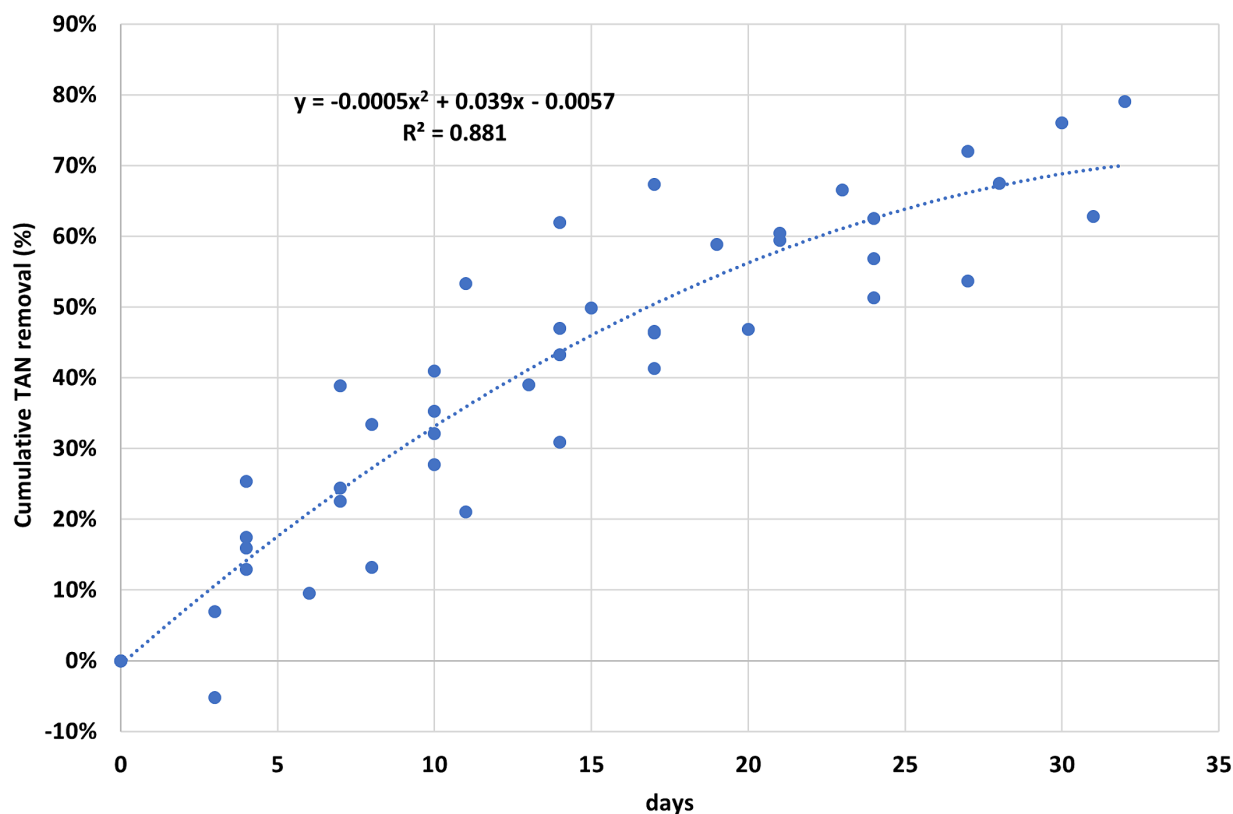


FIGURE 2
TAN removal trend in the trials conducted with reactors heated to 40°C.

the TAN release rate is lower than that in the initial stage. Therefore, each unit of N removed in this stage will have a higher unit cost.

3.1.4 Performance at 2 weeks of functioning

Considering the trend in the removal of TAN, as shown in Figure 2, and based on the increased knowledge gained during the trials conducted in the pilot plant, a treatment duration of 2 weeks resulted as a tradeoff between the achievable N removal rate and consumption/costs. In detail, after 2 weeks of treatment, the performances shown in Figure 3 were achieved. In trials with reactors heated to 40°C, the digestates showed an average TAN removal rate of 54%, compared with 35% for cattle slurry and 34% for pig slurry. Without considering the Pig2 trial, which was characterized by high N mineralization, the average removal rate of TAN from pig slurry was 47%. The trials conducted at ambient temperature showed significant variability, with the Pig4.at trial close to 25% of TKN removal and the Cattle3.at trial characterized by high N mineralization. The trials with NaOH addition showed higher values than the other trials, with an average TAN removal rate of 76%. It should be noted that the addition of NaOH allowed a TAN removal rate of 64% to be achieved after just 1 week of treatment.

3.1.5 Characteristics of the obtained AS solution

The amount and N concentration of the AS solution showed variability among the trials conducted, due to treatment duration and the ambient temperature during each trial.

In the trials with high slurry/digestates volume reduction, the water evaporated from the reactors condensed in the scrubber

diluting the AS solution. The level of the scrubber tank also increased the necessity of emptying the AS solution before it became saturated with NH_3 to prevent spilling liquid from the scrubber.

In trial Pig1 conducted with the heated reactors and low slurry volume reduction, the N concentration of the AS solution reached 69.7 g kg^{-1} (AS solution concentration of 33.2% w/w). Therefore, in the trials with high slurry volume reduction, the concentration of N in AS was low ($5.7\text{--}44.0 \text{ g kg}^{-1}$ of N).

Due to the high N removal rate of the Pig5.sh trial, conducted by adding NaOH and at ambient temperatures during the summer period (approximately 30°C of slurry temperature), the N concentration of the AS solution reached the maximum value of 85.9 g kg^{-1} (AS solution concentration of 40.9% w/w).

In addition to the N content, the AS solution was also characterized for total solids and major and trace element contents, which are reported in Table 4.

3.2 Technical–economic assessment: electricity consumption, acid requirements, operational costs

The electricity consumption of the pilot plant related to electricity usage, such as reactor mixing, pumping, and airflow generation, was determined during the trials conducted at ambient temperature and was found to be an average of $0.25 \text{ kWh}_d \text{ m}^{-3}$ treated per day. This consumption was considered for each trial.

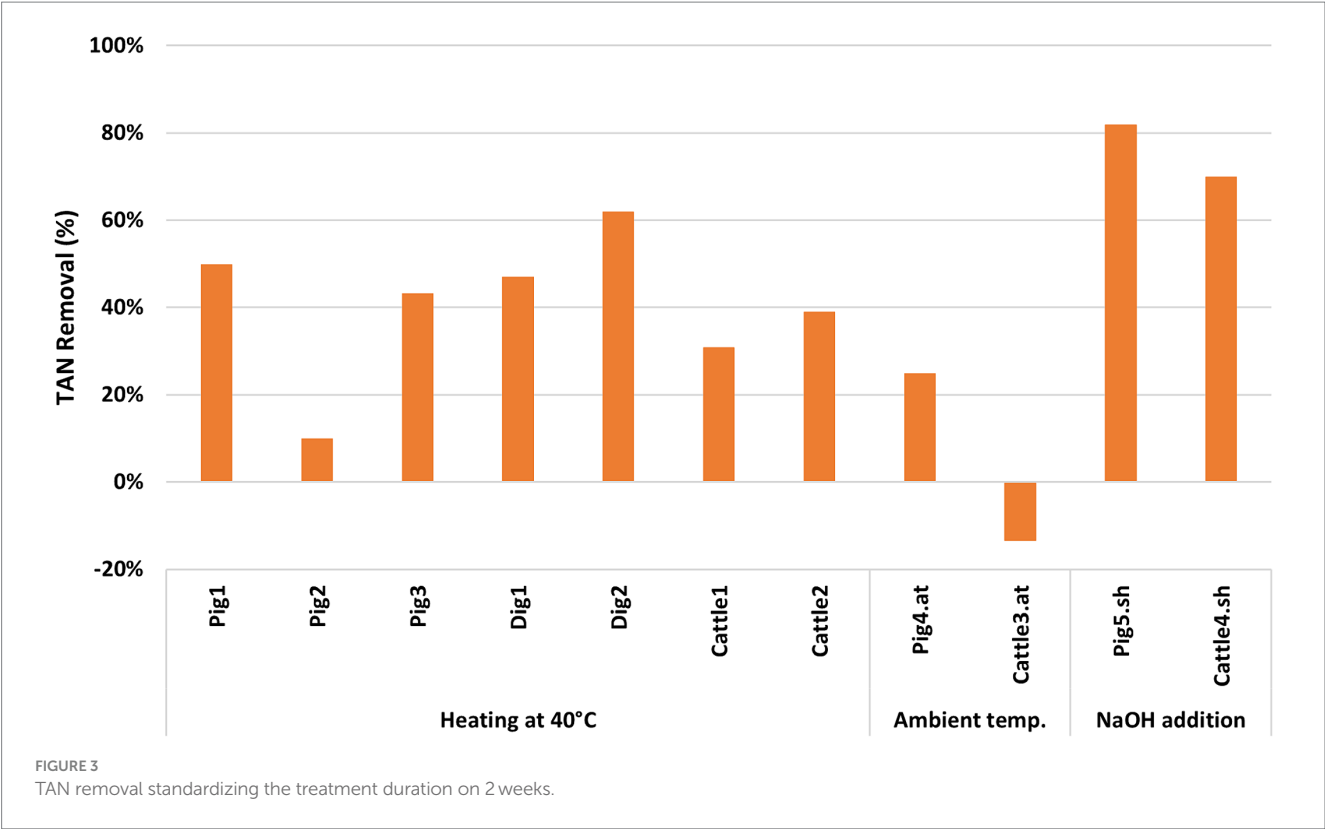


TABLE 4 Characteristics of the AS solution obtained, in terms of total solids, major elements, and trace elements.

	Parameter	Mean ± (SE)
Major elements	TS (%)	18.0 ± (7.9)
	Ca (mg kg ⁻¹)	4.1 ± (3.2)
	K (mg kg ⁻¹)	4.1 ± (1.8)
	Mg (mg kg ⁻¹)	14.3 ± (1.1)
	Na (mg kg ⁻¹)	5.4 ± (2.3)
	P (mg kg ⁻¹)	130.8 ± (19.5)
Trace elements	Al (mg kg ⁻¹)	16.9 ± (8.3)
	As (mg kg ⁻¹)	n.d.
	Cd (mg kg ⁻¹)	< 0.5
	Co (mg kg ⁻¹)	n.d.
	Cr (mg kg ⁻¹)	< 0.5
	Cu (mg kg ⁻¹)	9.7 ± (0.8)
	Fe (mg kg ⁻¹)	3.1 ± (0.4)
	Mn (mg kg ⁻¹)	n.d.
	Mo (mg kg ⁻¹)	< 0.5
	Ni (mg kg ⁻¹)	< 0.5
	Pb (mg kg ⁻¹)	n.d.
	Se (mg kg ⁻¹)	n.d.
	Zn (mg kg ⁻¹)	2.8 ± (1.4)

Mean ± Standard Error (SE). (n.d = not detectable).

Regarding the daily electricity consumption related to TKN removal, in the trials with the heated reactors, the electricity consumption ranged between 0.11 and 0.33 kWh_{el} kgN⁻¹ removed per day for the Dig1 and Cattle2 trials, respectively. The lowest value in the Dig1 trial was due to the high TKN removal efficiency (72%) of a digestate with a TKN of 3.05 g kg⁻¹, compared with a TKN removal efficiency (46%) of a cattle

slurry with a TKN of 1.67 g kg^{-1} . In the trials at ambient temperature with the addition of NaOH, the electricity consumption ranged between 0.07 and $0.18 \text{ kWh}_{\text{el}} \text{ kgN}^{-1}$ removed per day for the Pig5.sh and Cattle4.sh trials, respectively, due to the ambient temperature of approximately 26°C . In the trials conducted without the addition of NaOH, the electricity consumption ranged between 0.18 and $2.62 \text{ kWh}_{\text{el}} \text{ kgN}^{-1}$ removed per day for the Pig4.at and Cattle3.at trials, respectively. This large difference was related to the higher removal of TKN in trial Pig4.at (26%) compared with the 3% found in trial Cattle3.at.

The energy required for heating the reactors was highly dependent on the season during which the trial was conducted. In the summer period with ambient temperatures from 23.2°C to 27.2°C (Pig1, Dig1, and Cattle1), the thermal energy consumption was within the range of $0.37\text{--}0.44 \text{ kWh}_{\text{th}} \text{ m}^{-3}$ treated per day. The maximum value was obtained in trial Dig1, which lasted for 32 days, leading to a reduction in TKN of 72%, whereas the minimum value was obtained in trial Pig1, which lasted for 15 days, leading to a reduction in TKN of 42%. Increasing the treatment time to achieve higher N removal rates leads to higher electricity consumption expressed per unit of volume treated per day. This is because the TAN release rate slowed down as the treatment time increased (Figure 2).

The thermal energy consumption referred to daily N removal between 0.20 and $1.56 \text{ kWh}_{\text{th}} \text{ kgN}^{-1}$ removed per day, where the minimum consumption was observed in Dig1 and Pig1 trials and the maximum was observed in Cattle1. In the Cattle1 trial, the initial TKN content of 1.25 g kg^{-1} was lower than that in the Dig1 and Pig1 trials with TKN contents of 3.05 and 4.25 g kg^{-1} , respectively. This means that it is energetically prejudicial to treat diluted slurry.

In trials conducted with colder ambient temperatures between 11°C and 17°C (Cattle2, Dig2, Pig2, and Pig3), the heating electricity consumption ranged between 0.62 and $1.42 \text{ kWh}_{\text{th}} \text{ m}^{-3}$ treated per day, respectively, for trials Pig2 (temperature 17.3°C , duration 28 days, and TKN removal rate of 44%) and Cattle2 (temperature 11°C , duration of 31 days, and TKN removal rate of 46%). Regarding the colder season, this resulted in consumption ranging from 0.60 to $1.88 \text{ kWh}_{\text{th}} \text{ kgN}^{-1}$ removed per day for trial Pig2 and Cattle2, respectively. The lower temperature and the longer treatment time required in trial Cattle2 led to much higher energy consumption compared with trial Pig2.

Overall, regarding N removal, the electricity consumption varied between 2.69 and $55.01 \text{ kWh}_{\text{el}} \text{ kgN}^{-1}$ removed in the trials at ambient temperature, $1.04\text{--}3.74 \text{ kWh}_{\text{el}} \text{ kgN}^{-1}$ removed in the trials with NaOH addition, and $2.04\text{--}10.21 \text{ kWh}_{\text{el}} \text{ kgN}^{-1}$ removed in the trials with the reactors heated, respectively, for trials Pig1 and Cattle2. The thermal energy consumption of all the trials with heated reactors ranged from 3.04 to $58.31 \text{ kWh}_{\text{th}} \text{ kgN}^{-1}$ removed for trials Pig1 and Cattle2, respectively.

The acid requirement depends on both the amount of N retained and the amount of condensed water in the scrubber (see par. 3.1.2). The theoretical requirement calculated based on the stoichiometric ratio in the AS formation reaction is equal to 3.5 kg of H_2SO_4 for each kg of N removed.

The amount of acid used (expressed as pure acid) varied between 5.7 and $18.9 \text{ kg acid per kgN}^{-1}$ removed or $5.0\text{--}20.2 \text{ kg acid m}^{-3}$ treated in the trials with heated reactors and between 5.3 and $12.5 \text{ kg acid per kgN}^{-1}$ removed or $11.6\text{--}12.3 \text{ kg acid m}^{-3}$ treated in the trials with the reactors at ambient temperature and in the trials with NaOH addition.

The trials Pig1, Dig1, and Pig5.sh conducted at warm ambient temperatures ($23.2^\circ\text{C}\text{--}27.2^\circ\text{C}$) showed that the acid requirement per unit of N removed was between 5.3 (Pig5.sh) and 7.2 (Dig1) $\text{kg acid per kgN}^{-1}$

removed compared with trial Cattle2, which had a requirement of 18.9 kg of acid per kgN^{-1} removed as the low ambient temperatures (11.4°C).

Overall, the treatment costs varied between 1.66 and 4.20 € kgN^{-1} removed in the tests with the reactors heated, between 0.93 and 12.80 € kgN^{-1} removed in the tests with the reactors at ambient temperature, and between 4.43 and 7.93 € kgN^{-1} removed in the tests with NaOH addition. The cost of the stripping process could be partially recovered from the sale of the AS solution. The obtainable benefit, expressed as the surrogate value of N recovered as AS solution, was assumed to be 1.08 € kgN^{-1} . This income could even exceed the operating costs, making the treatment profitable. Specifically, in the Pig4.at trial, the potential profit was 0.15 € kgN^{-1} .

Furthermore, the treatment costs have been split between electricity consumption and H_2SO_4 and NaOH requirements to evaluate the share of individual cost items on the overall cost.

The costs related to electricity consumption varied between 0.47 and 2.34 € kgN^{-1} removed in the trials with the reactors heated, between 0.62 and 12.64 € kgN^{-1} removed in the trials with the reactors at ambient temperature, and between 0.24 and 0.86 € kgN^{-1} removed in the trials with NaOH addition. Specifically, the lowest costs were obtained in trials Pig1, Pig4.at, and Pig5.sh, where a high efficiency in N removal was achieved within 2 weeks of treatment.

Regarding the H_2SO_4 requirements, the costs varied between 1.01 and 2.41 € kgN^{-1} removed in the trials with the reactors heated, between 0.17 and 0.31 € kgN^{-1} removed in the trials with the reactors at ambient temperature, and between 0.80 and 2.47 € kgN^{-1} removed in the trials with NaOH addition. The lowest costs were obtained in trials Pig2, Cattle3.at, and Pig5.sh.

The costs resulting from the use of NaOH varied between 3.40 (Pig5.sh) and 4.60 (Cattle4.sh) € kgN^{-1} removed.

4 Discussion

4.1 Nitrogen removal efficiencies and mineral fertilizer production

4.1.1 TKN and TAN removal and volume reduction

The results of the trials conducted show TKN removal rates between 40 and 70% and TAN removal rates between 50 and 80%. These removal rates were similar to those found in other studies at the laboratory scale based on the slow-release stripping process. Provolto et al. (2017) found that the highest TAN removal efficiency (87%) was obtained at 40°C with pH adjusted to 9 using sodium hydroxide. However, at 40°C without pH adjustment, removal efficiencies of 69% for TAN and 47% for TKN were obtained in 10 days. Heidarzadeh Vazifehkhora et al. (2022) reached TAN removal rates of 92, 83, and 67% with 20 days of hydraulic retention time (HRT) and 83, 60, and 41% with 12 days of HRT from the digestates, pig slurry, and dairy cattle slurry, respectively.

Regarding the stripping process at the pilot scale, there are studies in the literature (Baldi et al., 2018; Brienza et al., 2023; Vingerhoets et al., 2023), where the treatment was performed by treating the slurry in stripping towers. Vingerhoets et al. (2023) treated a liquid fraction of a digestate for 2–3 h and obtained significantly improved N removal efficiency, from 27% to 40%, by raising the pH from 8 to 10 at a temperature of 30°C and an airflow rate of $1,440 \text{ m}^3 \text{ h}^{-1}$. At ambient temperature, Brienza et al. (2023) found a TKN removal rate of 21% (32% TAN removal) from a liquid fraction of anaerobically digested

pig slurry with a pH of 8, treated during three periods between spring and autumn, in a pilot plant with the capacity to process approximately 1 ton per hour. In the present study, treating pig slurry with a pH of 7.9, a comparable TAN removal rate of 25% was obtained at ambient temperature of 30°C (Pig4.at), while a higher TAN removal rate of 82% (Pig5.sh) was obtained by increasing pH from 7.9 to 10.7 (Figure 3). In both cases, 2 weeks of treatment were necessary, much longer than the few hours reported by Brienza et al. (2023) and Vingerhoets et al. (2023).

Using our pilot plant, the addition of NaOH allowed TAN removal rates 1.9 times higher than those achieved when heating the reactors to 40°C, consistent with the findings of Baldi et al. (2018), who reported TAN removal rates of 1.6 times higher in trials with pH adjusted to 10.5 using NaOH addition, compared with trials without pH modification. Baldi et al. (2018) obtained on a full-scale plant a removal efficiency of 59–66% on digestate with pH increased to 10.5 by adding NaOH and treated for 6 h at 56°C–58°C in a continuous process, and they achieved a higher removal efficiency of 79% in a semi-batch process on digestate with pH increased to 10.1, treated for 6 h at 52°C. In the trials without NaOH addition, conducted at a temperature of 45–55°C and a pH of 9.2–9.5, they obtained a lower N removal rate (23–39%). A similar result was found by Bolzonella et al. (2018), who reached a removal efficiency of 22% when performing a stripping trial on digestates at a temperature of 60–70°C. In our study, the trials conducted at 40°C achieved TAN removal rates, ranging from 10% (Pig2) to 62% (Dig2), but after 2 weeks of treatment.

Compared to the pilot-scale slow-release stripping process tested in this study, the cited studies that treated slurry in stripping towers achieved comparable N removal rates, although with shorter treatment times. The main difference in the process was due to the lower surface of liquid exposed to air in the slow-stripping process, as the slurry was just mixed in the reactor without creating drops or enhancing the surface with packed beds. In fact, similar results were obtained but using a higher overall quantity of air. Considering the G/L ratio, defined by Palakodeti et al. (2021) as the ratio of the total volume of used gas to the volume of the stripped liquid, in our case, the value was approximately 44,000 compared to values well below 10,000 for packed beds (Palakodeti et al., 2021). The disadvantage of stripping towers is the treatment of slurry in columns with packed beds, which have a high risk of clogging and foaming, making this process more difficult to manage than the slow-release stripping process. Additionally, stripping towers require more intensive slurry solid/liquid separation to avoid clogging, increasing overall energy consumption and treatment costs.

4.1.2 Characteristics of the obtained AS solution

The results obtained highlight the possibility of obtaining AS with 69.7 g kg⁻¹ of TAN in the trial Pig1, with the heated reactors and 85.9 g kg⁻¹ of TAN in the trial Pig5.sh, with the addition of NaOH. In the AS obtained in these trials, the sulfur trioxide (SO₃) contents (stoichiometrically determined) were 199.2 and 245.6 g kg⁻¹ for heated and NaOH-added trials, respectively. These AS solutions reached the compositional requirements of Fertilizing Product Regulation (EU) 1009/2019, which establishes rules for placing EU fertilizing products on the market (Regulation (EU) 1009, 2019). This regulation defines a liquid inorganic macronutrient fertilizer [PFC 1(C)(I)(b)(ii)] as a substance that contains only one declared main macroelement [N, phosphorus pentoxide (P₂O₅), or potassium oxide (K₂O)] for at least 15 g kg⁻¹ by mass and one or more declared secondary macroelements [calcium oxide (CaO), magnesium oxide (MgO), sodium oxide (Na₂O), or sulfur trioxide (SO₃)] for at least

7.5 g kg⁻¹ by mass. The sum of all declared contents of major and minor macronutrients must be at least 70 g kg⁻¹ by mass.

Overall, the AS solutions obtained had a quality in line with Sigurnjak et al. (2019), who reported trials on two full-scale plants, where the NH₃ was removed from biogas or digestates in the treatment plant or at the end-of-pipe pathway, in which AS with 53–74 g kg⁻¹ of TAN was obtained. Similar results were reported by Ledda et al. (2013), who obtained AS with 51–61 g kg⁻¹ of TAN in a digestate treatment plant characterized by sequential integration of solid–liquid separations, ultrafiltration, reverse osmosis, and cold NH₃ stripping. Pigoli et al. (2021) obtained an AS with 71.7 g kg⁻¹ of TAN in a full-scale plant where organic wastes were transformed by high-solid thermophilic anaerobic digestion coupled with N stripping technology.

The results obtained in this study are in line with the literature when considering the trials of limited water addition to the AS. As explained in the results, this condition was not achieved in some cases due mainly to the set-up of the pilot plant but could be easily improved in full-scale installations.

The average TS content (18.0% ± 7.9%) of the AS solution obtained in this study was consistent with literature findings of 20% ± 10% reported by Pigoli et al. (2021).

Contents of the major and trace elements in the AS solutions of all the trials were consistent with the data, as shown by Pigoli et al. (2021) and Brienza et al. (2021). In addition, the Cu and Zn contents of the AS solution were lower than the threshold established in Regulation (EU) 1009/2019 (Cu ≤ 600 mg kg⁻¹ DW and Zn ≤ 1,500 mg kg⁻¹ DW).

Regarding TOC content in the AS solutions of all the trials, its value was calculated as the difference between TS content attributable to AS and TS contents attributable to residual acid and was always under the threshold of Regulation (EU) 1009/2019 (≤ 10 g kg⁻¹ FW).

4.2 Technical-economic assessment: electricity consumption, acid requirements, and operational costs

The lowest electricity consumption found in the pilot plant trials (1.04–2.04–2.69 kWh_{el} kgN⁻¹ removal in the NaOH addition, heated reactors, and ambient temperature trials, respectively) was obtained when treating concentrated slurry. The highest electricity consumption (10.21–55.01 kWh_{el} kgN⁻¹ removal, respectively, in the trials with reactors heated and at ambient temperature) was obtained when diluted slurry was treated because the recoverable N is less than a concentrated slurry for the same treated volume and treatment time. The lowest electricity consumption values are lower than those reported in Flotats et al. (2011), which showed an energy consumption for the stripping column of approximately 28 kWh kg⁻¹ of stripped N; or in Brienza et al. (2023) that showed a need for 12 kWh_{el} kgN⁻¹ recovered in a stripping plant operating at ambient temperature in different seasons; as well as in Brienza et al. (2021), which reported a need for approximately 3.8 kWh_{el} and 59 kWh_{th} to recover 1 kg of N as AS, through a stripping column operating at 90°C.

Regarding pure acid requirement in terms of N removed as AS, the pilot plant requirements were 5.3 and 7.2 kg acid per kgN⁻¹ removed in the summer period (optimal conditions due to the limited condensate accumulation in the AS) or 11.1–18.9 kg acid per kgN⁻¹ removed in the winter period (critical conditions due to the relevant condensate accumulation in the AS). Elsewhere, Vingerhoets et al. (2023) highlighted lower needs with 3.5 kg H₂SO₄

kgN⁻¹ removed from digestate and cattle slurry by considering a H₂SO₄ concentrated at 98%.

Brienza et al. (2021) reported the use of 7.3 kg of 50% H₂SO₄ to recover 1 kg of N in different full-scale NH₃ stripping units, which is slightly lower than the acid requirement of the pilot plant when it was operating in optimal conditions.

The operational costs achieved in the pilot plant in the optimal conditions were 1.66 € kgN⁻¹ removed and 0.93 € kgN⁻¹ removed for the trials conducted with reactors heated and with reactors at ambient temperature, respectively. These results were consistent with the findings of Brienza et al. (2021) who showed that the operational costs of the stripping treatment were 0.52 € kgN⁻¹ removed from digestate processed in a full-scale plant operating at 47–76°C, in which, gypsum was used to recover NH₃ in the scrubbing unit. In addition, Brienza et al. (2023) reported costs of 2.65 € kgN⁻¹ removed, for a stripping unit treating digestate at ambient temperature in combination with an aerated constructed wetland. Bolzonella et al. (2018) provided a technical-economic assessment of a stripping system recovering less than 40% of the influent digestate N, indicating a processing operational cost of 3.34 € kgN⁻¹ removed.

The operational costs of the trials with NaOH addition were 4.43–7.93 € kgN⁻¹ removed for trials Pig5.sh and Cattle4.sh and were consistent with the findings of Vingerhoets et al. (2023) that reported costs within the range of 3.66–4.49 € kgN⁻¹ removed. This was observed while treating cattle slurry with a comparable N removal rate, albeit with an increase in pH to 10. Considering the potential benefits, it might be valuable to assess alternative additives such as lime, which is cost-effective but requires proper management due to the sediment it generates (Cattaneo et al., 2019). Alternatively, given that the Pig5.sh and Cattle4.sh trials yielded comparable results to those with heated reactors but in just 1 week instead of 2, there is a potential to increase the pH less significantly. This approach would result in savings on NaOH usage and

would extend the treatment duration. Another option to consider is the volatility of CO₂ compared with NH₃, with CO₂ volatilization being approximately five times higher than NH₃ volatilization within the first 4 h during aeration. Consequently, introducing NaOH a few hours after treatment initiation could reduce the required quantities as CO₂ stripping contributes to a pH increase of 0.5–1 point (Husted et al., 1991).

4.3 Pilot plant performance assessment

The mass and N balance shown in Figure 4 describes an optimal operating condition considering a limited amount of water evaporated from the stripping reactors and condensed in the AS solution of no more than 1%. Based on the TAN removal trend shown in Figure 2, a TAN removal of 45% (corresponding to a treatment time of 2 weeks) was selected. Consequently, a TKN removal rate of 40% was assumed. Three types of slurry with different TKN contents of 1.5, 3, or 4.5 g kg⁻¹ were considered for treatment in the pilot plant, while a fixed TAN/TKN ratio of 65% was considered regardless of the TKN concentration. Considering a 10% N mineralization of the initial TKN of the treated slurry (organic N converted to TAN), the resulting total removed TAN was higher than the TAN initially contained in the input slurry. Therefore, the sum of the TAN content in the starting slurry and the AS solution was 115%. With the same TAN removal rate, as the initial concentration of TKN increases, the concentration of N in the AS aqueous solution increases from 21.2 g kg⁻¹ for the most diluted slurry to 59.6 g kg⁻¹ for the most concentrated one.

Moreover, the European Commission proposal Ares (2024) 2885619–19/04/2024, to update the Nitrate Directive 91/676/EEC, considers the use of certain fertilizing materials from livestock manure, including AS, above the amount of 170 kg N per hectare per year up to an additional limit of 100 kg N per hectare per year. This

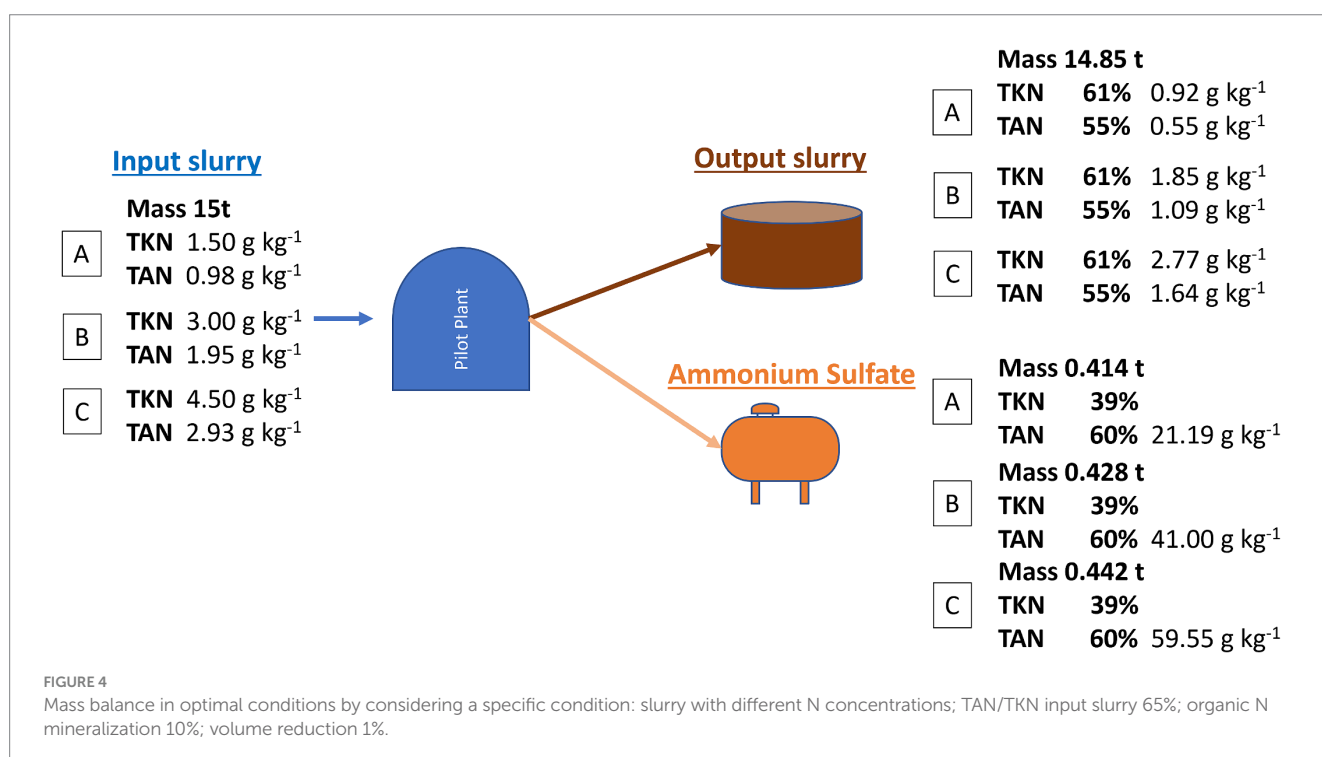


TABLE 5 Achievable performance when upscaling the pilot plant to a full-scale plant.

Parameter	Optimal value
TAN removal	60%
Days	14
Electrical consumption (kWh _{el} kgN ⁻¹ removed)	0.52
Acid requirement (kg kgN ⁻¹ removed)	3.5
Acid cost (€ kgN ⁻¹ removed)	0.74
Electricity cost (€ kgN ⁻¹ removed)	0.12

applies if the AS has a mineral N to total N ratio of at least 90% and the fertilizing materials do not exceed 300 mg kg⁻¹ dry matter and 800 mg kg⁻¹ dry matter for Cu and Zn, respectively.

The target values to make the pilot plant technically and economically sustainable with the aim of its upscaling are shown in Table 5. The optimal conditions shown in Table 5 can be achieved by considering the following:

- To treat slurry or digestate with TKN content of approximately 4–5 g kg⁻¹;
- The valorization of heat generated by the CHP engines of a biogas plant;
- An optimized acid requirement of 3.5 kg pure acid kgN⁻¹ removed (Vingerhoets et al., 2023);
- Electricity consumption of 0.52 kWh_{el} kgN⁻¹ removed. This value has been obtained starting from the reduction in electricity consumption obtained from the upscaling of the slow-release NH₃ stripping process from a laboratory scale equal to 2.1 kWh_{el} kgN⁻¹ removed, with the reactors filled to 0.04 m³ (Provolo et al., 2017), to pilot scale considering the lowest consumption achieved of 1.04 kWh_{el} kgN⁻¹ removed, with the reactors filled to 4 m³. Considering the same scale factors (halving the electricity consumption by increasing the treated volume by 100 times), the process upscaling at full scale will result in electricity consumption of 0.52 kWh_{el} kgN⁻¹ removed with reactors filled with 400 m³ of slurry/digestate.

From the perspective of the process upscaling, a possible reduction of the treatment times could be achieved by increasing the surface-to-volume ratio of the manure to accelerate NH₃ release. The pilot plant had a surface-to-volume ratio of 10 cm² dm⁻³, but by introducing a technical solution to increase this ratio to 60 cm² dm⁻³, the NH₃ removal in 14 days can reach 60% of the initial content (Trotta, 2020).

A full-scale plant with these characteristics could be a sustainable solution and competitive with other treatments, as the operating cost of 0.86 €/kg of N in the AS is similar to the market cost of mineral N. The process does not require any pretreatment of the slurry other than the usual separation of the coarse solid fraction and can be easily managed by the farmer as no special skills are required.

5 Conclusion

The slow-release NH₃ stripping process has proven to be easily manageable at a pilot scale, allowing a TKN removal rate of up to 72%, confirming the expected performance based on laboratory tests. Moreover, the process is modular and enables

the adjustment of the N removal rate according to the needs of the livestock farm.

Under optimal conditions, when slurry or digestates had a high TKN concentration (4–5 g kg⁻¹) and by limiting the accumulation of condensed water in the scrubber, high-quality AS was obtained. It reached an N concentration between 69.7 and 85.9 g kg⁻¹, classifying it as a mineral fertilizer according to the thresholds specified in EU Regulation 1009/2019.

The treatment studied can be economically sustainable. The operational cost (acid and electricity) of an optimized upscaled plant is 0.86 € kgN⁻¹ removed, which is lower than case studies treating slurry or digestate in stripping towers. This holds true in situations where excess heat is available for valorization. Conversely, ambient temperature treatment with NaOH addition is sustainable by implementing management solutions that reduce the use of this additive (e.g., CO₂ stripping or a minor pH increase compensated by a longer treatment time).

Introducing the treatment in farms with an N surplus or those needing to transport effluents over long distances improves overall effluent management. Within the same farm, AS becomes available, reducing the need for mineral fertilizers (if allowed by the threshold of the Nitrates Directive), and the separated solid can be applied in more distant fields. Alternatively, both products are more easily transportable to other farms compared with liquid manure.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

AF: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. OF: Data curation, Investigation, Writing – review & editing. ER: Data curation, Writing – review & editing. GP: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This research was funded by the Lombardy Region as part of the Rural Development Program 2014–2020, Measure 16 – “Cooperation,” Sub-measure 16.1—“Support for the establishment and management of the EPI Operational Groups in the field of productivity and sustainability of agriculture,” Operation 16.1.01—“EPI Operational Groups.” Project “Conserva—Innovative Plant for the Conservative Removal of Nitrogen from Livestock Slurries and Digestate.” The authors acknowledge support from the University of Milan through the APC initiative.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY

Harald Menzi,
Independent researcher, Bern, Switzerland

REVIEWED BY

Elio Dinuccio,
University of Turin, Italy
Joachim Deru,
Louis Bolk Instituut, Netherlands

*CORRESPONDENCE

Karin Andersson
✉ karin.i.andersson@slu.se

RECEIVED 29 February 2024

ACCEPTED 02 July 2024

PUBLISHED 26 July 2024

CITATION

Andersson K, Dahlin AS, Sørensen P and
Delin S (2024) Bedding material properties and
slurry C/N ratio affect the availability of
nitrogen in cattle slurry applied to soil.
Front. Sustain. Food Syst. 8:1393674.
doi: 10.3389/fsufs.2024.1393674

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Bedding material properties and slurry C/N ratio affect the availability of nitrogen in cattle slurry applied to soil

Karin Andersson^{1*}, A. Sigrun Dahlin², Peter Sørensen³ and
Sofia Delin¹

¹Department of Soil and Environment, Swedish University of Agricultural Sciences, Skara, Sweden,

²Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala,
Sweden, ³Department of Agroecology, Aarhus University, Tjele, Denmark

Introduction: Cattle slurry used as fertilizer in crop production is a mix of feces, urine, water, and bedding material from the housing system. Previous studies have shown that slurry nitrogen (N) availability to crops is dependent on C/N ratio. As the bedding material can contribute a significant part of total slurry carbon (C), its characteristics may affect the C/N ratio of the slurry. There is increasing interest in using the solid fraction from mechanical slurry separation as bedding material, and therefore this study investigated the potential effect of this fraction on slurry N availability, compared with more commonly used bedding materials such as straw and sawdust.

Methods: In two parallel 28-day laboratory incubations, net mineral N release and C mineralization from slurries applied to sandy loam soil were measured. The slurries comprised a liquid fraction (LF) from mechanical cattle slurry separation with a screw-press and different added bedding materials. Liquid fraction was mixed with two types of bedding material, solid slurry fraction (SF) and chopped straw, in different proportions, resulting in C/N ratios of 10, 12, and 14 in the slurry. In additional treatments, two other bedding materials, ground straw and sawdust, with slurry C/N ratio 12, were used.

Results: For SF and chopped straw, similar negative linear correlations were seen between slurry C/N ratio and net mineral N release after 28 days. Carbon mineralization, expressed as a percentage of total C added, was higher from the mixture containing SF than that containing straw, while no clear relationship with C/N ratio was found. At slurry C/N ratio 12, net release of mineral N was 28–39% of total N and decreased in the order: sawdust>chopped straw=SF=ground straw. Net C mineralization at the same slurry C/N ratio was 33–46% and decreased in the order: SF=ground straw>chopped straw>sawdust.

Discussion: For bedding materials with similar fiber composition (i.e., SF and straw), differences in C availability due to particle size or degree of degradation by microorganisms did not influence slurry N availability measurably. For sawdust, with high lignin content, the results indicate that limited C availability may lead to lower slurry N immobilization.

KEYWORDS

bedding material, solid fraction, straw, sawdust, slurry separation, soil incubation experiment

1 Introduction

Animal manure is an important source of nitrogen (N), a vital plant nutrient in crop production. However, only around 50% of the N in cattle slurry is present as easily available ammonium (NH_4^+), while the rest is in organic form (Webb et al., 2013). The fraction of slurry N available for plant uptake as NH_4^+ or NO_3^- is influenced by several factors, including animal type (Lukehurst et al., 2010), feed (Sørensen et al., 2003; Powell et al., 2006), pre-treatments such as solid-liquid separation (Fangueiro et al., 2015), anaerobic digestion (Möller and Müller, 2012), or acidification (Fangueiro et al., 2013), and slurry storage (Sørensen, 1998). The 1st-year availability of slurry N is negatively correlated with its carbon/nitrogen (C/N) ratio (Sørensen et al., 2003; Delin et al., 2012). Organic bedding materials are rich in C and poor in N, and may hence increase the C/N ratio in slurry. However, organic bedding materials differ in e.g., lignin content, particle size, and degree of degradation of organic matter, which may influence C availability and thereby mineralization/immobilization of N. Commonly used bedding materials include sawdust, sand, woodchips, straw, and peat (Ferraz et al., 2020; Frondelius et al., 2020). With pressurized filtration of slurry using a screw-press separator, a fibrous solid fraction suitable as bedding material can be produced (Hjorth et al., 2010). There is interest in using this solid fraction (SF), also called recycled manure solids (RMS), as bedding material in dairy housing systems (Husfeldt et al., 2012; Lybæk and Kjær, 2019; Leach et al., 2022).

In Sweden, free stall barns with cubicle housing is the most common housing system in dairy production (Växa Sverige, 2024), with concrete passageways cleaned with automatic scrapers, transporting the slurry to storage tanks outside the building. Storage tanks for slurry require coverage, either with a roof or a naturally formed cover crust, to reduce ammonia emissions (Swedish Board of Agriculture, 2021). Swedish standard figures state that the slurry production per dairy cow is 26–29 tons per year (Andersson et al., 2023) and the amount of bedding material needed is 400 kg (Agriwise, 2023), which implies ~ 13 – 15 g bedding kg^{-1} slurry. Depending on the need for and use of the solid fraction, the amount of slurry that is separated may differ between farms with slurry separation. At the farm where the material for this study came from, untreated slurry collected from a pumping pit between the cow shed and the slurry separator had an average DM content of 8.5% which is close to the Swedish standard value of 9% (Andersson et al., 2023), while separated slurry collected from the storage tank had an average DM content of 5.3% ($n = 7$ over 3 years; Andersson and Delin, unpublished¹). The C/N ratio of untreated and separated slurry was 10.7 and 6.4, respectively.

When SF is used as bedding material, partly degraded material in the form of animal feces is re-circulated in the system. With the most easily degradable C already degraded in the rumen, SF as bedding material could potentially reduce slurry N immobilization, due to a lower C availability than with other bedding materials, but knowledge in this area is limited. For slurry application in

spring and summer, less immobilization could enhance crop N uptake and hence reduce the risk of N re-mineralization and nitrate leaching after harvest, since nitrate leaching is strongly dependent on whether there is a crop present to take up the nitrogen (Sørensen et al., 2023). Compared with untreated slurry, solid-liquid separation reduces ammonia emissions from field application of the liquid fraction, while the overall ammonia mitigation effect when including emissions from the solid fraction is more uncertain (Pedersen et al., 2022). Slurry separation can also reduce greenhouse gas emissions (Amon et al., 2006; Holly et al., 2017). Solid-liquid separation thus potentially has important environmental benefits, but a better understanding of the individual and overall effects is needed.

The aim of this study was to investigate if the type and properties of the bedding material added to cattle slurry affects the N and C turnover of the slurry within the 1st month after slurry application, and thus the short term potential of the slurry to supply N to a crop. The results could be used to improve advice to farmers about the 1st year fertilizer value they can expect from their slurry, so that they can adjust their crop fertilization rates accordingly. In all treatments, the liquid fraction from mechanical slurry separation (LF) was used, representing cattle slurry without bedding material. In a two-factor experiment with the factors bedding material and C/N ratio, chopped straw and SF was added in different amounts to obtain certain slurry C/N ratios, and the slurry mixtures were compared to determine whether the relationship between slurry C/N ratio and N availability differed between the two bedding materials. In additional treatments, aiming at the same slurry C/N ratio, addition of different bedding materials (chopped straw, ground straw, solid fraction, and sawdust) to LF was compared, in order to investigate their effect on slurry turnover rate and N availability. The hypotheses were that: (1) carbon dioxide (CO_2) production increases with increasing chemical and physical C availability of the bedding material to microbial utilization, where C availability was assumed to be lowest for sawdust (due to high lignin content), followed by SF (where easily degradable compounds in the consumed feed has been digested in the rumen, increasing the proportion of recalcitrant compounds in the SF), higher for chopped straw with large particle size, and highest for ground straw with small particle size; (2) net mineral N release is related to the C availability of the bedding material, with lower N immobilization, and thereby higher net N mineralization, from slurries with a lower content of easily available C; and (3) in chopped straw and SF bedding materials, there is a negative linear relationship between slurry C/N ratio and net mineral N release and the slope of this relationship is steeper for chopped straw, which is a more easily available C source than solid fraction.

2 Materials and methods

2.1 Soil, manure, and bedding materials

The soil used in laboratory incubation experiments was a sandy loam with 17% clay, 61% sand, 3% organic matter, and pH (1:5 H_2O) 6.7. It was collected from a field at SLU's experimental farm Götala close to Skara in western Sweden ($58^\circ 22' \text{N}$, $13^\circ 29' \text{E}$). The crop rotation at the farm includes both 3–4 years grass/clover

¹ Andersson, K., and Delin, S. (unpublished). *Nitrogen Efficiency of Cattle Slurry Depending on Slurry Treatment and Application Method in Different Crops*.

forage leys and annual crops, with frequent addition of slurry and farmyard manure from beef cattle. Top soil was collected from a plowed field after a cereal crop in late autumn, ~2 months before experiment start and stored in open containers at cool temperature (0–5°C) until use. Two separate incubation experiments, one on N turnover and one on C turnover, were performed. These started on different dates (February 8 and 22, 2022, respectively), so soil preparation was performed using two separate batches, one for each experiment. The procedure used was the same for both batches, and started 10 and 13 days before the start of the N and C turnover incubation, respectively. To reduce the water content, soil was air-dried at 16–18°C for 2 days, with gentle mixing twice a day. The soil was sieved through a coarse (7 mm) mesh sieve and water-holding capacity (WHC) was determined using the method described in Jansson (1958). Water content after drying was 56 and 51% of WHC, respectively, for the two batches. Both soil batches were pre-incubated at 15°C in a climate chamber (Termaks, KB 6395, Nordic Labtech AB, Fjärås, Sweden), for 8 and 11 days, respectively.

Liquid (LF) and solid (SF) fractions from screw-press separated cattle slurry were collected from an organic dairy cattle farm (Otterslättns Lantbruk, Hova, Västra Götaland County, Sweden) 2 weeks before the start of the first experiment. Both fractions were collected directly from the slurry separator (CRI-MAN SM 260/75 FA DM, CRI-MAN, Correggio, Italy). The fractions were stored at cool temperature (0–5°C) until the start of the experiment.

Winter wheat straw was collected from a conventional farm. To obtain chopped straw, the straw was cut with scissors into ~0.5–1.0 cm pieces. To obtain ground straw, pieces of straw were milled in a grain mill to maximum particle size 0.8 mm. Sawdust (spruce and pine in unknown proportions) was collected from a commercial sawmill and sieved through a 2 mm mesh sieve.

2.2 Analyses of manure, bedding material, and soil samples

Samples of LF from slurry separation and the bedding materials used in the experimental slurries were analyzed for dry matter content (DM), total C, total N, and ammonium-N at the Soil and Plant Laboratory, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. Total C and organic N were analyzed by combustion of dried samples followed by elemental analysis [928 Series Macro Determinator (model CN928), LECO, St. Joseph, MI, USA]. Ammonium-N was extracted with 2M potassium chloride (KCl) and analyzed using a flow injection analyzer [FIAsstar Analyzer (5000), FOSS, Hillerød, Denmark]. Total N was calculated as the sum of organic N and ammonium-N.

After the incubations, an additional analysis of fiber fractions [neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL)] was performed on stored samples of the original bedding materials, at the laboratory of the Department of Applied Animal Science and Welfare, SLU, Uppsala, using the method described by Van Soest et al. (1991). Acid detergent lignin was analyzed by the Klason lignin method, using sulfuric acid (72%). Hemicellulose content was calculated as the difference between NDF and ADF, and cellulose as the difference between ADF and ADL. As shown in Table 1, the concentrations of fiber

fractions (hemicellulose, cellulose, and lignin) were similar in solid fraction and straw, while sawdust had a higher content of both cellulose and lignin, and higher total NDF content.

Soil samples from the N incubation were analyzed for ammonium-N and nitrate-N at the Soil and Plant Laboratory, SLU, Uppsala. Milled, frozen soil samples were extracted with 2M KCl and then analyzed by colorimetry on a Seal AA3 auto-analyzer (SEAL Analytical Inc., Mequon, Wisconsin, USA).

2.3 Treatments

In all treatments, the bedding materials were added to the LF from slurry separation, which was assumed to represent “pure” cattle manure without any bedding material added. This allowed differences in slurry properties related to the degree of slurry separation to be analyzed. Using LF (rather than unseparated slurry with higher DM content) as the basis for the treatments also resulted in greater variation in C/N ratio in the mixtures, without reaching unrealistically high C/N ratios in relation to that on real farms. The mixtures of LF and bedding materials are referred to hereafter as “slurry” or “slurries.”

The amount of slurry added to the soil was based on supplying 50 kg total N ha⁻¹ in all treatments, i.e., 26 tons ha⁻¹ with LF, in this experimental setting with a soil depth of 50 mm corresponding to 102 mg kg⁻¹ dry weight (DW) of soil. To obtain the different desired C/N ratios, the relative proportions of LF and bedding material were varied between the treatments. Straw and sawdust contributed very little N to the slurries, and in these treatments the proportion of bedding material was low and the amount of LF was more or less constant between C/N ratios. For SF, with higher ammonium N concentration, the amount of LF was reduced by at most 23%. Ammonium N content relative to total N in the slurries varied slightly between treatments and was on average 35% (Table 2). Due to the differences in N concentration, the amount of bedding material that needed to be added varied widely, between 12 and 127 g kg⁻¹ LF, and there was also variation in the amount of total C applied to the soil (Table 2).

2.4 Incubation for analysis of mineral N

Small plastic flowerpots (height 50 mm, volume ~98 cm³) were used as soil containers. On the day before experiment start, a filter paper was placed at the bottom of each pot to prevent soil loss through the drainage holes, and 70 g of pre-incubated soil were added and lightly packed down. The pots were placed in plastic boxes (36 x 24 x 13 cm), each containing pots for three replicates of one treatment and all sampling times, and placed in the same climate chamber where the soil was pre-incubated. The temperature was uniform in all parts of the chamber and was kept at 15 ± 0.3°C throughout the experiment. At the start of the experiment, one box at a time was removed from the climate chamber. The bedding material was added first to each pot and evenly distributed over the soil surface, and then LF was added. The separate weighing and addition of bedding material and LF was done to ensure that accurate C/N ratios were obtained. For ground

TABLE 1 Properties of the different bedding materials used in experimental slurries.

	Liquid fraction (LF)	Solid fraction (SF)	Straw	Sawdust
Number of samples (<i>n</i>)	2	1	1	1
Dry matter (%)	3.4	33.3	83.7	90 ^a
Total C (g kg ⁻¹ fresh weight)	15.2	160	396	500 ^a
Total N (g kg ⁻¹ fresh weight)	1.96	4.58	1.86	0.6 ^a
C _{tot} /N _{tot} ratio	7.8	35	212	833
Ammonium N (g kg ⁻¹ fresh weight)	0.71	0.72	0	0
Total NDF (% of dry weight)	-	76.3	77.7	87.2
Hemicellulose (% of dry weight)	-	31.1	32.3	15.3
Cellulose (% of dry weight)	-	37.5	40.3	49.2
Lignin (% of dry weight)	-	7.6	5.1	22.7

Cells with “-” indicate that the parameter was not determined.

^aFrom the literature (i.e., not analyzed).

straw and sawdust, the manure layer was stirred gently, taking care not to disturb the soil beneath, in order to create a slurry from the hydrophobic bedding material and the LF. Different amounts of water was added to obtain the same soil water content (70% of WHC) in all treatments and then another 45 g of pre-incubated soil were added to each pot, on top of the slurry, and gently packed down. The pots in their plastic box were then returned to the climate chamber. The reason for adding the experimental slurry as a layer in the middle, rather than thoroughly mixing it with the entire soil volume, was to mimic a real-life situation with animal slurry incorporated by plowing or injection, resulting in a more uneven distribution of slurry within the soil.

The lids of the plastic boxes were kept closed to prevent the soil from drying out. To ensure enough oxygen supply and avoid anaerobic conditions, the boxes were aerated at intervals of 3–4 days by opening the lid for a few minutes. On days 3, 7, 14, and 28, the three replicates of each treatment were destructively sampled for mineral N analysis. The entire soil volume from each replicate was placed in a plastic bag and frozen within 30 min for later analysis. To ensure that the water content remained constant during incubation, the boxes were weighed on each sampling occasion and, when necessary, deionized water was sprayed over the pots to replace the weight loss.

2.5 Incubation for analysis of CO₂ production

The procedure for preparing the soil and adding LF and bedding materials in incubation for analysis of CO₂ production was similar to that used in incubation for N analysis. However, the water content was kept slightly lower, at 65% of WHC, since the 70% used in the N incubation proved to be slightly too high, causing mold in some pots. Three replicates of each treatment were prepared. Each replicate was placed in a glass jar (outer diameter 120 mm, height 185 mm, volume 1,660 cm³) with metal screw-lid, together with a CO₂ trap. The CO₂ trap consisted of a 50 mL plastic scintillation vial with 30 mL of 0.3 M KOH for the first measurements and 0.2 M

KOH from day 10, absorbing the CO₂ produced in the jar. To ensure that all CO₂ produced was captured, the jar lids were kept closed, except when the traps were changed and CO₂ production measured. The KOH concentration was changed in order to permit the desired sampling frequency throughout the experiment, since the activity was expected to be lower during the later stage of incubation and a sufficiently high saturation level in the traps was needed to allow precise measurements. On days 2, 5, 10, 15, 21, and 28, the CO₂ traps were changed and electrical conductivity (EC) in the KOH solution was measured using a conductivity meter (ProfiLine Cond 3310 Portable Conductivity Meter, WTW, Weilheim, Germany). At each sampling occasion, the jars were aerated for a few minutes, thereafter a new CO₂ trap was placed in the jar, the lid was immediately closed and the jar was returned to the climate chamber. To correct for background CO₂ in the air and for basic soil respiration, the set-up included one blank treatment with only a CO₂ trap and one control treatment without any slurry added to the soil.

2.6 Calibrations for CO₂ determination

For calibration of the conductivity meter, solutions with different ratios of KOH and potassium carbonate (K₂CO₃) were prepared in order to imitate absorption of different amounts of CO₂, following the procedure described by Smirnova et al. (2014). Constant A, describing the theoretical maximum amount of CO₂ absorbed, was calculated for the 0.3 M and 0.2 M KOH solutions as:

$$N(\text{CO}_2) = A \frac{C(t_0) - C(t_1)}{C(t_0)} \quad (1)$$

where $C(t_0)$ is conductance before any absorption of CO₂, $C(t_1)$ is conductance at time t_1 and A is empirically determined.

Temperature calibrations for the 0.3 M and 0.2 M KOH solutions at temperatures between 15 and 30°C revealed a negative linear correlation between conductivity and temperature. The resulting equations, obtained by fitting a straight line to the data points in Excel, were used for temperature correction in the conductivity calculations.

TABLE 2 Properties of the liquid fraction (LF) from separated slurry and of mixtures of LF and different bedding materials [solid fraction (SF), straw, and sawdust] used in experimental slurries.

Treatment code	C _{tot} /N _{tot} ratio	C _{tot} /N _{org} ratio	Bedding material added	Amount bedding added (g kg ⁻¹ LF ⁻¹)	C from bedding (% of tot C)	N from bedding (% of tot N)	NH ₄ -N (% of tot N)	Tot C added to soil (mg kg ⁻¹ DW soil)
LF8	8	12.2	None	-	-	-	36.2	794
LF+SF10	10	15.3	SF	38	29	8	34.5	1,022
LF+SF12	12	17.9	SF	79	45	16	33.0	1,226
LF+SF14	14	20.4	SF	127	57	23	31.5	1,430
LF+straw10	10	15.6	Straw—chopped	12	23	1	35.8	1,022
LF+straw12	12	18.6	Straw—chopped	22	37	2	35.5	1,226
LF+straw14	14	21.6	Straw—chopped	33	46	3	35.1	1,430
LF+straw12-ground	12	18.6	Straw—ground	22	37	2	36.0	1,226
LF+sawdust12	12	18.8	Sawdust	17	36	1	36.7	1,220

2.7 Data treatment

2.7.1 Carbon

As a first step, a temperature correction to 20°C was made for the measured conductivity data, using the temperature calibration described above. The equations used for correction (obtained from Excel) were: $y = -0.261x + 69.181$ for the 0.3 M KOH solution and $y = -0.1774x + 47.142$ for the 0.2 M solution, where x is temperature (°C) and y is conductance (mS cm⁻¹). From the conductivity values obtained, CO₂ production for each sampling period was calculated using Equation (1), with constant A being 346.7 for 30 mL 0.3 M KOH and 235.2 for 30 mL 0.2 M KOH. Checks for outliers revealed that in a few cases one of the three replicates had considerably higher CO₂ production than the other two, indicating potential leaks between the glass jar and the lid. When the standard deviation within a treatment was more than 25% of the mean, the deviant value was omitted. This was the case for one of the blank samples on sampling days 2, 5, and 10.

For each sampling occasion, the average value of the blank samples was subtracted from all treatment values, to remove effects of background CO₂. To correct for basic soil respiration, the average value of the control treatment replicates with unfertilized soil was subtracted from the CO₂ values for the slurry treatments. As a last step, cumulative CO₂ production and amount of CO₂ produced per day, as a percentage of total added C, were calculated for each treatment. The amount of CO₂ absorbed by the traps was in the range 0.2–0.6 mg day⁻¹ for the blank samples and 1.5–2.4 mg day⁻¹ for the control treatment with unfertilized soil, with no clear pattern over time. For the fertilized treatments, CO₂ production (before correction for blank and control) decreased from 20.4–26.0 mg day⁻¹ at the first sampling occasion to 3.6–6.2 mg day⁻¹ after 28 days.

2.7.2 Nitrogen

The amount of mineral N supplied from slurry addition to soil is hereafter referred to as *Net mineral N release*, expressed as % of added total N. It gives a measure of potentially plant-available N from slurry, and was calculated according to Equation (2):

$$\text{Net mineral N release} = \frac{\text{Mineral N in soil with slurry} - \text{Mineral N in control soil}}{\text{Total N added with slurry}} \quad (2)$$

Net mineralization of slurry N over a given period (day 0– x), which describes the mineralization-immobilization processes, was calculated according to Equation (3):

$$\begin{aligned} \text{Net N mineralization} \\ = \text{Net mineral N release (day } x) - \text{Net mineral N release (day 0)} \end{aligned} \quad (3)$$

where net N mineralization is expressed as a percentage of total N added. It was also recalculated as a percentage of mineral N and organic N added.

2.7.3 Statistical analyses

For statistical analyses, the software Minitab (Version 21.3.1, Minitab18, Ltd., Coventry, UK) was used. To test the main

effects and potential interactions of the factors *bedding material* (levels chopped straw and SF) and *C/N ratio* (levels 10, 12, and 14) on net mineral N release and CO₂ production, two-way ANOVA analyses were performed using the General Linear Model function. Response variables were mineral N content at day 3, 7, 14, and 28 and cumulative CO₂ production at day 2, 5, 10, 15, 21, and 28. One-way ANOVA (using the General Linear Model) was used to test for effects of different bedding materials at C/N ratio 12. To test whether a transformation of data was needed, results from the Box-cox transformation options “No transformation” and “Optimal λ ” in Minitab were compared. Since data transformation in none of the cases significantly affected the results, the ANOVA and regression analyses on non-transformed data are presented. Pairwise differences were tested with a Tukey test at significance level 5%. To test whether the slope of the regression between net mineral N release and slurry C/N ratio at days 14 and 28 differed between SF and chopped straw, regression models were fitted, with *C/N ratio* as a continuous variable and *bedding material* as a categorical variable. An interaction for *C/N ratio* × *bedding material* implies significant differences in the regression coefficient (describing the slope of the regressions for the two bedding materials).

3 Results

3.1 Effect of C/N ratio and differences between chopped straw and SF

3.1.1 Net mineral N release

The two-way ANOVA revealed increased net mineral N release with decreasing slurry C/N ratio, except at day 3 (Supplementary Table 1, Figure 1A). The analyses showed no interaction between the factors C/N ratio and bedding material, except at day 7, when net mineral N release from slurry with straw was higher at C/N ratio 14 than at C/N ratio 12 (Supplementary Table 2). Initial net N immobilization occurred at all C/N ratios, but from day 3 the net mineral N release patterns diverged between C/N ratios (Figure 1A). For C/N ratio 8 and 10, initial immobilization changed to net N mineralization from day 3, while for C/N ratio 12 the immobilization phase lasted somewhat longer. Slurry with C/N ratio 14 showed net N immobilization throughout the incubation, while the net mineralization declined for slurry C/N ratio 10 and 12 toward the end of the experiment (Figure 1A). Averaged over C/N ratios 10–14, chopped straw as bedding material had higher net mineral N release (i.e., less N immobilization) than SF at day 7 and 14, while there was no difference after 28 days (Supplementary Table 1, Figure 1B).

By the end of the experiment, average net mineral N release from the experimental slurries amounted to 43, 36, 30, and 26% of total N added for C/N ratio 8, 10, 12, and 14, respectively (Supplementary Table 2, Figure 1A). This corresponded to net immobilization of 13 and 21% of added mineral N (i.e., 5 and 7% of added total N) for C/N ratio 12 and 14, respectively. At C/N ratio 10, the amount of soil mineral N was similar at the start and end of the experiment, while LF without any bedding material added and C/N ratio 8 resulted in net N mineralization, corresponding to 10% of added organic N (i.e., 6% of added total N).

There was a negative linear correlation between slurry C/N ratio and net mineral N release from the slurries with straw or SF addition after both 14 and 28 days (Supplementary Table 1, Figure 2). On day 14, the correlation was stronger for SF than for straw (Figure 2A), and there was a tendency for a steeper slope of the regression for SF ($p = 0.109$). By day 28, there were no longer any differences between SF and straw (Supplementary Table 1, Figure 2B).

3.1.2 CO₂ production

The two-way ANOVA for CO₂ production showed interaction between the factors C/N ratio and bedding material at days 2, 5, and 10, and a tendency for interaction at days 15, 21, and 28 (Supplementary Table 3). The rate of CO₂ production from both the SF and chopped straw slurries was highest during the 1st days, followed by considerably lower and slowly declining CO₂ production during the rest of the incubation (Figure 3). The decline in CO₂ production rate during the 2 last weeks of the experiment was most pronounced for C/N ratio 8 (Figure 3). Comparing C/N ratios, cumulative CO₂ production at the end of the experiment was lowest for C/N ratio 14, while no difference was seen between the other C/N ratios ($p = 0.002$; Supplementary Table 4). Comparing the two bedding materials, cumulative net CO₂ production by day 28 was higher for slurry with SF than with straw ($p < 0.001$), corresponding to 45 and 39% of total added C, respectively (Supplementary Table 4, Figure 3).

3.2 Effect of bedding material particle size and C availability

3.2.1 Net mineral N release

All four slurries at C/N ratio 12, with different bedding materials, showed initial net immobilization, followed by different patterns for the net mineral N release (Figure 4). Sawdust as bedding material resulted in higher net mineral N release than from the other bedding materials from day 7 (Supplementary Table 5, Figure 4). On day 28, net mineral N release was 39% of total N added for sawdust, compared with 28–30% for the other bedding materials (Supplementary Table 5). Net N immobilization corresponded to 14% of added mineral N for SF, 16% for chopped straw, and 23% for ground straw, while slight net N mineralization occurred for sawdust (corresponding to 3% of added organic N).

3.2.2 CO₂ production

In the comparison between treatments with four different bedding materials, all with C/N ratio 12, CO₂ production (% of C input) at day 2 was highest for SF, followed by ground straw and sawdust, and lowest for chopped straw ($p < 0.001$; Supplementary Table 6, Figure 5A). From day 5, SF and ground straw had, or tended to have, higher CO₂ production per day compared with chopped straw and sawdust (Figure 5A). Accordingly, cumulative CO₂ production at day 28 was highest for SF and ground straw (46% of added total C), followed by

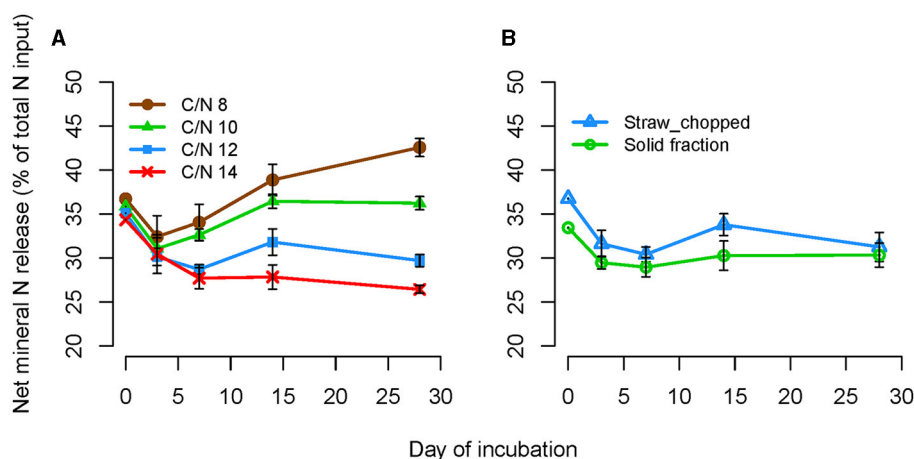


FIGURE 1

Effect of (A) C/N ratio and (B) bedding material type on net mineral N release from experimental slurries after 3, 7, 14, and 28 days of soil incubation. Values at day 0 are the calculated amounts added for each treatment. In (A), the values for C/N ratio 10, 12, and 14 are mean values for the bedding materials solid fraction and chopped straw ($n = 6$). In (B), the values for the bedding materials are mean values for C/N ratios 10, 12, and 14 ($n = 9$). C/N ratio 8 was not included in the two-way ANOVA, but is shown in the figure for comparison ($n = 3$). Error bars represent standard error of the mean (SEM). The two-way ANOVA showed a significant effect of C/N ratio ($p < 0.05$) for day 7, 14, and 28. For the factor bedding material, $p < 0.05$ at day 7 and 14. An interaction between the factors was seen at day 7 ($p < 0.05$).

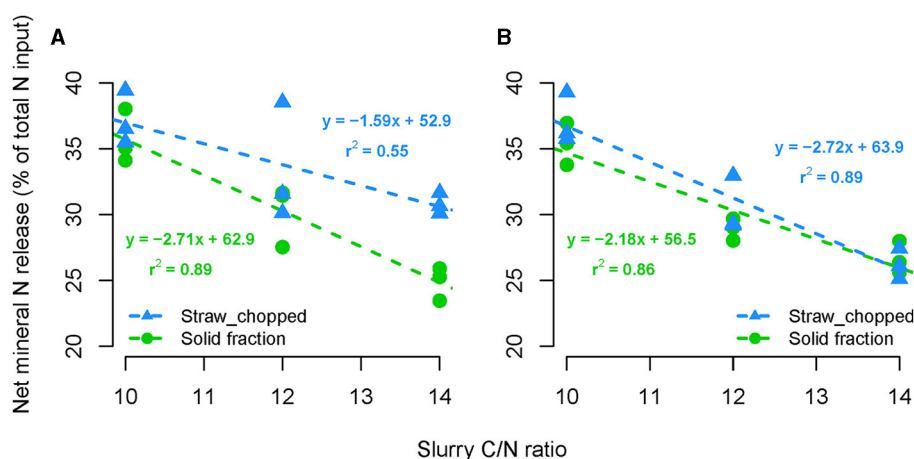


FIGURE 2

Relationship between C/N ratio and net mineral N release from experimental slurries with the bedding materials chopped straw and solid fraction after (A) 14 and (B) 28 days of incubation. Regression analysis showed a p -value < 0.001 for the factor C/N ratio at both day 14 and 28. For bedding material, the effect was non-significant ($p > 0.05$) both at day 14 and 28. For the interaction between C/N ratio and bedding material, the p -value was 0.109 at day 14 and 0.292 at day 28.

chopped straw (38%), and lowest for sawdust (33%; $p < 0.001$; Supplementary Table 7, Figure 5B).

4 Discussion

4.1 C and N turnover during the 1st days of incubation

The high initial CO_2 production rate in all treatments was most likely an effect of breakdown of short-chain volatile fatty acids (VFA) from LF, as they are an easily available C source that is

quickly consumed during the 1st days of incubation (Kirchmann and Lundvall, 1993). The higher initial CO_2 production from slurries with lower C/N ratios (Supplementary Table 5, Figure 3, day 2) can be explained by the smaller amount of “extra” C added with bedding material in the slurry mixes at low C/N ratios (Table 2). Hence, those slurries contained a higher proportion of easily degradable C components, including VFAs.

Slurry with solid fraction had the highest initial CO_2 production of the different bedding materials at C/N ratio 12 (Supplementary Table 6, Figure 5A), probably as a result of an already established micro fauna, due to its previous contact with animal feces. Chopped straw had the lowest initial CO_2 production,

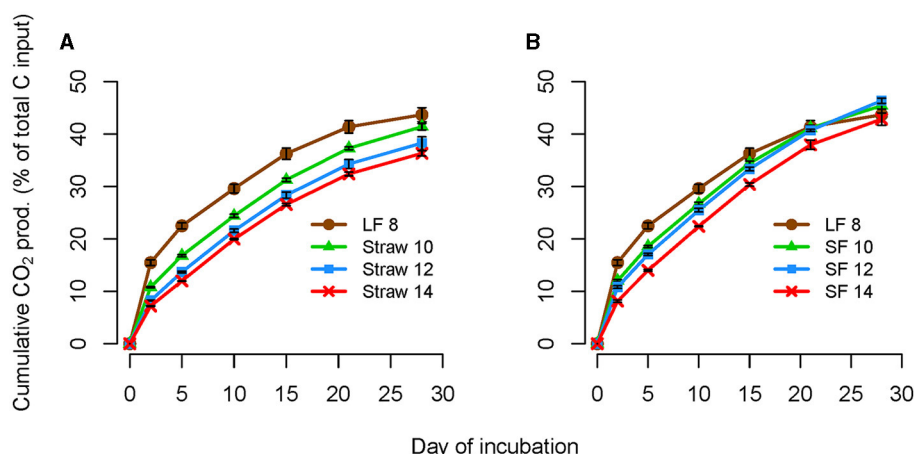


FIGURE 3

Cumulative net CO₂ production from experimental slurries at different C/N ratios and with the bedding materials (A) straw and (B) solid fraction during soil incubation ($n = 3$). C/N ratio 8 (without bedding material) was not included in the two-way ANOVA analysis, but is shown in the figure for comparison ($n = 3$). Error bars represent standard error of the mean (SEM). The two-way ANOVA showed significant main effects ($p < 0.05$) for both C/N ratio and bedding material at all sampling occasions. The interaction between C/N ratio and bedding material had p -values < 0.05 for day 2, 5, and 10.

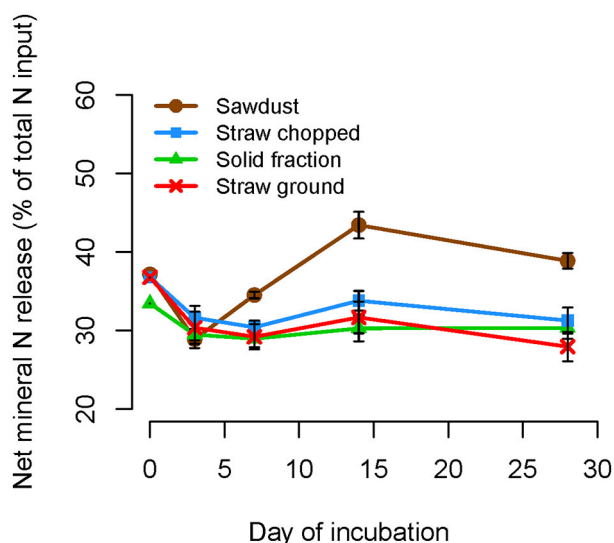


FIGURE 4

Net mineral N release during soil incubation from experimental slurries with different bedding materials, all with C/N ratio 12. Values at day 0 are the calculated amounts added for each treatment. Error bars represent standard error of the mean (SEM). The one-way ANOVA showed p -values < 0.05 for day 7, 14 and 28.

probably owing to its larger particle size and thereby smaller specific surface area compared with sawdust and ground straw. Sawdust had higher initial CO₂ production rate than chopped straw despite lower chemical C availability (more lignin), likely as a result of the smaller particle size making the most easily degradable C fractions accessible from the start (Sørensen et al., 1996).

Rapid initial N immobilization, similar to that in this study (Figures 1, 5), has been observed in a previous study which found a strong correlation between N immobilization and initial VFA

content of slurries (Kirchmann and Lundvall, 1993). All treatments in the present study contained similar amounts of LF (the main source of VFAs) and showed similar initial N immobilization. This indicates that mineralization/immobilization processes during the 1st days after soil application were mainly driven by the properties of LF, while the added bedding materials had little influence.

4.2 Later C and N mineralization patterns

From day 5, the CO₂ production rate followed a slowly declining pattern that continued during the rest of the incubation. During this phase, the most easily available C sources had been depleted and the processes controlling C turnover seemed to be dominated by the properties of the bedding materials added. This was reflected e.g., in sawdust giving the lowest CO₂ production through having the highest lignin content, and in higher cumulative CO₂ production from ground straw than chopped straw. Higher CO₂ production from ground wheat straw compared with chopped straw during the 1st weeks after soil application was also observed by Angers and Recous (1997), and was hypothesized to be a result of higher N accessibility with finer particles. In studies with low C/N ratio amendments such as rye residue (Angers and Recous, 1997) and pea residue (Jensen, 1994), where N content in the residues was less limiting for decomposition, the opposite relationship was seen. This was attributed to more intimate association with soil mineral particles for the ground residues, leading to physical protection of C in microbial biomass and by-products. In the present study, CO₂ production rate (averaged over C/N ratio 10–14) was consistently higher from SF compared with chopped straw, despite similar fiber composition, which was likely a combined effect of differences in particle size and degradation by microorganisms, as discussed in the section above for initial CO₂ production. For nitrogen, the decline in net mineral N release at C/N ratios 8, 10, and 12 from

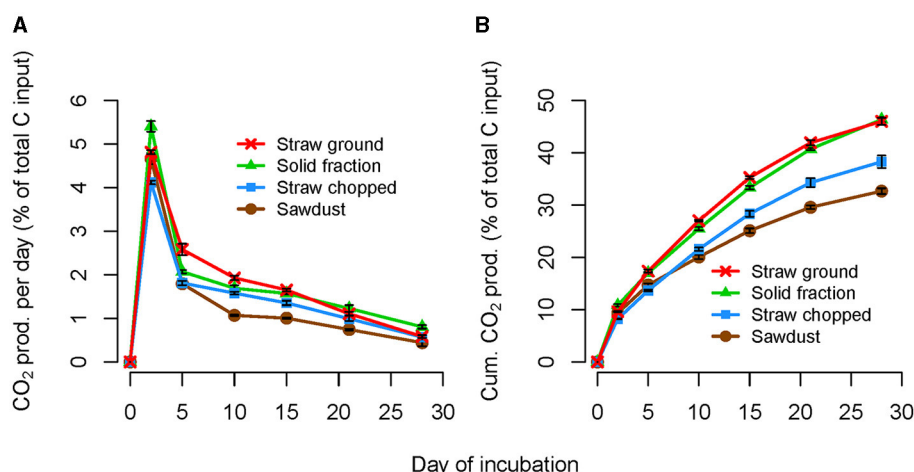


FIGURE 5

(A) Carbon dioxide production per day and (B) cumulative CO₂ production from soil incubation of experimental slurries with different bedding materials, all with C/N ratio 12. Error bars represent standard error of the mean (SEM). In both one-way ANOVA analyses, the *p*-values were <0.05 for all sampling occasions. Note different scales on Y-axis.

day 14 to day 28 (Figure 1A) could potentially be explained by re-immobilization of mineralized N and/or gaseous N losses through denitrification (not measured).

4.3 Nitrogen turnover and slurry C/N ratio

The negative linear relationship between C/N ratio and net mineral N release (Figure 2) is in line with hypothesis 3. The slope of the relationship was anticipated to be steeper with chopped straw than SF, based on the assumption that the straw contained more un-degraded and easily available C, resulting in greater N immobilization. This was not the case, and analysis of the fiber fraction revealed very similar content of hemicellulose, cellulose, and lignin in SF and straw (Table 1). The tendency for a steeper slope of the relationship for SF at day 14 (Figure 2A) is likely because microorganisms were already integrated with that material, whereas the straw had to be colonized, slowing down the straw degradation process and hence the N demand and immobilization. In addition, chopped straw had larger particle size, making the C less easy to access, which likely also delayed the degradation process (Sørensen et al., 1996).

A negative relationship between C/N ratio and N availability has been reported previously, for different organic fertilizers by Delin et al. (2012) and for cattle slurry by Sørensen et al. (2003). Sørensen et al. (2003) found that N availability was positively correlated with ammonium N content of the slurry, but in the present study the ammonium N content was within a narrow range (Table 2) and the relationship could not be evaluated. Norris et al. (2019) found that prediction of N supply from slurries could be improved by considering the ratio of total C to water-extractable N (rather than total N), but that considering more labile C fractions rather than total C weakened the predictions. This suggests that the amount of bioavailable N in organic material has a stronger influence on N mineralization/immobilization processes than amount of bioavailable C.

4.4 Bedding material particle size

The hypothesis that higher C availability due to smaller particle size would increase N immobilization was neither supported nor refuted. Ground straw showed higher cumulative CO₂ production than chopped straw (Figure 5) and a tendency for greater net N immobilization (Figure 4), but the difference in net mineral N release was not significant. However, on comparing ground and chopped barley residues added to soil, Ambus and Jensen (1997) found greater N immobilization with finer particle size after 2 weeks, and the difference remained throughout their 60-day incubation. Microbial growth was similar regardless of residue particle size in that study, leading to the conclusion that despite grinding, some C in the residues was still physically protected by lignin. In the present study, there were two different interacting sources of added C (LF and bedding material), which makes the results more difficult to interpret.

4.5 Relationship between C and N turnover

After 28 days, net mineral N release was similar for SF and chopped straw, despite differences in CO₂ production. In a study by Jensen et al. (2005), net N mineralization from a wide variety of mostly low-lignified plant materials was most closely correlated with concentrations of total plant N and neutral-detergent soluble N. In the same study, C mineralization was most closely correlated with holocellulose (hemicellulose + cellulose) content, which is in line with our observation that sawdust had both the lowest cumulative CO₂ production and the lowest holocellulose content. According to Chen et al. (2018), cellulose is rapidly degraded after soil incorporation of straw, while lignin is not broken down, but accumulates in the soil.

With regard to our three starting hypotheses, the results showed that: (1) C availability (as indicated by cumulative CO₂ production) depended on a combination of bedding material

particle size, fiber composition, and probably previous degradation by microorganisms; (2) for bedding materials with a relatively low degree of lignification (i.e., straw and solid fraction) there was no relationship between C and N availability, while for sawdust, with higher lignin content, high net mineral N release was associated with low CO₂ production; and (3) for both SF and chopped straw, there was a negative linear relationship between slurry C/N ratio and net mineral N release, with similar slope for both bedding materials. The contrasting results for sawdust, with substantially higher net mineral N release than from the other bedding materials, show that in addition to slurry C/N ratio, bedding material fiber composition is an important factor affecting slurry N availability.

4.6 Practical and environmental implications

The results indicate that bedding materials with similar fiber composition (i.e., SF and straw) give slurries with similar short term fertilizer value, when applied in amounts resulting in the same slurry C/N ratio. Applied at farm scale, this means that since SF has a lower C/N ratio than straw (Table 1), addition of similar amounts of SF as with straw would result in a lower slurry C/N ratio and hence potentially in higher N availability. The results also indicate that sawdust as bedding material could result in higher N availability, provided that the amount used gives the same slurry C/N ratio as with other bedding materials. This study confirms the relationship between C/N ratio and N availability, suggesting that slurry C/N ratio can be used as a predictor for N use efficiency in crop production (Delin et al., 2012). The results indicate that a higher N use efficiency can be expected from slurry containing sawdust, compared with SF and straw.

The environmental effects of slurry separation are more complex and difficult to predict. For the liquid fraction, the lower DM content increases the infiltration rate and hence reduces ammonia emissions after slurry application (Pedersen et al., 2022). When applied in spring or summer, the lower C/N ratio and thereby increased N availability can also improve the synchronization between N supply and crop N uptake. This potentially reduces the risk of mineral N surplus in autumn and subsequent losses during winter, when crop N uptake is low or no crop is present to take up the nitrogen (Delin and Stenberg, 2021; Sørensen et al., 2023). Simultaneous application of easily available carbon and nitrogen, as with all animal slurries, may stimulate denitrification and increase the risk of nitrous oxide emissions (Senbayram et al., 2012). For soil application of the liquid fraction from separated slurries and digestates, studies have shown both similar (Holly et al., 2017) and higher (Fangueiro et al., 2008b; Meng et al., 2022) nitrous oxide emissions, compared with raw slurries.

With covered storage of the liquid fraction, reducing the ammonia emissions, the overall environmental effect of slurry separation is largely dependent on how the solid fraction is treated during storage. With composting of the solid fraction, the total ammonia emissions are increased, while greenhouse gas emissions are reduced (Amon et al., 2006; Fangueiro et al., 2008a). Storage of the solid fraction without adding oxygen by turning, could either increase (Holly et al., 2017) or reduce (Hansen et al., 2006) the

emissions of nitrous oxide. A system where the solid fraction is used as bedding material is probably most comparable with composting, with good oxygen supply and hence increased ammonia emissions. However, an evaluation of that system would need to include a comparison of the in-house ammonia emissions with other types of bedding materials.

5 Conclusions

There was a negative linear relationship between slurry C/N ratio and net mineral N release after 28 days, with similar slope of the regression line for the bedding materials straw and solid fraction. Differences in C availability between these two bedding materials, as indicated by cumulative CO₂ production, did not affect N availability. The particle size of straw used as bedding material also had no effect. However, the relationship for sawdust was different, with higher net mineral N release than from SF and straw, in combination with lower CO₂ production, indicating limited C availability due to high lignin content. Therefore, if farmers use C/N ratio as an indicator of short term N availability in slurry, the same relationship can be used for calculation regardless of whether straw or SF is used as bedding material and regardless of particle size of the straw. If the same amount of bedding material is added, SF would result in a lower slurry C/N ratio, and hence potentially in higher N availability. If sawdust is used, N availability may be 24–39% higher at similar C/N ratio.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

KA: Conceptualization, Formal analysis, Investigation, Resources, Visualization, Writing – original draft, Writing – review & editing. AD: Formal analysis, Resources, Writing – review & editing. PS: Conceptualization, Writing – review & editing. SD: Conceptualization, Investigation, Resources, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. The work has been funded by Region Västra Götaland, Grant Number: RUN 2021-00020.

Acknowledgments

The authors thank Bo Stenberg for acquiring financial support for the project and for taking part in the conceptualization. Thanks also to Stina Larsson for practical assistance with the incubations.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2024.1393674/full#supplementary-material>

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EDITED BY

Tom Misselbrook,
Rothamsted Research, United Kingdom

REVIEWED BY

Mohd Huzairi Mohd Zainudin,
University of Putra Malaysia, Malaysia
Amita Shakya,
Amity University, Chhattisgarh, India

*CORRESPONDENCE

Asma Sikander
✉ asma.sikander@tuhh.de

RECEIVED 15 May 2024

ACCEPTED 01 August 2024

PUBLISHED 13 August 2024

CITATION

Sikander A, Deegener S and Körner I (2024)
Decomposition of biodegradable plastic bags
for kitchen waste collection in industrial
composting.
Front. Sustain. Food Syst. 8:1433460.
doi: 10.3389/fsufs.2024.1433460

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Decomposition of biodegradable plastic bags for kitchen waste collection in industrial composting

Asma Sikander*, Stefan Deegener and Ina Körner

Hamburg University of Technology, Institute of Wastewater Management and Water Protection, Hamburg, Germany

With the growing awareness of microplastics in the environment, the question of microplastics as remnants of biodegradable plastic (BDP) products is gaining momentum. BDP bags for kitchen waste collection are often certified as fully compostable as proof of industrial compostability. Frequently certification is done using under standardized laboratory conditions within 12 weeks. Composting plants often process biowaste into compost in a shorter time under inhomogeneous conditions. This study investigates the decomposition behavior of commercially available BDP bags for kitchen waste collection at an industrial composting plant within a practically realistic time frame of 6 weeks. The research determines the decomposition behavior under practice conditions. Four bag types, two made from thermoplastic starch (TPS) blends and two from polylactic acid (PLA) blends underwent composting. The decomposition was evaluated by examining the remaining macro- (> 8 mm), meso- (2–8 mm), and microparticles (1–2 mm) in the compost with different methods. After 6 weeks, the mass-based decomposition rate of TPS blend bags exceeded 95%. In contrast, PLA blend bags failed to reach a decomposition rate of 90%. The study did not depict very small particles <1 mm. However, they are present. The study findings indicate that composting time and conditions, inhomogeneities, as well as the bag type are crucial factors influencing the decomposition of BDP bags. Concerns are raised about the usefulness of DIN 13432 as certification scheme for industrial conditions.

KEYWORDS

biowaste management, kitchen waste collection, biodegradable plastic bags, industrial composting, certification, microplastic

1 Introduction

Rising environmental concerns associated with conventional plastic waste have prompted a shift towards the utilization of biodegradable alternatives. For biowaste collection from households, biodegradable plastic (BDP) bags have been designed to undergo microbial decomposition under specific environmental conditions. They are marketed as promising for a more sustainable waste management (VERBUND, 2023).

In Germany, household biowaste can be disposed in collection bins (bio bin) using BDP bags in accordance with the Biowaste Ordinance (BioAbfVO, 2022), given compliance with specified requirements. Beside acceptance of the local waste management company, it includes certification in accordance with DIN EN 13432 (2000, 2020) but goes a step further by

considering a shorter timeframe of 6 weeks for decomposition (DINplus, 2020). The DIN 13432 standard attests to complete the defined BDP decomposition goals within a maximum period of 12 weeks. It defines a material as fully compostable if 90% of the organic matter biodegrades under standardized laboratory composting and disintegrates into particles <2 mm.

The quality requirements related to foreign matter in compost are regulated also by the Biowaste Regulation (BioAbfVO, 2022) in §4 (4). It stipulates that deformable plastic foils are allowed to be present to a maximum of 0.1% of the compost dry matter (DM). This value is also expressed in the fertilizer regulation (DüMV, 2021) in § 4 (3). Remaining BDP particles would contribute to the foreign matter in compost. Many compost plant operators raise the question of the practical feasibility to achieve complete decomposition within the stipulated six-week period and have concerns of BDP residues in the compost. They also express apprehension regarding potential compost contamination by especially small BDP particles. The need to address these concerns becomes crucial in light of evolving awareness of microplastic-related issues in all environments including composting facilities.

This paper aims to investigate the decomposition behaviour of BDP bags under industrial conditions. In the context of this study, the term “decomposition” is defined as the process involving both biological degradation into carbon dioxide and water and disintegration into smaller particles. These processes occur concurrently and may influence each other. Our investigations encompass the recording of both together and due to their interdependence, these processes cannot be reported separately. Consequently, statements on decomposition in this study encompass both processes collectively. To assess the decomposition, areas and masses of the remaining BDP bag particles after composting were examined. The methods employed were evaluated for their informative value in determining the degree of decomposition. A comprehensive understanding of the BDP decomposition and the resultant impurities including the methodological proofs and their uncertainties is imperative to assess the overall effectiveness and environmental compatibility of these materials.

2 Materials and methods

2.1 Materials

2.1.1 Household biowaste collection bags

In the conducted experiments, four distinct biowaste collection bag types (denoted as S, G, B, R) were employed (Figure 1). The G-series employed two bag variants, G1 and G2, varying in their maximum filling volumes. These bags were produced from BDP, certified as compostable according to DIN EN 13432 (2000). The BDP bags, sourced from various manufacturers, were chosen to encompass a broad representation of the material compositions prevalent in Germany.

One major compound in all bag types was polybutylene adipate terephthalate (PBAT), the second polylactic acid (PLA) or thermoplastic starch (TPS). In the following, the respective materials were termed as PLA-blend or TPS-blend. This information on the major compounds was provided by the companies. Information on additives were not available.

Table 1 presents a comprehensive overview of the primary characteristics of the bags. They differed in the maximum filling volume and the foil thickness and therefore also in the foil area and the bag mass. Details regarding determination of the parameter are available in Deegener et al. (2022).

2.1.2 Biowaste in the bags

The biowaste used for filling the bags was a mixture from food waste and bio bin waste in an approximate ratio of 1:2. The mixing was done using a shovel until the mixture had no watery consistency anymore. The bio bin waste consisted mostly of green waste from gardens with some food waste. During mixing and bag filling (Section 2.2.1 Filling of BDP bags), the biowaste underwent a manual removal of larger impurities, such as plastic films and coarse plastic contaminants. This process eliminated large undesirable elements from the biowaste to a large extent.

2.1.3 Biowaste around the bags inside the nets

Twenty filled bags (Section 2.2.1 Filling of BDP bags) formed one batch and were enclosed by bio bin waste (Section 2.1.2 Biowaste in the bags). The materials were held together by a net. Details are to be found in the Supplementary Table 1. From the bio bin waste, the larger impurities were removed as described before. In total, a set of 32 nets was crafted for the experiment. These nets were designed to be stable, allowing for permeability of air and liquids while remaining resistant to decay. Additionally, they were intended to prevent substantial amounts of biowaste loss during transportation. To meet these specifications, a three-layered structure was produced using various net materials. The “bird protection net” and the “industrial net” were interlinked with a nylon cord, and the “football goal net” was introduced as a third layer to enhance overall strength.

2.1.4 Biowaste around the nets

Bio bin waste consisting mainly from green waste with a few food waste compounds was used to enclose the nets. Furthermore, some sieving residues from former industrial composting processes were contained. This mixture contained impurities. It represented the common substrate used in the composting facility and not the investigated samples from the net contents.

2.2 Method

2.2.1 Filling of BDP bags

Impressions from the filling are shown in Figure 2. For each bag type, a total of 160 BDP bags were filled with the food-waste rich mixture (Section 2.1.2 Biowaste in the Bags). For each bag, 1.5 to 2.0 kg of such waste was used. For the G-series, half of the bags were from type G1, the other from G2. Generally, half of the bags were left unsealed, while the remaining half were divided into two groups. For bag types B, G and S, one group was closed with a single knot, and the other with a double knot. For bag type R, which features a drawstring, half of these bags were left unsealed, while the other half were closed using the drawstring mechanism.

For one experimental batch, 20 filled bags from one type (S, G, B, R) were merged to one batch and their cumulative masses and volumes

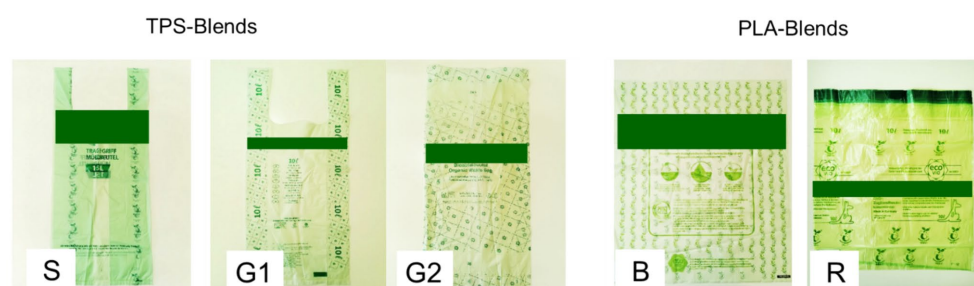


FIGURE 1
Classification of BDP bags by type designation (based on Deegener et al., 2022).

TABLE 1 Characteristics of employed BDP bags (based on Deegener et al., 2022).

Material	TPS-blend		PLA-blend	
Bag Coding	S	G1 G2	B	R
Maximum filling volume (L/bag)	15	10 30	20	12
Foil thickness (μm)	17	18 17	20	20 30*
Foil area (cm^2/bag)	4,200	3,900 5,800	4,600	3,600
Bag mass (g/bag)	9	8.5 12.5	11	9
Foil density (g/cm^3)	1.26	1.24 1.20	1.20	1.21

*Bag drawstrings from 30 μm thickness.

were determined. The average batch mass was 37 kg with a biowaste density of approximately 0.5 kg/L. For details see [Supplementary Table 1](#).

2.2.2 Filling of mesh nets

The process of net preparation is depicted from [Figure 3](#). The 20 filled bags from one batch were placed in the three-layered net along with the bio bin waste (Section 2.1.3 Biowaste around the bags inside the nets). The average content per net was 97 kg with more than one third consisting of filled bags ([Supplementary Table 1](#)).

A total of 8 nets were prepared for each bag type. Each net was labelled (S1-S8, G1-G8, B1-B8, R1-R8) and attached with a rope for easy identification and handling. Subsequently, the nets were weighed and stored overnight outside before placed into the composting containers (Section 2.1.3 Biowaste around the Bags inside the Nets).

2.2.3 Intensive rotting in containers

The overall composting time was 6 weeks. In the first 2 weeks, an intensive rotting process in containers took place. Impressions of the container loading and before emptying are to be found in [Figure 4](#). The filled nets (Section 2.2.2 Filling of mesh nets) were positioned within four industrial 20 m³ containers, totalling two nets from each type (S, G, B, R) in one container. Nets were placed at the centre of each container and surrounded with the biowaste described in Section 2.1.4 Biowaste around the nets.

This supplemental biowaste was deposited into the containers using a wheeled loader. Once the containers were filled, they were sealed and weighed before being transported to the rotting site. There, the containers were connected to the aeration and temperature measurement system. The aeration rate was maintained at approximately 220 m³/h per container, representing the typical air flow in the company. More details on the company are to be found in [Deegener et al. \(2022\)](#).

After week 2, the containers were disconnected from the aeration system and subjected to another round of weighing. The container contents were disposed of in the rotting hall and all of the nets were recovered. It was at this juncture that the first round of sampling took place with one net from each type (Section 2.2.3 Intensive rotting in containers).

2.2.4 Post rotting in a windrow

The remaining 28 nets were embedded in the rotted biowaste from the containers. They underwent a subsequent phase of naturally ventilated post-rotting within a hall. Impressions of this post-rotting phase are depicted in [Figure 5](#). It took place for 4 weeks making the whole composting process 6 weeks. The post-rotting pile was turned after week 3, 4 and 5, each time connected with mass determination of the nets and with removal of a part of the nets for sampling (Section 2.2.3 Intensive rotting in containers). Following the completion of week 4, a defined irrigation took place on the remaining nets since dry out zones were detected. The degree of dryness of the nets was visually categorized into different levels, ranging from very dry to moist. The calculated water addition per net ranged from 6 to 44 L to adjust for optimum composting conditions. Also, the region below and above the nets was irrigated. The composting was complete in week six with the recovery of the remaining 8 nets.

2.2.5 Sampling from nets

Throughout the rotting phase, five samplings were conducted, occurring in the 2nd, 3rd, 4th, 5th, and 6th weeks. In the 2nd and the 4th week one net per batch was sampled, in all others two. Impressions of the net sampling are illustrated in [Figure 6](#). Upon opening the nets, their contents were emptied onto a tarpaulin (5 × 3 m). All clearly visible BDP fragments were collected by hand from the rotting material until no fragments were visible anymore. The gathered BDP



Food waste rich biowaste for bags



Filling of BDP bags with biowaste



20 filled BDP bags for one net

FIGURE 2

Preparation of filled BDP bags for composting in nets (partially based on Deegener et al., 2022).



Net content with biowaste and filled BDP bags



Closure of the inner mesh layers



Determination of net mass with a crane scale

FIGURE 3

Preparation of nets for the composting experiment (based on Deegener et al., 2022).

fragments were air-dried over a week, weighed and stored for subsequent investigations.

The rotting material remaining after BDP fragment removal was reduced to a sample size of 10–20 L in order to achieve a representative sample for further analytics. The material was mixed using shovel and shaped into a flat square. This square was divided into four segments. Two opposite segments were selected and the other two removed (Figure 6). This process was repeated until the desired sample quantity was achieved. All samples were stored under cooled conditions for future analysis.

3 Measurement and analysis

3.1 Aeration, temperature and water content determination

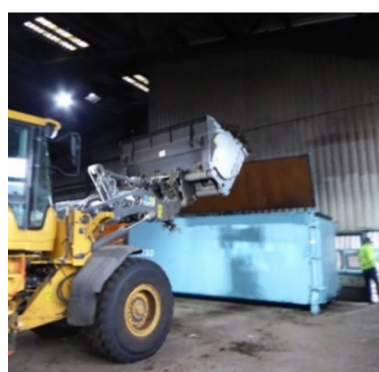
The intensive rotting containers were actively aerated with 220 m³/h following the company's standard while the post-rotting windrow was naturally ventilated.

During intensive rotting, container temperatures were continuously monitored using the company's standard plug-in sensors. In the post-rotting period, windrow temperatures were measured weekly with laboratory sensors after net removal. After the recovery of nets from the windrow the inside temperatures were determined by a lance sensor. Additionally, surface temperatures were partially assessed with a FLIR thermal imaging camera.

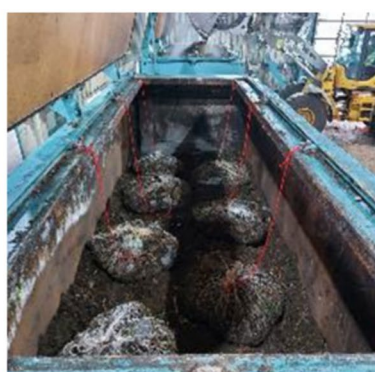
The water content of the representative samples of the nets rotting materials was determined by drying a 20 g sample at 105°C following (DIN EN 15934, 2012).

3.2 Separation of BDP particles from representative samples

The BDP particle separation was carried out with the representative samples received after removing the large BDP fragments (Section 2.2.3 Intensive rotting in containers) from net contents. Sieving was conducted using a mechanical screening machine (Retsch, type AS 200 basic B). Approximately 100 g of an air



Filling of container with biowaste



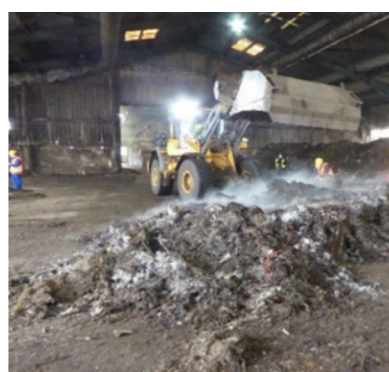
Placement of nets in a container



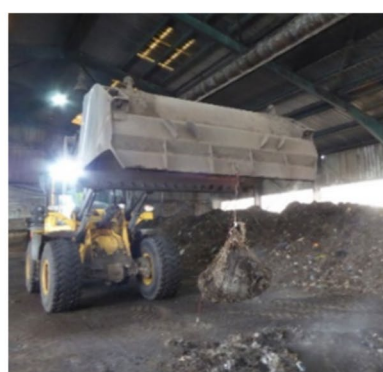
Rotted biowaste after 2 weeks

FIGURE 4

Images from the container filling for the intensive rotting phase (based on Deegener et al., 2022).



Formation of post-rotting windrow



Placement of a net



Removed nets at end of post-rotting

FIGURE 5

Images from the post-rotting phase till finalizing of the composting process (based on Deegener et al., 2022).

dried (room temperature, 24 h) test sample was sieved for 5 min using a sieve tower including the following mesh sizes: 16, 8, 4, 2, and 1 mm. The sieving was performed as double determination. After sieving, the various sieve fractions were weighed using a laboratory balance.

BDP bag fragments were manually removed from the various sieve fractions. Approximately 5 g of each sieve fractions material was evenly spread onto a petri dish. Utilizing an illuminated magnifying glass and tweezers, BDP particles within these fractions were manually sorted, enumerated, and subsequently stored in 2 mL Eppendorf tubes for subsequent analysis.

3.3 Determination of BDP bags decomposition

3.3.1 Classification of BDP fragments and particles

The large removed BDP fragments from the nets content (Section 2.2.3 Intensive rotting in containers) and the smaller particles

removed from the sieve fractions (Section 3.2 Separation of BDP particles from representative samples) were segregated into three categories based on size: macroparticles (> 8 mm), mesoparticles (2–8 mm), and microparticles (1–2 mm). The majority of macroparticles were isolated from the entire contents of the net through manual procedures as previously described (Section 2.2.3 Intensive rotting in containers). Since BDP particles from sieve fractions measuring >8 mm occurred very seldom, these fractions were not subjected for further evaluation. All macroparticles therefore are assigned to the first removal step directly from the net's contents. The mesoparticle from 2–4 and 4–8 mm were analysed separately, but for further evaluation merged to one fraction. Furthermore, the microparticles <1 mm were removed, but not considered in this evaluation due to the large uncertainty of the results.

3.3.2 Mass determination of BDP samples

Mass determinations were determined for three types of samples, each using a specific method. Therefore each determination method has different uncertainties.



FIGURE 6
Preparation of samples for further analytics (partially based on Deegener et al., 2022).

3.3.2.1 Mass determination of the original BDP bags

The mass determination of the original BDP bags involved a dual approach: Firstly, several bags were weighted individually. Secondly, the mass of bag rolls was measured, and subsequently divided by the number of bags per roll. For subsequent calculations the mean value for each bag type was employed. Twenty bags per batch were used with the initial masses documented in the [Supplementary Table 1](#). The amount of BDP material in one net was around 0.2 kg with slight variations between the BDP bag types.

3.3.2.2 Mass determination of remaining BDP macroparticles

The determination of the mass of the BDP macroparticles removed directly from the nets content followed a multiple-step procedure. Initially, the separated particles underwent air drying for 1 week. Subsequently, manual efforts were employed to eliminate adhering dirt ([Figure 6](#)). The resultant was termed as “pre-cleaned macroparticle” and their mass was recorded. However, visual inspection indicated remaining impurities. A previously established cleaning protocol involving the use of different cleaning agents was implemented, ultimately selecting distilled water as the most suitable cleaning agent. In order to receive “cleaned macroparticles,” the pre-cleaned macroparticle sample was placed within an aluminium tray, weighed, subjected to oven drying at 40°C for about 24 h until a constant weight was achieved. The choice of 40°C was deliberate, aimed at safeguarding the integrity of BDP by avoiding the potentially adverse impacts of higher temperatures. The macroparticles were immersed in a beaker filled with distilled water, ensuring complete submersion. Continuous stirring for 10 min (100 rpm, room temperature) was performed using a magnetic stirrer. Post-cleaning, the sample was withdrawn from the water, relocated to the aluminium foil, and placed in the oven at 40°C for drying. Subsequent to the drying process, the samples were once again weighed to ascertain the final mass of the remaining macroparticles. The method has a lower accuracy compared to the method for original BDP bag mass. On one side the particles remained light brownish, suggesting some remaining impurities leading to a slight overestimation of mass. On the other side, small

particles might detach from the large particles, leading to an underestimation.

3.3.2.3 Mass determination of remaining meso- and microparticles

Their masses were approximated using the counted particle numbers (Section 3.3.4), their area sum (Section 3.3.5) and the foil density of the original BDP bags ([Table 1](#)). This is the method has the lowest accuracy. The foil density is likely overestimated due to decomposition effects. The particle counts have their limitations in the inhomogeneities of the material also with careful representative sample preparation. And the area sum is also an approximation as explained in Section 3.3.4.

3.3.3 Area number determination of remaining BDP macroparticles

In order to compare the remaining BDP macroparticles of each batch, the “area number” of air-dried and pre-cleaned was determined. The macroparticles were loosely arranged without overlap to a rectangle onto a white 1 × 1 m surface. In cases where an exceptionally large quantity of macroparticles was present, they were distributed across two of such surfaces. The area covered with particles arranged in a rectangle was calculated as illustrated in [Figure 7](#) and designated as area number. A photograph of the resulting rectangle was captured as well.

3.3.4 Particle number determination of remaining BDP meso- and microparticles

The particles counted at each sieve fraction (Section 3.2 Separation of BDP particles from representative samples) were converted into number of particles per 100 g dry matter of the rotting material. This conversion utilized the mass of the rotting materials and the particles found in each sieve fractions as well as the moisture of the initial material. The BDP particles from 2–4 mm and 2–8 mm were subsequently merged to mesoparticles of 2–8 mm. Although microparticles in the fraction <1 mm were counted, the values were not further used for evaluation. However, 1–2 mm particles were assessed and classified as microparticles.

3.3.5 Area sum determination of remaining meso- and microparticles

The common methodology for the area sum determination used in the German compost quality assurance system (BGK, 2006) considers microplastic particles >2mm, separated in a way as described in Section 3.2 Separation of BDP particles from representative samples and using an image processing system for particle area calculation. The result is given in mm²/L compost fresh matter. To ascertain the “area sum” of BDP micro and mesoparticles in this experiment, two different procedures were implemented. Both were applied on the particles removed from the rotting material (Section 3.2 Separation of BDP particles from representative samples).

3.3.5.1 Image processing

Particle areas were determined for 2–8 mm mesoparticles with the SketchAndCalc app. The irregular areas of the particles depicted in

images of samples were determined as shown in Figure 8. To ensure the acquisition of clear images, paper was positioned beneath an inverted petri dish. The samples were arranged on the dish and subsequently flattened using tweezers, with the utilization of a magnifying glass when deemed necessary. Images were then imported into the app and the individual sample outlines delineated within the app interface facilitating the computation of their respective areas based on the defined scale. To gauge the accuracy of the procedure a calibration was carried out using the calibration image from BGK (2006) and Methodenbuch Kapitel II.C3.8 (2006).

3.3.5.2 Approximation based on particle numbers and their sieve sizes

To allow an easier routine determination in practice, an approximation was carried out based on the particle numbers. Mesoparticles from 2 to 8 mm were approximated to possess a square shape with an average side length of 5 mm, resulting in an average area of 25 mm² / particle. The “area sum” was then the multiplication result of the particle number and the average area per particle fraction. For microparticles from 1 to 2 mm, a side length of 1.5 mm was assumed yielding an average area of 2.25 mm²/particle.

The area sum for the particle numbers from one count was then recalculated with both methods referring to 100 g compost DM as well as to 1 L compost FM.

4 Results

4.1 Mass loss, temperature and water content

Mass loss, temperature and water content are the main parameters which describe a composting process.

4.1.1 Mass loss

Figure 9 illustrates the mass losses within the nets after intensive and post rotting. The highest mass losses were achieved during the intensive rotting phase. Nets removed after 2 weeks showed a slightly higher mass loss of their content (23–31% of the initial mass, Supplementary Table 3) compared to total container contents (16–23%, Supplementary Table 2).



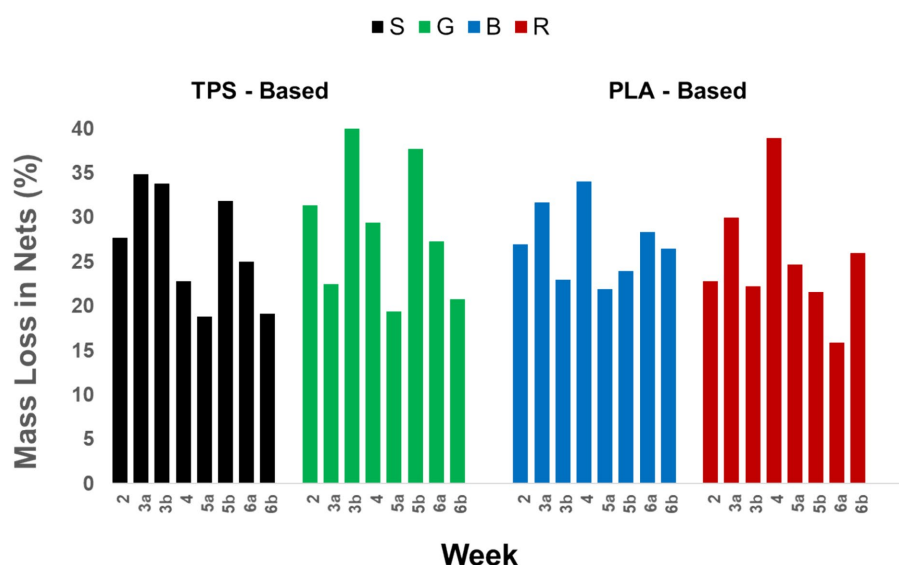


FIGURE 9

Mass losses within the nets during the experimental period (a/b referring to two nets from one type removed at the same time).

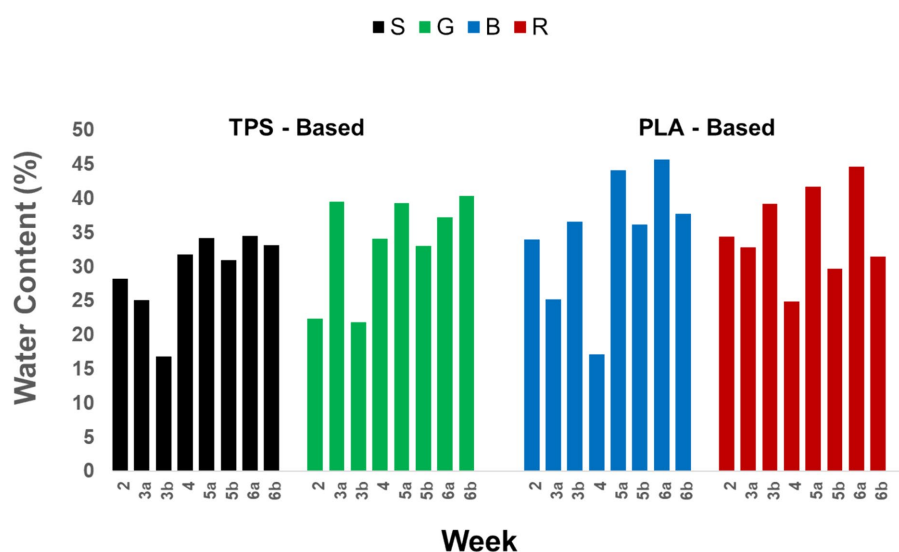


FIGURE 10

Average water content of the rotting material within the nets during composting. (a/b referring to two nets from one type removed at the same time; after week 4 water was added).

The average mass loss of 27% (nets) compared to 20% (container) can mainly be explained by the higher food waste content in the nets.

The mass losses of the nets in the post rotting phase ranged between 18 and 42% (Supplementary Table 3) with an average across different nets at 27%. Food waste was the primary contributor to biodegradation, followed by green waste, with negligible mass loss attributed to BDP degradation. Besides the first 2 weeks, the composting time was not the major parameter determining the mass loss. The parallel nets (a/b) clearly show, that at a specific time degradation results can be very different due to the inhomogeneity of the material and the specific degradation conditions (Figure 9).

4.1.2 Water content

The water content of the initial food waste was approximately 72% on a fresh matter (FM) basis, while biowaste exhibited a water content of around 63% FM. The water content within the bags and nets were originated from the compositions described in Section 2.1 Materials. For biowaste adjacent to the nets it was around 47% FM. In conclusion, the average water content in the nets were within a range conducive to composting with a higher water content in the bags compared to the material around.

The average water contents within the nets after their removal from the intensive or post-rotting are documented in the Supplementary Table 3. Figure 10 illustrates this water contents.

The significant reduction in water content observed during intensive rotting resulted from the implementation of intensive aeration (Section 2.2.3 Intensive rotting in containers) in connection with high temperatures. A water content below 25% can strongly inhibit the composting process, whereas levels below 20% can essentially halt it, as the composting microorganisms become inactive. Since in several nets such situation occurred, it was decided to moisten the net contents, so that optimum conditions for composting were established again. The subsequent increase in water content after week 4 was attributed to this deliberate water addition as outlined in Section 2.2.4 Post rotting in a windrow. At the end of composting, water content was in a suitable range for compost marketing. BGK (2023) gives guidance values in the range from 35 to 45% FM.

A comparison of Figures 9, 10 shows the relation of water content to mass loss. Specifically high mass losses were connected with low water contents and vice versa, meaning that the water release contributes significantly to the mass loss and also to the development of inhomogeneities. The water contents displayed in Figure 10 were originated from the representative samples presenting the net average. The distribution of the specific water contents in the nets was very inhomogeneous ranging partly from dry out zones to wet zones. This emphasises the occurrence of zones with different composting conditions in small areas.

4.1.3 Temperature

Details to the temperature developments within the containers are to be found in Supplementary Table 2. The temperatures within the nets after their removal from the containers or the pile are documented in the Supplementary Table 3.

During the intensive rotting phase in all containers maximum temperatures exceeded 75°C with a cooling till the container opening. However, at the end of the intensive rotting the temperatures remained above 65°. The temperature development in containers 1, 3 and 4 was quite similar, in container 2 temperatures were around 5° higher. The temperatures in the post rotting weeks 3–5 varied strongly between 22 and 70°, indicating inhomogeneity in the composting process. After week 4 the temperature increased partially in the specifically dry nets after moistening. However, generally a tendency of cooling was detected with temperatures between 42 and 56° at the end of the composting time of 6 weeks. These high temperatures show that the material was still active, but suitable rotting degrees for marketing of the compost were reached.

The inhomogeneity of the temperatures and the wide range of composting conditions in one net is shown in Figure 11. Formation of inhomogeneities initiates early due to the heterogeneous nature of the decaying material, and it progresses during composting as a result of diverse degradation processes stemming from material disparities. Additionally, varying water content levels arise due to aeration via unevenly distributed airflow, further increasing the inhomogeneities.

4.2 Determination of BDP bag decomposition

4.2.1 Remaining BDP macroparticles

Figure 12 presents the macroparticles residual mass after washing with distilled water related to the original BDP mass and Figure 13 provides the area number as a parameter for the visual impression of the remaining BDP impurities. Supplementary Table 5 gives the respective data needed for their calculation as well as data to the dirt removal by washing with distilled water.

4.2.1.1 Impact of washing

Washing of pre-cleaned macroparticles with distilled water led to a significant mass loss indicating that a substantial amount of dirt was removed. It was highest in the samples S4, S5, G2, R2, R6 and R7, where more than 30% of the mass of the pre-cleaned was assigned to dirt removable by washing. However, the removal percentages exhibited significant variability across different nets, spanning from 3–36%.

The results show that washing of particles is necessary if decomposition of BDP bags is to be evaluated by means of comparison to the initial BDP bag mass. Therefore, the masses of the cleaned macroparticles were determined individually for each net with a separated washing procedure for each sample.

4.2.1.2 Remaining BDP macroparticle masses

Figure 12 presents the remaining macroparticle mass for the different BDP bag types in dependency of the composting time. It is notable that the TPS-blends showed a higher decomposition compared to the PLA-blends.

The best PLA-blend bag decomposition result was found in net B5a with a residue of 2% macroparticles related to the initial bag material. The best results for TPS-blend bags exhibited nets S4, G5a,

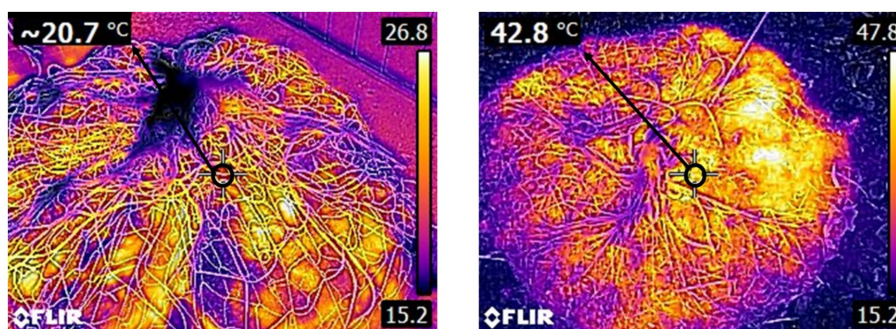


FIGURE 11

Examples for the surface temperature distribution in the nets before placement in a container (left) and after removal from a container (right).

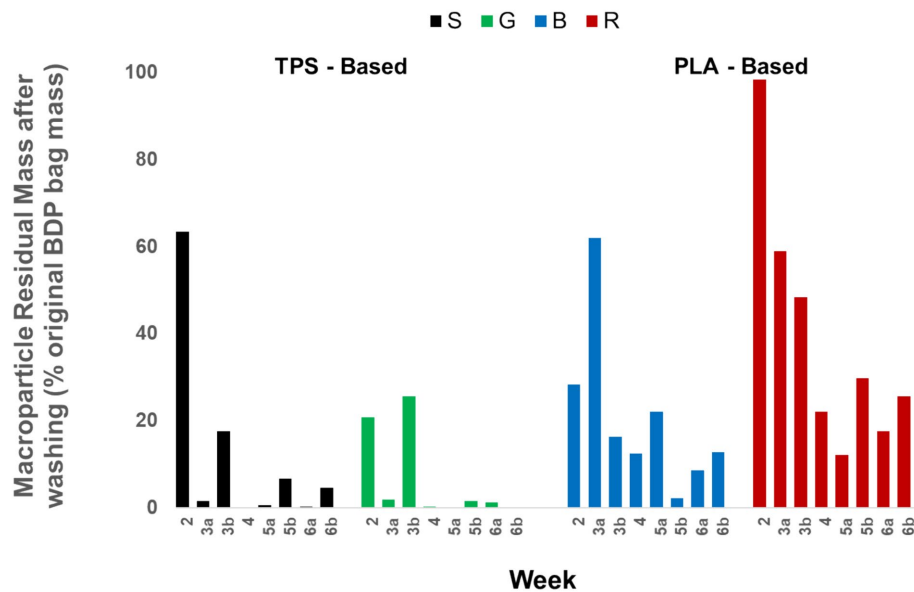


FIGURE 12

Mass share of cleaned macroparticles remaining after composting in relation to the original BDP bag mass per net (a/b referring to two nets from one type removed at the same time).

and G6b with minimal remaining macroparticles ($< 0.1\%$). However, the parallel nets show larger remains.

In conclusion, a complete decomposition was not achieved for either bag type. For TPS-blend bags a decomposition with only minimal visual residues seems in principle possible, but it requires stringent, homogeneous conditions, which may be challenging to replicate at practical scales without substantial effort.

4.2.1.3 Remaining BDP macroparticle area numbers

The macroparticle residues have a strong impact on the visual impression of a compost. The area number (Section 3.3.3 Area number determination of remaining BDP macroparticles) of the manually removed BDP macroparticles was therefore determined for each net. The photographic documentation for each net depicting the top view of the macroparticles areas, which are resulting in the area numbers per net is available in [Supplementary Figure 2](#) and for the TPS-blend bags and [Supplementary Figure 3](#) for the PLA-blend bags. This documentation includes also information about the composting time, as well as the average water content and the temperatures in the nets rotting material. The temporal evolution of the macroparticles area numbers is illustrated in [Figure 13](#).

Comparing [Figure 12](#) with the mass-related determination and [Figure 13](#) with the area-related determination, the same trend is obvious. Considering the large efforts needed for washing, the area number has advantages in the simplicity of the method. It is suitable for an optical evaluation and comparison of composts. However, if data for the decomposition rates or particle mass residues are needed, the mass determination is the better method.

4.2.2 Remaining BDP meso- and microparticles

[Figure 14](#) presents the numbers of meso- and microparticles related to compost DM. [Supplementary Table 6](#) gives the respective data and additionally the data related to compost FM. Data for the

area sum of mesoparticles (2–8 mm) in one net determined with the image processing method and the approximation method are compared in [Supplementary Table 4](#). In [Figure 15](#) the area sums of micro- and mesoparticles related to the compost volume determined by the approximation method are visualized with the respective data shown in [Supplementary Table 6](#). Particle mass data can be found in [Supplementary Table 5](#) and are visualized in [Figure 16](#).

4.2.2.1 Particle numbers

The numbers of the meso- and microparticles depicted in [Figure 14](#) show that both types were present in all nets. At week 6, the number of microparticles significantly exceeded the number of mesoparticles. Also particles < 1 mm were counted in [Deegener et al. \(2022\)](#). However, they are not displayed here since the uncertainty in the counts is high due to their small size. However, the trend towards an increase in number with a decrease in particle size was also observed there.

The microparticle number (1–2 mm) per 100 g of composting material typically varied between 100 and 400 for PLA blends and between 50 and 300 for TPS blends, with occasional deviations. The mesoparticle number was commonly smaller with a few exceptions (S5b, G5b, B4, R3a/b, and R5a/b). An accumulation of the number of mesoparticles occurred in the R series, which can presumably be attributed to the drawstrings in the bags.

Over time, an observable trend emerges wherein mesoparticles exhibit a decline while microparticles display an increase, indicating the fragmentation of mesoparticles. Notably, there existed significant individual variability within this comparison. The mesoparticle contribute also to the optical impression of a compost, but not the microparticles. However, the microparticles cannot be neglected. Due to their small size, they could easily be ingested by organisms with impacts on organisms and ecosystems. Re-determinations of numbers from the stored particles lead partly to slightly different results

concluding that during storage and by handling larger particles might break down and rather small particles might easily get lost.

4.2.2.2 Area sums

The area sums of mesoparticles determined with the image processing method and with the approximation method are compared in [Supplementary Table 4](#). The results show, that although there is a deviation between the two methods, the scales match. Therefore, the

approximation method based on the sieve fractions and a square particle with side lengths of the average from the upper and the lower sieve seems to be suitable for the comparison of the remaining small BDP particles with the advantage of easy determination. The area sums related to compost volume were therefore calculated for the mesoparticles as well as for the microparticles as shown in [Figure 15](#).

The results show that mesoparticles are the main contributor to the area sum. On average across all batches, the area numbers of

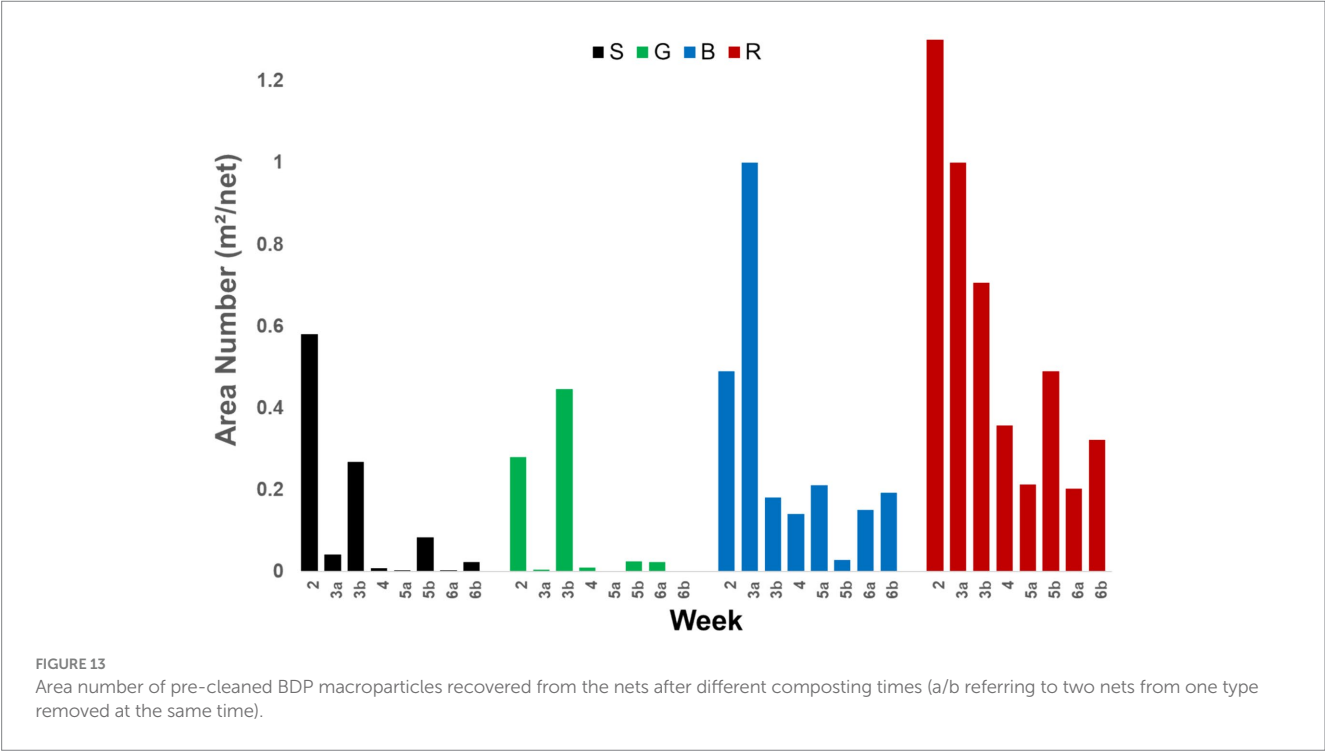


FIGURE 13
Area number of pre-cleaned BDP macroparticles recovered from the nets after different composting times (a/b referring to two nets from one type removed at the same time).

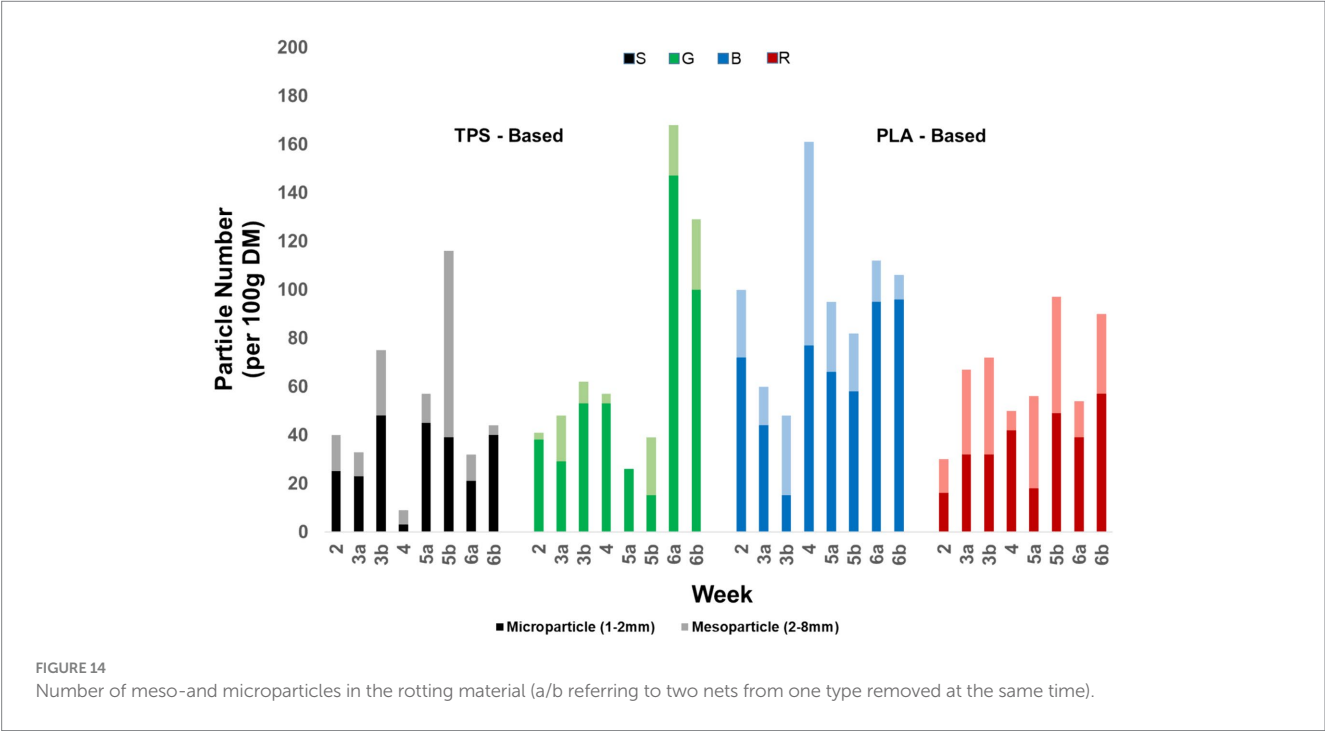


FIGURE 14
Number of meso- and microparticles in the rotting material (a/b referring to two nets from one type removed at the same time).

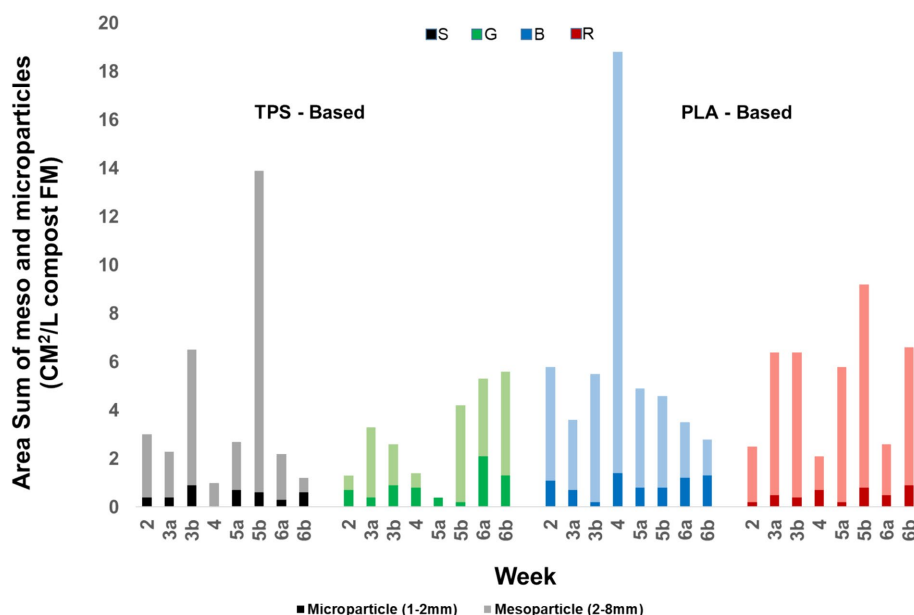


FIGURE 15

Area sum of meso-and microparticles in the rotting material (a/b referring to two nets from one type removed at the same time).

mesoparticles were approximately fivefold greater than those of microparticles. However, the area sum provided by microparticles is also significant and cannot be neglected. The limitation values for microplastic >2mm provided from BGK (2023) are 15 cm²/L for fresh and matured composts and 10 cm²/L for substrate composts. Considering the data from Figure 15, particles from BDP bags might be a significant contributor beside conventional plastic particles.

4.2.2.3 Particle masses

Since a washing procedure for meso-and microparticles would be error-prone and a considerable labor is involved, the masses were also approximated using the procedure described in Section 3.3.2 Mass determination of BDP sample. It is based on the particle area and the foil density of the original bags as shown in Table 1. However, the foil density serves as an estimate and may be slightly lower for degraded BDP bags due to a loss of compounds. Consequently, the mass values for micro- and mesoparticles are likely somewhat overestimated. The calculation results are shown in Supplementary Table 5 and further evaluated in Figure 17. In tendency the mesoparticle masses were larger than the microparticles masses with a few exceptions.

4.3 Decomposition levels of BDP bags

Decomposition levels of BDP bags during composting under industrial conditions were assessed by considering BDP bag's residual masses. The cumulative residual fractions of micro-, meso-, and macroparticles, relative to the original BDP bags, were calculated from the masses described in Section 4.2.1 Remaining BDP macroparticles for macro- and in Section 4.2.2 Remaining BDP

meso- and microparticles for meso-and microparticles. The decomposition levels were determined by quantifying the mass difference from 100%. The decomposition level determined based on mass analysis is illustrated in Figure 16. The distribution of residual mass across cleaned macroparticles as well as based on area determined meso-and microparticles is depicted in Figure 17 as a percentual distribution. Detailed results are provided in Supplementary Table 5.

The highest levels of degradation reached were observed in samples S4, G4, and G5b, with approximately 99% degradation. A few further batches reached degradation levels above 95%. However, they all could be assigned to TPS-blend bags. None of the PLA-blend bags reached such high levels. The most favorable outcomes were achieved by sample B5b, with nearly 95% degradation. There was also a clear difference between the B- and the R-series with a lower decomposition level in the R-series due to the drawstring which seemed problematic to degrade due to their higher foil thickness. In terms of composting time, the first 3 weeks had the most significant impact on decomposition for TPS-blend bags. However, with PLA-blend bags, an increase in decomposition level was observed for a longer duration, as depicted in Figure 17.

During decomposition rate calculations, microparticles (1–2 mm) were observed to be relatively minor compared to meso-and macroparticles. However, in the context of mass-based BDP decomposition analysis, they are not insignificant. Often macroparticles contributed strongest to the residual BDP bag mass, but with a lower trend with increasing composting time. All particle types are to be considered as relevant. They should be considered together in evaluation, since larger particles can easily be converted in to smaller ones by mechanical movements. In industrial composting this often is the case for instance during turnings, transports and sieving procedures.

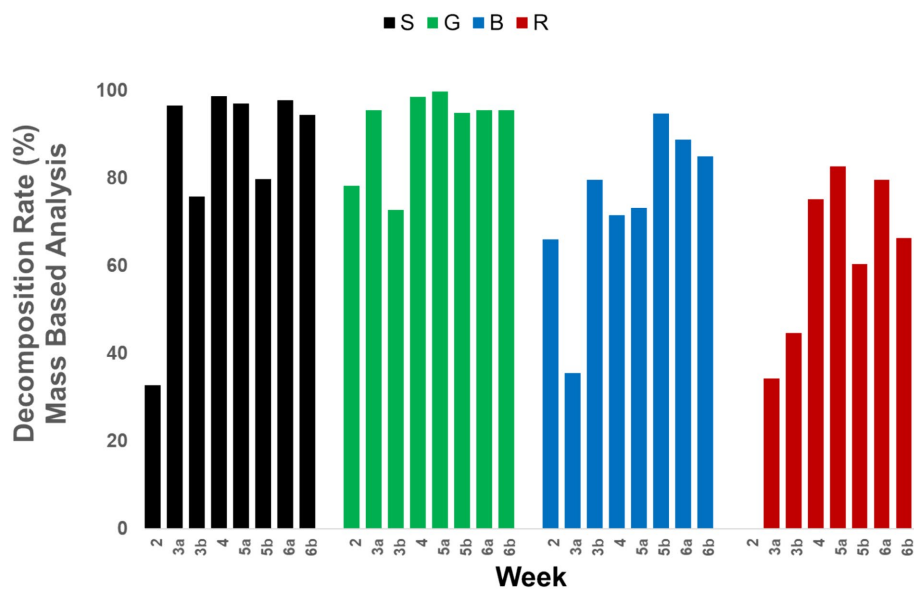


FIGURE 16
Mass based decomposition level of BDP bags considering macro-, Meso- and microparticles (a/b referring to two nets from one type removed at the same time).

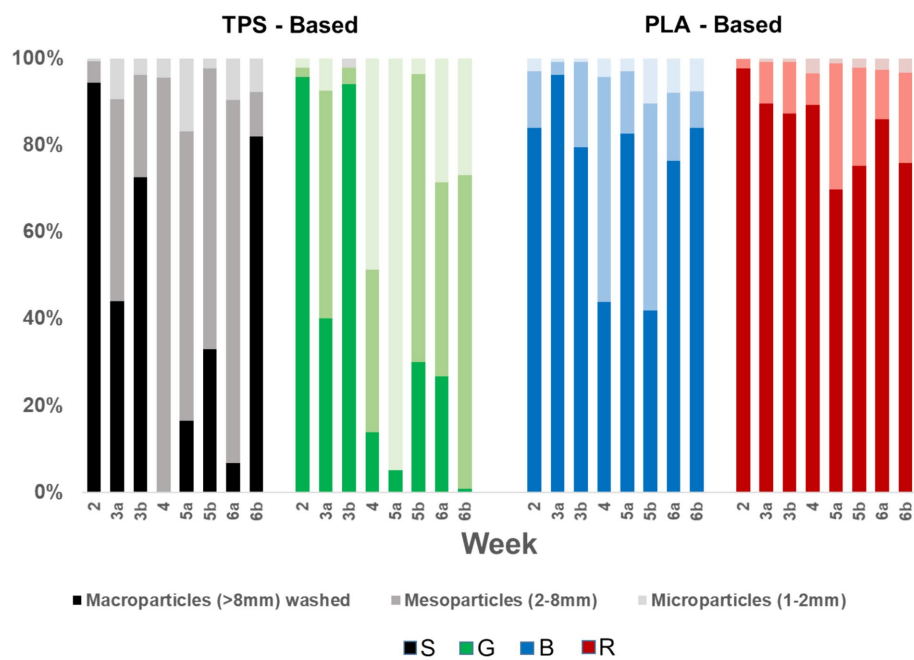


FIGURE 17
Distribution of residual BDP bag mass across macro-, meso- and microparticles (a/b referring to two nets from one type removed at the same time).

5 Discussion

5.1 Evolution of temperature and water content during composting

Composting time, water content, and temperature are paramount factors influencing decomposition of compostable materials. During

the experiments composting period, the evolution of temperatures and water contents followed typical patterns observed in the composting of biowaste, albeit with some expected variations and inhomogeneities.

In all containers, the recorded temperatures consistently surpassed the threshold of 75°C, indicative of robust microbial activity and effective decomposition processes. Towards the end of

intensive rotting phase, temperatures remained elevated, exceeding 65°C. This sustained thermal profile not only met the hygiene standards outlined by the [BioAbfVO \(2022\)](#) regarding material sanitization but also underscored the vigorous degradation activity within the containers. However, the rotting material degradation was still ongoing.

Maintaining appropriate water content throughout composting is crucial for effective decomposition, as indicated by guidelines set forth by [BGK \(2017, 2018\)](#). Initially, the water content of the incorporated rotting material fell within the recommended range of 50–65%, aligning with optimal conditions for composting. Instances of drying out connected with interruption of microbial degradation in general occurred in certain nets, where the average water contents dropped to approximately 20%, accompanied by lower temperature levels. This drying phenomenon can be attributed primarily to the intense aeration during the active decomposition phase. Additionally, water transfer occurred due to the higher water contents within the nets compared to the surrounding material. To address suboptimal water content levels, a targeted humidification process was implemented during the post-rotting phase. While this intervention positively influenced activity within the composting material, it did not result in significant changes in BDP decomposition rates. This lack of pronounced improvement in decomposition rates post-humidification suggests a potential disruption of the established BDP-relevant microbial biocenosis, likely induced by abrupt cooling coupled with heightened humidity levels.

Nets with lower average water content in the rotted material ([Figure 10](#)) tended to exhibit more remaining BDP particles, i.e., a lower decomposition level ([Figure 16](#)). This is obvious when comparing the parallel nets (a/b) from one type and time, where nets with lower water content resulted mostly in a lower total decomposition level. The only exceptions are G6a/b and B5a/b. Only a few nets (S3a/b, G3b, B3a, B4, and R4) has exceptionally low water contents $\leq 25\%$. Nonetheless, noteworthy instances of effective decomposition were also observed in some of these nets. The potential impact of water contents exceeding 40% remains uncertain, as investigations did not conclusively address this aspect.

These observations underscore the intricate relationship between water content, temperature, and microbial activity in determining the decomposition kinetics of BDP within composting environments. To receive [DIN EN 13432 \(2000\)](#) certification of biodegradability the BDP materials are tested under homogeneous and well-defined conditions. Commonly it worked with a temperature of 58%, without dry out zones and undisrupted microbial consortia. With such adjusted optimal ranges for BDP decomposition an advanced BDP decomposition under homogeneous conditions can be reached, as the multitude of certified BDP products prove. Under realistic industrial conditions the initial inhomogeneity of the material and during the process the development of multiple zones with different temperatures and water contents has to be kept in mind. Further investigations are warranted to elucidate the degree of acceptable inhomogeneity, the ranges of water contents in connection with the temperature profiles and the impacts of disruptions of microbial communities. Such insights are vital for refining composting protocols aimed at mitigating environmental impacts associated with plastic pollution.

5.2 Decomposition of BDP bags—particle numbers and sizes analysis

For compost quality, plastic particles from the meso- and microparticle size are relevant, since in the industrial composting practice, macroparticles often would be removed, e.g., by sieving, before the compost is marketed. However in the case of BDP macroparticles such mechanical procedures may result in their mechanical breakdown leading to an increase in meso- and microparticle numbers. This has to be kept in mind, when evaluating the meso- and microparticle numbers in this study.

The sieving process conducted on representative samples aimed to elucidate the presence of BDP meso- and microparticles within the composting material. Upon completion of sieving, it was observed that the number of BDP mesoparticles was relatively low compared to microparticles. In the study, the decision to exclude the analyses of the very small microparticles (<1 mm), although present in large number ([Deegener et al., 2022](#)), was justified based on the inherent uncertainties on the type of removed particles and the potential to overlook some. However, their presence as a result of decomposition is not considered to be irrelevant, but has to be studied with more advanced methods.

[Figure 14](#) provides insights into the relationship between composting duration and particle size number and distribution considering mesoparticles (2–8 mm) and microparticles (1–2 mm). These findings corroborate the notion that ongoing decomposition processes result in a reduction in the size of remaining BDP particles and an increase in number. However, the S-series shows that at a certain point the particle number will start to decrease due to advanced biodegradation. This reversal point was in the S-series between week 5 and 6. In the other three series (G, B, R) it was not reached within the 6-week study period. From [Figure 16](#) it can be estimated, that macroparticle handling can have a large impact on the meso- and microparticles in composts in practice.

When evaluating plastic particle numbers in composts, the sample homogeneity and representative sampling is crucial for ensuring the reliability and reproducibility. However, even when done with care as in this experiment, multiple determinations in one representative sample often varied by more than 100% ([Deegener et al., 2022](#)). Larger sample sizes would be helpful, however are connected with a huge additional work and effort. Evaluating composts plastics impurities by particle numbers is the most common method in research as an evaluation of [Porterfield et al. \(2023\)](#) showed.

5.3 Decomposition of BDP bags—area based analysis

The evaluation of decomposition through area-based analysis involved all particle fractions with exception of the very small microparticles (<1 mm), albeit employing diverse methodologies. The parameter “area sum,” as defined by [BGK \(2006\)](#) (Section II.3) is already in practical use in Germany for particles >2 mm and serves to quantify impurities in compost, expressed as cm^2/L of compost. In this study, it was also used and simplified to quantify the area sum of BDP meso- (2–8 mm) and microparticles (1–2 mm; [Figure 15](#)). Additionally, the macroparticle top view area ([Figure 13](#)) was determined with a different procedure and termed as area number.

The determination of macroparticle area numbers (Supplementary Figure 1; Figure 2) faces challenges in direct comparison with the initial bag areas due to dimensional variations. The initial bags were evaluated for two-dimensional (2-D) single-layer areas, whereas macroparticle samples from composting often exhibited multilayer agglomerates, including knots, drawstrings, and three-dimensional (3-D) wrinkling. Discrepancies arise from overlaps, voids, and missed fragments during manual readouts, leading to an underestimation of the actual macroparticle area. Consequently, the area number is not comparable with the initial bag area, and also not with the meso- and macroparticle area. However, determination is simple and quick and gives a good method to compare decomposition of BDP bags. The results show similar trends as the mass-based analysis of macroparticles. But to avoid misinterpretations of the decomposition, the smaller particle fractions have to be considered as well. For example, G6b showed no macroparticles, but the mass-based BDP-decomposition level was 95.5%.

The residual area of mesoparticles were determined via image processing, utilizing the SketchAndCalc app. A calibration provided an accuracy of the particle area measurement of 96%. This inexpensive and user-friendly app facilitates irregular area calculations based on the photographic images taken from the counted particles (Section 5.2 Decomposition of BDP bags—particle numbers and sizes analysis). This method was compared to a calculative approximation based on the particle numbers. The particle shapes were assumed to be square or a circle with side length or diameter as average of the sieve sizes used. The squares gave a better fit than the circles to the areas determined by image processing. The approximation method struggles to accurately determine BDP areas due to the irregular shape of the particles. However, the uncertainty in particle number counting is larger than the one due to the simplification of the particle morphology and size.

Thus, in this study, the approximation method was used to determine the area sum for the mesoparticles (2–8 mm) as well as for the microparticles (1–2 mm). This method offers an easy and robust comparison of different BDP batches. The area occupied by mesoparticles was clearly larger than that of microparticles (Figure 15). Cumulating all particles to one value makes a comparison of batches easy. Considering mesoparticles area sum as suggested by BGK (2006) is enough to characterize a product by the visual impression. However, considering the microparticles additionally is completing the picture and useful for the comprehensive mass-based analysis.

5.4 Decomposition of BDP bags—mass based analysis

The determination of the degree of decomposition of BDP bags requires analyzing both the masses before and after composting. In mass-based analysis, one focus lies on weighing the macroparticles. However, dust adhering to the particles during composting pose a significant challenge as they contribute to the mass of BDP residues. To mitigate this, a washing step is to be conducted to obtain more accurate measurements. It's worth noting that washing was performed solely for the macroparticles, as the small size of meso- and microparticles rendered washing impractical. For such particles

masses were calculated based on particle areas and foil densities. Since the mass determination of BDP bags before composting can be considered as quite accurate, the other methods have certain uncertainties.

During the macroparticle washing process, observations revealed challenges with stirring connected with particle disruption and particles adhering to surfaces. These issues underscore the importance of careful adjustments of the washing process to mitigate their influence on experimental accuracy. However, despite all care, the washed particles still had some brownish color. The procedure is laborious, time consuming and necessary for each batch individually, since the dust attachments can largely vary.

Direct mass determination of meso- and microparticles was omitted due to the impurity problems, low particle counts per sample, and the small size of the particles. Instead, higher accuracy was achieved by determining particle numbers, approximating their sizes and calculating their residual masses using the densities and thicknesses of the original bags. However, it's important to note that the densities of original bags and real residual particles may not be identical. Therefore, the mass fractions of micro- and mesoparticles remaining after mesh removal represent maximum values, as composting likely reduces particle density and thus residual mass.

However, the described determination procedure is considered suitable to evaluate the decomposition level of BDP bags. The relevance of all three particle types is evident in Figure 16, highlighting the comprehensive description of BDP decomposition without neglecting specific BDP shares. Their distribution could be quite manifold. Microparticles <1 mm were neglected, since their estimated proportion was considered insignificant when compared to the methodological uncertainties and would contribute a maximum of 1%. However, with mass-based determination, there is a lack of information on compounds that may have dissolved from the BDP bags. More advanced methods would be necessary for such evaluations.

6 Conclusion

The study highlights the complex challenges, issues, and potential solutions regarding microplastics in composts, emphasizing the importance of assessing the decomposition of BDP bags. Compliance with regulatory standards such as DIN EN 13432 (2000), DINplus (2020), DüMV (2021), and BioAbfVO (2022) is crucial, with criteria defining complete decomposition within specified timeframes. However, the methods employed currently have some weaknesses and can mislead to the assumption of fully degradability of BDP materials under industrial conditions. This is partly due to certifications being conducted under laboratory conditions and partly because very small particle sizes are excluded from consideration.

The research assess the decomposition of BDP bags following a composting period of 6 weeks. Within this timeframe, certain batches of TPS blend bags (S, G) exhibited nearly complete decomposition, whereas PLA blend bags (R, S) did not decompose as extensively. Additionally, it was observed that the heterogeneous nature of the rotting material resulted in varying degrees of decomposition across different zones, some areas achieved thorough decomposition, while others did not.

Notably, the actual composting duration in large-scale composting facilities also often falls below the required 6 weeks, with some plants

marketing compost after only 2 weeks. It's important to note that the study specifically pertains to four types of BDP bags and the conditions at the Neumünster composting plant, thus, extrapolating conclusions to other facilities may not be definitive.

When assessing the decomposition of bags, factors such as BDP type, water content of the rotting material, and temperatures and composting phases played significant roles. Decomposition was most vigorous during the initial 3 weeks, correlating with the highest BDP decomposition. Over the subsequent 3 weeks, BDP decomposition persisted, albeit to a lesser extent. The study solely focused on BDP particles, excluding other types of foreign matter, although occasional inclusion of conventional plastic particles cannot be entirely dismissed. This determination is based on the manual removal of conventional plastic films prior to composting and the optical distinctiveness of BDP particles.

The proportion of BDP bags present in biowaste affects the levels of BDP residues in compost. This proportion depends on citizen usage frequency and the ratio of kitchen waste to green waste processed in facilities. Therefore, the BDP residue values provided in the study should be viewed as indicative rather than absolute. Enhancing the precision of such assessments is feasible through further studies on particle contamination, particle residue densities, and assumptions regarding particle diameters. Nevertheless, the current level of accuracy effectively highlights trends.

In accordance with standards, the focus of many studies is on BDP plastics >2 mm. However, this study proved that 1–2 mm BDP particles might likely be present in composts as well. Although the impacts of microplastics in soils remain incompletely understood (Bertling et al., 2021), evidence suggests they impact soil chemistry, biology, and physics, with concerns about very small particles entering organisms' cells. However, analyzing particles <1 mm remain an analytical challenge.

The research findings underscore that BDP bags, from production to disposal, contribute to resource consumption and waste generation rather than waste reduction. Achieving 100% degradability of such bags poses practical limitations, and knowledge gaps regarding the behavior of particles in soil represent significant challenges. Given these considerations, the viability of employing BDP bags for biowaste collection warrants scrutiny. In this context, adopting a bagless approach for biowaste collection emerges as the most prudent strategy.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

Author contributions

AS: Conceptualization, Data curation, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. SD: Data curation, Funding acquisition, Investigation, Methodology, Resources, Validation, Visualization, Writing – review & editing. IK: Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. Composting and basic evaluations were financially supported by Verbund für kompostierbare Produkte e.V. Stadtwerke Neumünster facilitated composting activities and Entsorgungsbetriebe Lübeck. Verbund für kompostierbare Produkte e.V. supported in preparing and handling the nets. Extended laboratory works, evaluations and manuscript writing was made possible through the support of the following TUHH-projects: Calls for Transfer C4T531 (Hamburg Innovation), SOILCOM (EU Interreg North Sea Region Program), KUKOM (DBU) and CLOSECYCLE (EU Interreg North Sea Region Program). Publishing fees supported by Funding Programme Open Access Publishing of Hamburg University of Technology (TUHH).

Acknowledgments

The authors gratefully acknowledge the supporters from the Verbund für kompostierbare Produkte e.V. as well as from the Stadtwerke Neumünster and Entsorgungsbetriebe Lübeck. Special thanks also to the staff of the laboratory of the TUHH (Institute for Wastewater Management and Water Protection). The authors express their sincere appreciation to the staff of these institutions for their contributions throughout the execution and evaluation of the experiments. The authors are also deeply grateful to the before mentioned supporting organisations. Special acknowledgment is extended to M. Viramontes Espinosa and P. E. Borges from TUHH, whose dedicated efforts within the scope of their project and master's thesis, respectively. They significantly contributed to the success of this research. Many thanks are also extended to M. Viramontes Espinosa, P. E. Borges, IK, and SD for their contributions in preparing the photographs presented in this work.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2024.1433460/full#supplementary-material>

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OPEN ACCESS

EDITED BY

Tom Misselbrook,
Rothamsted Research, United Kingdom

REVIEWED BY

Shengyue Fan,
Minzu University of China, China
Zainab Oyetunde-Uzman,
Rothamsted Research, United Kingdom

*CORRESPONDENCE

Jianqiang Li
✉ Ljq9801@126.com

RECEIVED 14 June 2024

ACCEPTED 19 August 2024

PUBLISHED 04 September 2024

CITATION

Zhou K, Wang H, Zhang Z and Li J (2024) The impacts of transaction costs and peer effects on pig farmers' willingness to participate in a pig manure outsourcing treatment project. *Front. Sustain. Food Syst.* 8:1448874. doi: 10.3389/fsufs.2024.1448874

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The impacts of transaction costs and peer effects on pig farmers' willingness to participate in a pig manure outsourcing treatment project

Kun Zhou, Huan Wang, Zhenwang Zhang and Jianqiang Li*

College of Management, Sichuan Agricultural University, Chengdu, China

This study aimed to explore the key factors affecting pig farmers' willingness to participate in a pig manure outsourcing treatment project (PMOTP) from the perspectives of transaction costs and peer effects. Survey data from 512 pig farmers and the probit and Heckman probit models were used to estimate the impact of transaction costs and peer effects on farmers' participation in the PMOTP. The results demonstrate that more than 62% of farmers are willing to participate in the PMOTP, and the average willingness to pay reaches 7.2 yuan/ton. Farmers' willingness to participate in the PMOTP decreases with asset specificity, transaction frequency and uncertainty but increases with peer effects. Moreover, peer effects attenuate the inhibitory effect of transaction costs on farmers' participation in the PMOTP. Heterogeneity analysis showed that large-scale farmers without the self-breeding and self-rearing model are more affected by transaction costs and peer effects than other farmers. Moreover, asset specificity and peer effects are likely to increase farmers' willingness to pay for the project, which suggests that larger farms are more willing to invest in the PMOTP. These findings provide a useful reference for establishing a model for sustainable environmental service projects in the farming industry.

KEYWORDS

pig farmers, pig manure, outsourcing treatment, transaction costs, peer effects

1 Introduction

The scale of pig farming in China is growing due to increasing consumer demand (Huong et al., 2014). China is the world's largest pork producer, producing 55.41 million tons of pork in 2022 and accounting for 50% of the global pork production that year (Zhou et al., 2023). However, the manure and sewage produced during pig rearing cause considerable damage to soil, water and air (Shi et al., 2023). Some pig farmers have found it challenging to implement standardized treatment of livestock waste because of the need for more technology, funds and land, which has resulted in great damage to the ecological environment. In China, 37% of farms produce waste exceeding the maximum amount of animal waste that can be absorbed by farmland (Jin et al., 2020), and it is difficult to subject many small and medium-sized farmers to environmental supervision and enforce standardized treatment (Shi et al., 2023). Because of these factors, the environmental pollution problem of China's pig farming industry is becoming increasingly serious, posing a severe threat to rural production and the living environment and restricting the modern development of the livestock industry.

Understanding how to effectively address pollution from livestock production is a global concern for sustainable agricultural development. Policymakers need to take measures to address environmental pollution from livestock production without negatively affecting farmer welfare (Sims and Alix-Garcia, 2017). Outsource services are tools that rely mainly on market mechanisms to encourage environmental protection (Sun et al., 2017). The implementation of outsource services requires at least one buyer and one seller of ecological services in the market, and transactions and services are carried out in accordance with the voluntary trading principle. To alleviate the pollution caused by pig farming, the Chinese government has introduced a pig manure outsourcing treatment project (PMOTP). The PMOTP operates through voluntary transactions under a “polluter pays, third-party treatment” model. PMOTP providers offer services such as manure collection, transport, treatment, and return to farmland, and the farmers who benefit from environmental services pay the service providers, essentially creating an environmental protection market (Hou et al., 2021).

The purpose of the PMOTP is to help farmers deal with pig manure through professional organization, reduce the farmers' cost of pig manure treatment and improve the farm environment. However, although the Chinese government strongly supports the development of the PMOTP, it is very difficult to promote the application of such participation and payments. Economists believe that people have bounded rationality and that their behavior is influenced by both economic costs and social networks (Wang et al., 2021). In this way, farmers participating in the PMOTP consider not only the costs or benefits of participation but also social factors, such as the practices of their peers. The Chinese government has invested tens of billions of dollars to support the PMOTP to help farmers clean up their production environment. As the direct beneficiaries of the PMOTP, farmers' active participation in the project is highly important for the long-term operation of environmental protection programs.

Outsource services are an effective way to address environmental externalities (Fan et al., 2022), and understanding participants' behaviors helps improve the establishment of outsource service projects. First, the theory of planned behavior (TPB) can be applied to explain farmers' environmental protection behavior. The TPB suggests that individuals should first be willing to participate in a specific behavior, and their willingness is affected by their attitude, subjective norms, and perceived behavioral control (Ajzen, 1991). The TPB has been applied in environmental conservation studies on water resources (Chai et al., 2021), saltmarshes (Muenzel and Martino, 2018), forests (Pagdee and Kawasaki, 2021) and biodiversity (Ingram et al., 2014). Second, some scholars have conducted such analyses from the perspective of costs and benefits. Neoclassical economic theory proposes that people are rational and that the ultimate goal of their behavior is to maximize their economic profits. Studies have shown that participation in outsource service projects can significantly increase participants' income, but this effect varies across groups and can exacerbate income inequality (Sheng and Wang, 2022). Therefore, it is difficult to explain participant behavior from the perspective of expected benefits.

Scholars have also pointed out that human beings have bounded rationality. Simon (1955) argues that people struggle to obtain all the information they need to make decisions, so their behaviors are often not optimally useful. Tversky and Kahneman (1992) argue that people make decisions based on their knowledge, experiences, and circumstances, are influenced by their subjective feelings, and do not

always have the goal of achieving economic optimality. Bounded rational behavior is often considered in the study of consumer behavior (Luo et al., 2023; Zhang Q. et al., 2023). However, although previous studies have provided some guidance for the promotion of outsource service projects, little attention has been given to outsource services in the livestock sector or to farmers' participation in the PMOTP from the perspective of transaction cost theory and peer effects (PEs). Farmers have bounded rationality and oscillate between making perfectly rational and bounded rational decisions, and their behavior is constrained by a combination of economics and sociology. Therefore, considering the impact of transaction costs and PEs on farmers' participation in the PMOTP is of great theoretical and practical significance.

This paper explores the key factors affecting farmers' willingness to participate in the PMOTP from the perspective of transaction costs and PEs with the aim to provide theoretical support for the reasonable allocation of PMOTP costs and the construction of a payment mechanism for beneficiaries. It combines transaction cost theory and peer effects to construct an analytical framework for farmers' participation in the PMOTP and uses survey data from 519 pig farmers from Sichuan Province, China, to explain the main driving mechanisms and disturbance factors of farmers' participation. In addition, to better understand the factors influencing farmers' willingness to participate in the PMOTP and their level of participation so that the government can better establish the environmental payment service project, we explore the influence of group heterogeneity and the level of payment on farmers' participation in the PMOTP. The results of this study have important implications for the establishment of a model for sustainable payments for environmental protection services in the farming industry.

2 Theory and analytical framework

2.1 Transaction costs and the PMOTP

Transaction costs are a core factor determining whether a farmer participates in the PMOTP (Yao et al., 2022). Transaction costs are divided into *ex ante* and *ex post* transactions (Hautsch and Voigt, 2019). This paper takes farmers' willingness to participate in the PMOTP, which occurs before a transaction, as an example and thus focuses on *ex ante* transaction cost theory. According to transaction cost theory (Williamson, 1989), when deciding whether to participate in the PMOTP, bounded rational farmers should first consider the transaction costs generated in the transaction (Coggan et al., 2010). Under the assumption of a given benefit from project participation, when transaction costs are high, farmers usually choose to treat the animal manure themselves, while when transaction costs are low, farmers choose to treat the manure with the help of services provided by the external market (Liu and Li, 2023). Drawing on the analytical ideas of Williamson's transaction cost theory (Williamson, 1989), this paper analyses the impact of transaction costs on farmers' participation in the PMOTP in terms of asset specificity, uncertainty and transaction frequency.

2.1.1 Asset specificity

Asset specificity refers to the characteristic of an asset that is difficult to use for other purposes after being adapted to a specific purpose (Williamson, 2010). Asset specificity locks assets into a particular use structure or nature (Bluemling and Wang, 2018). If a

specific right of assets is separated and transferred, then in this case, pig farmers not only lose their overall right function but also bear higher transaction costs (Zhang X. et al., 2023). For example, pig farmers face higher costs for manure treatment equipment than do other types of farmers, and this strong asset specificity indicates a stronger trapping effect of pig manure treatment for these farmers, thus inhibiting their participation in the PMOTP.

2.1.2 Transaction frequency

Transaction frequency is an important part of transaction cost theory and an important factor affecting transaction costs (Williamson, 1989). In production, pig farmers need to buy many kinds of production materials (such as feed, vaccines, medicines, and disinfectants), and farmers likely connect with multiple transaction subjects or make frequent transactions when purchasing production materials. The higher transaction frequency than that associated with connecting to a single service subject results in higher transaction costs (Thomas and Vink, 2020). Therefore, when farmers have more frequent transactions, their transaction costs are relatively high (Sgroi and Sciancalepore, 2022), which may inhibit their participation in the PMOTP.

2.1.3 Uncertainty

The breeding industry often entails greater development risks than other industries (Taylor et al., 2020). In particular, the pig farming industry has been continuously impacted by risks, such as animal diseases, natural disasters and market fluctuations in the pig price (Xu et al., 2022). For example, African swine fever has had a severe impact on China's farming industry, decreasing the country's pig production by 30% in 2019 (Xu et al., 2022). To protect the safety of their animals, many farmers reduce their farms' interactions with the outside world. When pig farmers need to transact with a party outside the farm, they will doubtlessly be exposed to greater risk. These risks lead to greater uncertainty regarding pig farmers' production, operations and ability to participate in the PMOTP, with these farmers paying a high price to resist the impact of these risks. Natural disasters and market price uncertainty can also affect farmers' production decision-making behavior. Because most farmers have poor risk aversion, they tend to choose conservative production methods in the face of high risks (Li et al., 2023). Therefore, this study proposes the following research hypotheses:

Hypothesis 1: Increased transaction costs are not conducive to farmers' participation in the PMOTP.

Hypothesis 1a: Increased asset specificity has a restraining effect on farmers' participation in the PMOTP.

Hypothesis 1b: A higher transaction frequency has a disincentive effect on farmers' participation in the PMOTP.

Hypothesis 1b: Uncertainty has an inhibiting effect on farmers' participation in the PMOTP.

2.2 PEs and the PMOTP

PEs, known as "herd effects" in psychology (Sacerdote, 2011), refer to situations in which, due to their bounded rationality,

individuals cannot rely solely on their own information to make decisions and instead observe or learn from other individuals in the same region or industry (Eisenkopf, 2010). Behavioral economists believe that the "last mile" of people's behavioral decision-making is affected by cognitive biases (Diener et al., 2003). We can refer to this notion as people's behavior being affected by the costs of institutional change and information asymmetry (Chen et al., 2022; Ullah et al., 2020). Indeed, one of the prerequisites for the emergence of transactions is external economics, i.e., relying on external services to reduce production costs (Rindfleisch, 2020). However, information asymmetry creates an information gap between the consumer and the service provider. Especially in cases of information asymmetry, bounded rational individuals tend to overestimate the costs and risks of unknown behavioral decisions (Foramitti et al., 2021); thus, they prefer to maintain the status quo and choose options that are more familiar to them. Peer communication is a key channel through which farmers can obtain information and is an important reference for farmers in adopting particular behaviors (Yuan et al., 2021). Farmers tend to choose the same behaviors as their peers, especially when most of their peers choose a particular behavior due to the herd mentality (Ali-Rind et al., 2023).

The core of PEs is that human beings are both "economic" and "social." The behaviors of individuals are influenced by market economic factors and other peers (Sacerdote, 2011). Combining sociological and economic (Palm, 2017), there are three main aspects of PEs that can drive farmers to participate in the PMOTP. (1) Information exchange. PEs based on geographic proximity and kinship can reduce farmers' information-seeking costs, as the technological understanding transferred from peers is based on their own production experience and local conditions, which farmers find easier to understand and accept (Niu et al., 2022). For example, Zhang et al. (2022) found that due to the lack of communication channels with peers, farmers show hesitation in implementing government policies and green production. Thus, this aspect of PEs also helps farmers understand the PMOTP and reduces the risk expectation and uncertainty of participation. (2) Behavior imitation. The risks and costs of making innovation decisions independently are relatively high; thus, farmers may tend to overestimate expected risks and costs when weighing costs and benefits (Tran-Nam and Tiet, 2022). However, when other farmers participate in the PMOTP, farmers can directly observe the benefits and effectiveness of participation, as "seeing is believing," thus stimulating farmers' preference and significantly improving their assessment of the costs and benefits of participation (Li and Fang, 2022). On the other hand, peer farmers usually have similar resource endowments and social structure relationships, their behaviors align with their technical needs, and imitating each other's successful behaviors brings about a sense of security in behavioral decision-making; all of these aspects can change farmers' value judgment and prompt them to participate in the PMOTP (Zant, 2023). (3) Consumption cluster. Peer effects give rise to consumption agglomeration effects, which reduce transaction costs for farmers participating in the PMOTP (Gao et al., 2023). That is, farmers' consistent participation grants them higher levels of bargaining and negotiation power (Zant, 2023). When farmers produce consumption agglomeration effects, PMOTP providers may also choose to gather in areas with apparent consumption agglomeration because the transportation, time, and search costs can be reduced; this, in turn, attracts more farmers to participate.

Therefore, Hypotheses 2 and 3 are proposed. The theoretical research framework of this paper is presented below (Figure 1).

- Hypothesis 2:* Positive PEs can promote farmers' willingness to participate in the PMOTP.
- Hypothesis 2a:* Increased information exchange can promote farmers' willingness to participate in the PMOTP.
- Hypothesis 2b:* Positive Behavior imitation can promote farmers' willingness to participate in the PMOTP.
- Hypothesis 2c:* Greater consumption clustering can promote farmers' willingness to participate in the PMOTP.
- Hypothesis 3:* PEs can influence farmers' willingness to participate in the PMOTP by adjusting transaction costs.

3 Data source, variable selection, and research method

3.1 Data source

The research data came from a questionnaire survey of pig farmers in Sichuan Province, China. Sichuan Province is the largest pig-producing province in China, with its pig production volume ranking first among all provinces in China, reaching 63.14 and 65.48 million head in 2021 and 2022, respectively (NBS, 2023). Pig farmers are becoming increasingly concentrated in Sichuan Province; thus, this setting is typical and representative of pig farming (Li et al., 2023). We selected Jingyan County, Xuyong County, Nanxi District, Zhaohua District, and Yanting County in accordance with their volume of pig

farming and representative socioeconomic development characteristics, and we randomly selected 4–5 towns and 20–25 farmers in each county. The research method involved one-on-one household interviews. We sent out a total of 550 survey questionnaires. After screening and eliminating questionnaires missing important information or having inconsistent data, 519 valid questionnaires were obtained. A map of the survey sample locations is shown in Figure 2.

3.2 Variable selection

3.2.1 Dependent variable

The primary dependent variable of this study is farmers' willingness to participate in the PMOTP, expressed by their answer to the question "Are you willing to participate in the PMOTP?" Furthermore, we examined the farmers' willingness to pay for the PMOTP.

3.2.2 Independent variables

The core independent variables in this study are transaction costs and PEs. According to the definition of transaction costs (Rindfleisch, 2020; Williamson, 2010), ex ante transaction costs include mainly asset specificity, transaction frequency and uncertainty. In this paper, the amount invested in animal manure treatment equipment is used to measure asset specificity, and the transaction frequency occurring in the production process (including feed, medicine, etc.) and current pig-rearing risk are used to measure uncertainty. The peer effects in this paper came from three aspects: information exchange, behavior imitation and consumption cluster. "Number of peers in frequent contact" to represent information exchange; "If your peers participated in the PMOTP, are you willing to participate?" to represent behavior imitation; "how many of your peers adopt the PMOTP" to represent consumption cluster.

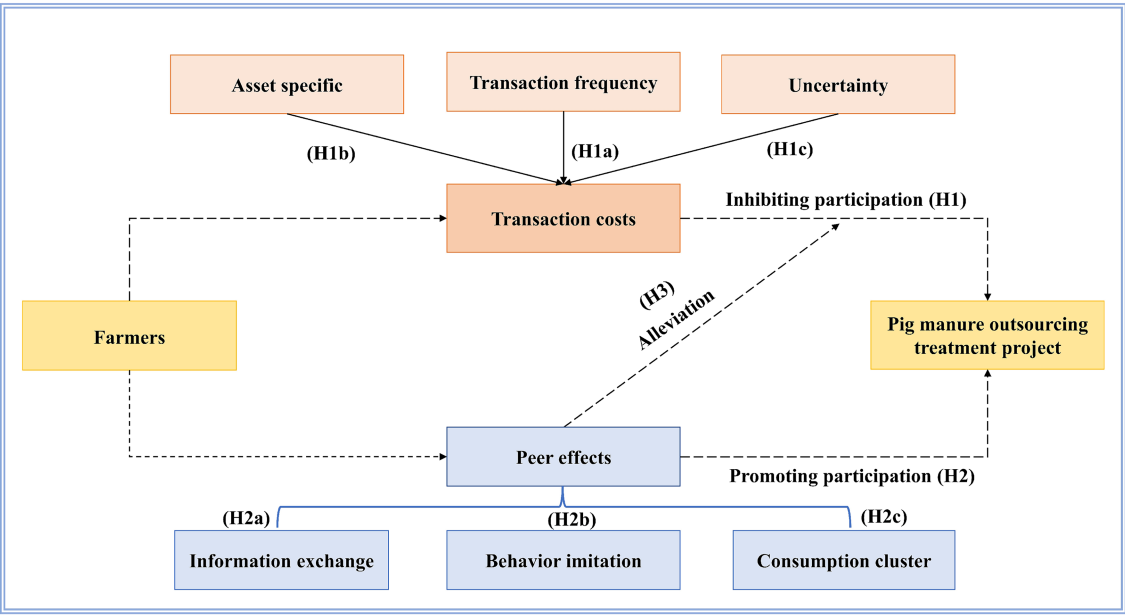
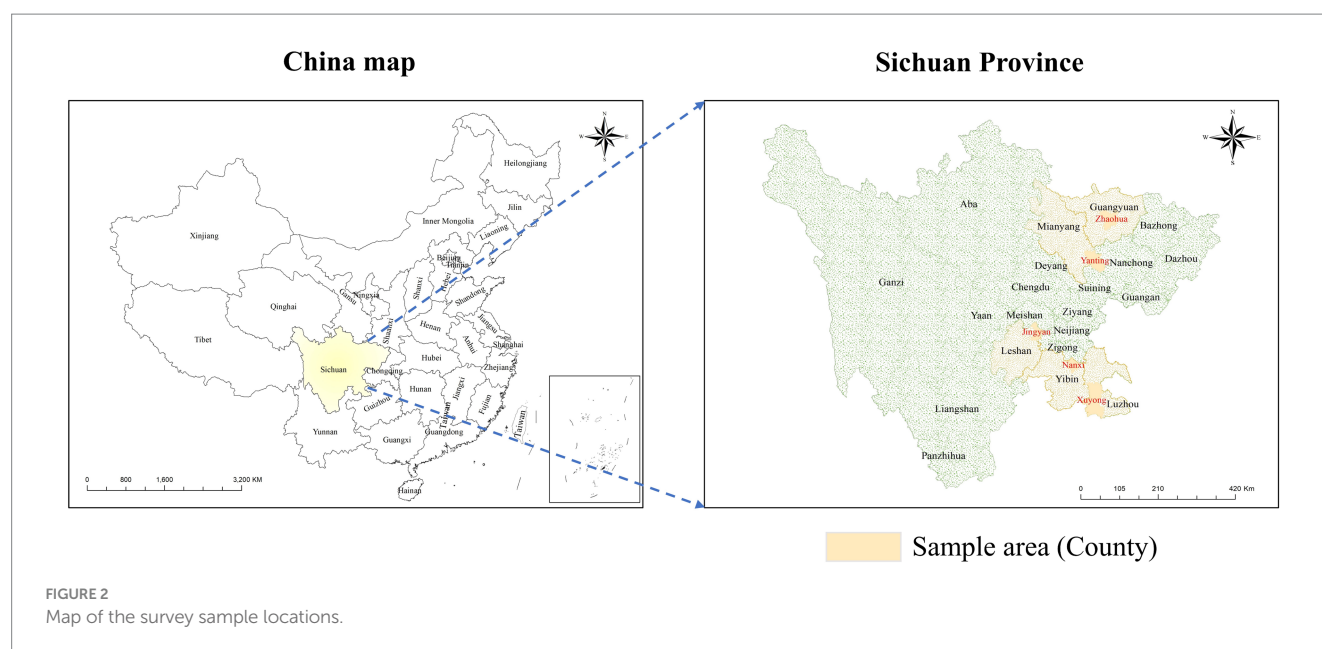


FIGURE 1
Theoretical research framework.



3.2.3 Control variables

To alleviate the problem of omitted variable bias, this study controls for individual, household, and production and business characteristics. Individual characteristics include mainly gender, age, education level, and risk preference (He et al., 2022); family characteristics include mainly the number of family members and the amount of income (Li et al., 2023); production and business characteristics include the number of rearing years, rearing scale, type of farming, and type of organization (Huong et al., 2020); and other variables include the intensity of governmental environmental supervision (Zhou et al., 2022). Table 1 shows the definitions of the variables.

Approximately 52% of the farmers were willing to participate in the PMOTP, with an average payment level of 7.2 yuan/ton (Table 1). Among the farmers in the sample, the ratio of male farmers to female farmers was approximately 8.3:1.7, the average farmer age was 50.62 years, the average time spent breeding was 13.59 years, and the average breeding scale was 728 heads. Most pig farmers were risk neutral and believe that the government's environmental supervision intensity is relatively strict (4.34), indicating that the current environmental pressure placed on farmers is burdensome. These farmers presented similar characteristics and structures to those of the pig farmers surveyed by Li et al. (2023) in Sichuan Province, China, which supports the bounded rationality of the samples to a certain extent.

Among the transaction costs, the average value of farmers' asset specificity reached 108,700 yuan, and the values of uncertainty and transaction frequency reached 3.68 and 3, respectively. The value of 2.71 obtained to measure the consumption cluster indicates that only a few peers have adopted PMOTP. Most farmers indicated they would be willing to participate in the PMOTP program if their peers participated.

3.3 Research methods

3.3.1 Probit model

This paper focuses first on farmers' willingness to participate in the PMOTP, using the dichotomous response variables of whether

farmers are willing or unwilling to participate. Therefore, a probit model is used to analyze farmers' willingness to participate in the PMOTP, and the probit mode can be expressed as Equation (1):

$$\begin{aligned} \text{Probit} \left(y = 1 \mid x_1, x_2, \dots, x_n \right) &= 1 - \Phi \left[- \left(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_n x_n \right) \right] \\ &= \Phi \left[\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_n x_n \right] \end{aligned} \quad (1)$$

where y denotes the farmers' willingness to participate in the PMOTP and is a standard normal cumulative distribution function; $x_1, x_2, x_3, \dots, x_n$ are independent variables; and $\beta_1, \beta_2, \beta_3, \dots, \beta_n$ are coefficients.

3.3.2 Heckman probit model

Furthermore, we wanted to analyze the farmers' willingness to pay. Farmers' participation in the PMOTP is divided into two stages: the first stage involved choosing one of two options (willingness or unwillingness to participate), and the second stage involved the level of payment (willingness or unwillingness to pay). The level of payments from this group of farmers could be observed only if they were willing to participate in the PMOTP. To achieve this goal, we required two steps: the willingness to participate and the willingness to pay. We assumed that farmers were willing to pay for PMOTP because they are willing to participate in the program; if farmers were not willing to participate in PMOTP, they would not pay for the program. Meanwhile, farmers' participation in the PMOTP is highly autonomous, and unobservable factors can have an impact on participation behavior. However, using only farmers participating in the PMOTP as a sample would produce biased estimates, i.e., sample selection bias.

The Heckman model can correct such sample selection bias through a two-stage estimate (Yarbaşı and Çelik, 2023). Therefore, we used the Heckman Probit model to analyze the payment decision-making behavior of farmers and solve the problem of sample selection

TABLE 1 Descriptive statistics of the variables.

Variable	Definition	Mean	S.D.
Willingness to participate (WTPAR)	Are you willing to participate in the PMOTP (0 = no; 1 = yes)?	0.52	0.50
Willingness to pay (WTPAY)	How much are you willing to pay to participate in the PMOTP (yuan/ton)?	7.20	9.63
Asset specificity (AS)	Investment amount in animal manure treatment equipment (10 ⁴ yuan)	10.87	3.40
Uncertainty (UNC)	Pig farming risks (1 = very low, 2 = relatively low, 3 = general, 4 = relatively high, and 5 = very high) ¹	3.68	1.09
Transaction frequency (TF)	Transaction frequency occurring in the production process (including feed, medicine, etc.) (1 = hardly frequent, 2 = less frequent, 3 = general, 4 = more frequent, and 5 = very frequent)	3	0.80
Information exchange (IE)	Number of peers in frequent contact (0–10 = 1, 11–20 = 2, 21–30 = 3, 31–40 = 4, over 40 = 5)	2.81	1.45
Behavior imitation (BI)	If your peers participated in the PMOTP, are you willing to participate? (1 = very reluctant, 2 = less reluctant, 3 = general, 4 = more willing, and 5 = strongly willing)	3.67	1.00
Consumption cluster (CC)	How many of your peers (pig farmers) adopt the PMOTP (1 = very little, 2 = relatively little, 3 = general, 4 = relatively much, and 5 = very much)	2.71	0.87
Age	Age of respondent (years)	50.62	8.53
Sex	Sex of respondent (0 = male; 1 = female)	0.83	0.38
Education (EdU)	Education of respondent (years)	8.97	3.28
Family size (FS)	Household resident population (unit: persons)	4.98	1.65
Rearing time (RT)	Rearing time of respondent (years)	13.59	9.63
Rearing scale (RS)	Rearing scale in 2022 (heads)	4.88	2,000.3
Rearing income as a proportion of total household income (RI)	Rearing income as a proportion of total household income	0.66	0.28
Rearing model (PS)	Rearing model adopted by farmers (1 = pig-breeding farm, 2 = self-breeding and self-rearing farm, 3 = weaned piglet farm, and 4 = piglet-finishing farm)	2.45	0.87
Organizational style (OR)	What kind of organization is your organization (1 = cooperative, 2 = breeding association, 3 = breeding community, 4 = company, and 5 = none)?	4.14	1.50
Risk appetite (RA)	Risk appetite type (1 = risk conservative, 2 = risk neutral, and 3 = risk aggressive)	2.01	0.65
Governmental environmental supervision intensity (GI)	Intensity of government regulation of standardized manure treatment (1 = very loose, 2 = less loose, 3 = general, 4 = tighter, and 5 = strongly tight)	4.34	0.95

Indicators 1–5 are evaluated on a 5-point Likert scale, with 1 being the weakest effect and 5 being the strongest effect.

bias. Referencing Heckman (1979), a probit model was used in the first stage to estimate the entire sample via the following Equation (2):

$$y_i = \begin{cases} 1, & y_i^* > 0 \\ 0, & y_i^* \leq 0 \end{cases}$$
$$y_i^* = Z_i'\beta_i + \mu_i \tag{2}$$

where y_i is the probability of farmers being willing to participate in the PMOTP, y_i^* is a latent variable that represents participation, Z_i' is an independent variable, β_i is a coefficient, and φ_i is a random error term.

In the second stage, ordinary least squares (OLS) regression was used to correct the sample selection bias problem and obtain consistent estimates (Yarbaşı and Çelik, 2023) via the following Equation (3):

$$g_i = X_i'\gamma_i + \alpha\hat{\lambda} + \varphi_i \tag{3}$$

where g_i is the payment level at which farmers are willing to participate in the PMOTP, X_i' is the independent variable, γ_i is the coefficient, α is the coefficient of the inverse Mill's ratio, λ is the

estimated value obtained from $P(Y_i = 1|w) = \Phi(w_i, \gamma)$, and φ_i is the random error term.

4 Results

4.1 Effect of transaction costs and PEs on farmers' participation in the PMOTP

4.1.1 Benchmark regression

Table 2 shows the benchmark regression results. Column (1) of Table 2 includes only the three indicators denoting transaction costs, while Column (2) includes all the control variables in the regression model. Columns (3) and (4) of Table 2 show the results of the stepwise regression of PEs on farmers' willingness to participate in the PMOTP. Column (5) to (7) shows the combined effect of transaction costs and PEs on farmers' willingness to participate in the PMOTP. As shown in Column (7) Table 2, transaction frequency, asset specificity and uncertainty are significantly negative at the 1% level, which fully supports Hypothesis 1 (a, b, and c) and is similar to the results of Hou et al. (2023). This finding suggests that the increase in farmers' transaction costs during the operation of the PMOTP is not conducive

TABLE 2 Benchmark regression results.

Variables	(1) WTPAR	(2) WTPAR	(3) WTPAR	(4) WTPAR	(5) WTPAR	(6) WTPAR	(7) WTPAR
TF	−0.284*** (0.076)	−0.275*** (0.079)			−0.287*** (0.095)	−0.271*** (0.097)	−0.169* (0.102)
AS	−0.027*** (0.007)	−0.051*** (0.010)			−0.061*** (0.010)	−0.071*** (0.012)	−0.072*** (0.013)
UNC	−0.208*** (0.055)	−0.260*** (0.057)			−0.239*** (0.068)	−0.272*** (0.071)	−0.281*** (0.074)
BI			0.860*** (0.086)	0.946*** (0.094)	1.043*** (0.107)	1.057*** (0.110)	1.227*** (0.111)
CC			0.171*** (0.036)	0.187*** (0.052)	0.196** (0.089)	0.204** (0.093)	0.173* (0.098)
IE			0.051 (0.052)	0.114** (0.056)	0.139** (0.061)	0.140** (0.064)	0.173*** (0.062)
Sex		0.026 (0.163)		−0.012 (0.009)		0.220 (0.218)	0.280 (0.238)
Age		−0.002 (0.008)		0.190 (0.191)		−0.010 (0.010)	0.003 (0.011)
EdU		−0.022 (0.020)		−0.058*** (0.022)		−0.065*** (0.025)	−0.051* (0.031)
FS		−0.057 (0.037)		−0.070* (0.039)		−0.074 (0.045)	−0.101** (0.051)
RT		0.006 (0.007)		0.003 (0.008)		−0.001 (0.008)	−0.008 (0.009)
RS		0.189*** (0.050)		−0.161*** (0.046)		−0.116* (0.063)	0.254*** (0.070)
RI		−0.096 (0.240)		−0.056 (0.257)		−0.136 (0.296)	−0.074 (0.340)
RA		−0.299*** (0.097)		0.057 (0.105)		0.077 (0.120)	−0.137 (0.125)
GI		0.037** (0.017)		−0.001 (0.018)		0.020 (0.020)	−0.005 (0.025)
PS	No	No	No	No	No	No	Control
OR	No	No	No	No	No	No	Control
Counties	No	No	No	No	No	No	Control
Towns	No	No	No	No	No	No	Control
Constant	1.867*** (0.310)	2.262*** (0.718)	0.860*** (0.086)	−2.123*** (0.786)	−2.550*** (0.544)	−1.671* (0.990)	−5.195*** (1.552)
Pseudo R ²	0.113	0.164	0.261	0.316	0.439	0.461	0.523
Observations	519	519	519	519	519	519	519

Column (1)–(6) standard errors in parentheses, and Column (7) robust standard errors in parentheses; *** $p < 0.01$, ** $p < 0.05$, and * $p < 0.1$.

WTPAR, willingness to participate; AS, Asset specificity; UNC, Uncertainty; TF, transaction frequency; BI, behavior imitation; CC, consumption cluster; IE, information exchange; EdU, education; FS, family size; RT, rearing time; RS, rearing scale; RI, rearing income as a proportion of total household income; RM, rearing model; OR, organizational style; RA, risk appetite; GI, Governmental environmental supervision intensity.

TABLE 3 Results of the robustness test.

Variables	(1) Probit	(2) Logit	(3) Logit	(4) OLS	(5) OLS
	WTPAR	WTPAR	WTPAR	WTPAR	WTPAR
TCs	−0.580*** (0.088)	−1.006*** (0.161)		−0.139*** (0.021)	
PEs	0.743*** (0.080)	1.268*** (0.145)		0.197*** (0.017)	
TF			−0.296*** (0.087)		−0.057*** (0.021)
AS			−0.126*** (0.026)		−0.002*** (0.001)
UNC			−0.479*** (0.137)		−0.066*** (0.016)
BI			2.122*** (0.218)		0.243*** (0.017)
CC			0.320* (0.174)		0.033* (0.019)
IE			0.315*** (0.111)		0.039*** (0.014)
Control variables	Yes	Yes	Yes	Yes	Yes
PS	Control	Control	Control	Control	Control
OR	Control	Control	Control	Control	Control
Counties	Control	Control	Control	Control	Control
Towns	Control	Control	Control	Control	Control
Constant	−0.176	−0.443	−9.281***	0.444	−0.275
	(1.041)	(1.822)	(3.115)	(0.290)	(0.288)
Pseudo R ² /R ²	0.333	0.334	0.520	0.374	0.498
Observations	519	519	519	519	519

Robust standard errors are in parentheses; *** $p < 0.01$, ** $p < 0.05$, and * $p < 0.1$.

WTPAR, willingness to participate; TCs, transaction costs; PEs, peer effects; AS, Asset specificity; UNC, Uncertainty; TF, transaction frequency; BI, behavior imitation; CC, consumption cluster; IE, information exchange; RM, rearing model; OR, organizational style.

to farmers' participation in the project. In contrast, PEs have a significant promoting effect. Among them, information exchange and behavior imitation promote the farmers' participation in the PMOTP at the significance level of 1%, and consumption cluster also has an incentive role on farmers' willingness to participate at the significance level of 10%. That is, when most of their peers participate in the PMOTP, respondents also show willingness to participate. Hypothesis 2 (a, b, c) was confirmed.

Control variables, the greater the share of rearing income in total household income is, the less likely the farmer is to participate in the PMOTP. However, uncertainty exists in the regression results for the rearing scale. When the rearing mode and organizational style are controlled, farmers' risk preference and rearing scale have opposite effects on their willingness to participate in the PMOTP, which indicates that the effects of farmers' risk preference and rearing scale may vary across different rearing modes or organizational styles. The specific roles of farmers' organizational style, rearing model and rearing scale were analyzed via a heterogeneity test.

4.1.2 Robustness test

In the benchmark regression (Section 4.1.1), we controlled for different types of rearing models, organizational styles, counties and towns and conduct robust standard error regression, which enhances the accuracy of the regression model. We changed the model and main explanatory variables to further ensure the correctness of the regression results. First, we combined the variables of three dimensions—asset specificity, uncertainty and transaction frequency—through principal component analysis (PCA) and used a probit model for regression.

Similarly, the PCA method was also used to calculate the comprehensive value of PEs. The regression results are shown in Column (1) of Table 3. The coefficient of transaction costs (TCs) is significantly negative (−0.580), and PEs are positive (0.743) and significant at the 1% level, which is consistent with the benchmark regression results.

Second, we used logit and OLS models to estimate the impact of transaction costs and PEs on farmers' willingness to participate in the PMOTP. Like probit models, logit models can also handle binary variables from cross-sectional data, and OLS regression is a common method used to verify linear relationships between variables. Columns (2) and (4) of Table 4 show that the sign direction of the coefficients of transaction costs and PEs does not change after OLS and Logit regression methods are adopted, and the p -value is less than 0.01. As shown in Columns (3) and (5) of Table 3, the transaction cost dimensions still have a significant negative impact on farmers' participation in the PMOTP ($p < 0.01$), while PEs dimensions maintain a positive role according to the logit and OLS approaches. In the results from the whole regression, we adopted the robust standard error regression method and control for the county and township, which can effectively improve the reliability of the regression results. By verifying via different methods, we still obtained results similar to those in Section 4.1.1, which indicates that the models and methods adopted are effective and reliable.

4.2 Moderating role of PEs

PEs can affect the behavioral choices of farmers, but in the context of high transaction costs, do PEs play a positive or negative moderating

TABLE 4 Moderating role of PEs.

Variables	(1) WTPAR	(2) WTPAR	(3) WTPAR	(4) WTPAR	(5) WTPAR
TCs	−0.567*** (0.092)				
PEs	0.766*** (0.086)	1.027*** (0.319)	0.938*** (0.097)	1.221*** (0.277)	0.829* (0.484)
TCs*PEs	−0.176* (0.099)				
TF		−0.316*** (0.089)			−0.188* (0.097)
AS			−0.046*** (0.012)		−0.045*** (0.011)
UNC				−0.312*** (0.066)	−0.281*** (0.066)
TF*PEs		−0.105 (0.097)			−0.081 (0.113)
AS*PEs			−0.027*** (0.010)		−0.026*** (0.009)
UNC*PEs				−0.124* (0.067)	−0.039 (0.068)
OR	Control	Control	Control	Control	Control
PS	Control	Control	Control	Control	Control
Counties	Control	Control	Control	Control	Control
Towns	Control	Control	Control	Control	Control
Control variables	Yes	Yes	Yes	Yes	Yes
Constant	−0.150 (1.045)	0.594 (1.054)	−0.464 (1.094)	0.942 (1.065)	1.414 (1.224)
Pseudo R ²	0.339	0.277	0.352	0.294	0.382
Observations	519	519	519	519	519

Robust standard errors are in parentheses; *** $p < 0.01$, ** $p < 0.05$, and * $p < 0.1$.

WTPAR, willingness to participate; TCs, Transaction costs; PEs, peer effects; AS, Asset specificity; UNC, Uncertainty; TF, transaction frequency; RM, rearing model; BI, behavior imitation; CC, consumption cluster; IE, information exchange; OR, organizational style; PE_{pc} , the comprehensive value of transaction costs.

role? In fact, farmers can obtain more information about the PMOTP by talking to their peers, which can affect their assessment of the value of participating in the project. Assuming that PEs can increase farmers' perceptions of the benefits of PMOTP participation, PEs can mitigate the adverse effects of transaction costs.

To verify this hypothesis, we first used the comprehensive index of transaction costs (TCs) and PEs (PEs) for interaction processing and conduct a regression. Moreover, to prevent possible collinearity of the interaction term, we decentralized the main variables. The results are shown in Table 4, Column (1). The interaction term TCs*PEs is not positive, indicating that PEs have a dampening effect on the role of transaction costs at 10% significance level.

Furthermore, we interacted transaction frequency, asset specificity and uncertainty with PEs and conducted regressions to verify the role of these interaction terms in farmers' participation in the PMOTP. The results, presented in Columns (2)–(5) of Table 4, show that only the AS*PEs coefficient is significantly negative ($p < 0.01$); i.e., PEs significantly weaken the negative effect of asset specificity. Additionally, the coefficients of the interaction terms UNC*PEs in Columns (4), respectively, are negative at 10% significance level. The coefficients of TF*PEs are negative but nonsignificant, indicating that PEs may inhibit transaction risk, but further tests are needed for verification. Therefore, following Otieno et al. (2023), we attempted to further analyze the moderating role of PEs through the use of grouped regression. We used the mean value of PEs as the basis for grouping. From Table 5, the negative effects of transaction frequency, risk and asset specificity on farmers' willingness to participate in PMOTP in the high-mean group (Column 2) are significantly weaker than the effects in the low-mean group (Column 1), which indicates that positive PEs can weaken the negative effects of transaction costs on farmers' willingness to participate in the PMOTP.

4.3 Heterogeneity analysis

4.3.1 Effect of the decision to join a cooperative organization on farmers' willingness to participate in the PMOTP

We further verified the effect of farmers' decision to join a cooperative organization on their participation in the PMOTP. Table 6 shows that farmers who do not join an organization are greatly affected by transaction costs and PEs (Columns 1 and 2). For example, asset specificity and uncertainty have stronger negative effects on PMOTP participation for farmers who are not involved in any cooperative than for farmers who are involved in at least one cooperative. A similar result is shown for PEs. Farmers not involved in any cooperative are more affected by PEs than those involved in at least one cooperative. Notably, in the process of heterogeneity analysis, we adopted a fixed effects model and conduct robust standard error regression; thus, the results are reliable.

4.3.2 Impact of different rearing modes on farmers' willingness to participate in the PMOTP

The main pig-rearing modes in China are self-breeding and self-rearing, pig-breeding, piglet-finishing and weaned piglet-selling farms. However, according to our survey results, the main pig-rearing modes in Sichuan Province are self-breeding and self-rearing farms and piglet-finishing farms,¹ while pig-breeding

1 In the self-breeding and self-rearing model, the farmer raises sows, breeds piglets, and then raises the piglets until they become commercial pigs for sale or eating. In the piglet-finishing model, piglets are moved from the nursery into the growing house to feed.

TABLE 5 Grouped regression results.

Variable	(1) WTPAR	(2) WTPAR
	PEs < Mean	PEs > Mean
TF	−0.316** (0.131)	−0.304** (0.128)
AS	−0.137*** (0.012)	−0.070*** (0.024)
UNC	−0.403*** (0.096)	−0.357*** (0.104)
Control variables	Yes	Yes
OR	Control	Control
PS	Control	Control
Counties	Control	Control
Towns	Control	Control
Constant	2.614 (1.997)	1.142 (1.424)
Pseudo R ²	0.345	0.439
Observations	244	275

Robust standard errors are in parentheses; *** $p < 0.01$, ** $p < 0.05$, and * $p < 0.1$.

WTPAR, willingness to participate; AS, Asset specificity; UNC, Uncertainty; TF, transaction frequency; PEs, peer effects; RM, rearing model; OR, organizational style.

TABLE 6 Effect of organizational style on farmers' willingness to participate in the PMOTP.

Variable	(1) WTPAR	(2) WTPAR
	Not join	Join
TF	−0.138 (0.103)	−0.425 (0.265)
AS	−0.063*** (0.014)	−0.052*** (0.019)
UNC	−0.290*** (0.076)	−0.586*** (0.214)
PEs	0.855*** (0.101)	0.766*** (0.192)
PS	Control	Control
Counties	Control	Control
Towns	Control	Control
Control variables	Yes	Yes
Constant	2.920* (1.597)	3.854 (3.194)
Pseudo R ²	0.378	0.476
Observations	380	139

Robust standard errors are in parentheses; *** $p < 0.01$, ** $p < 0.05$, and * $p < 0.1$.

WTPAR, willingness to participate; AS, Asset specificity; UNC, Uncertainty; TF, transaction frequency; PEs, peer effects; RM, rearing model; OR, organizational style.

farms and weaned piglet-selling farms are rare. Thus, we analyze only self-breeding and self-rearing farms and piglet-finishing farms. As shown in Table 7, farmers adopting the piglet-finishing model are generally more affected by transaction costs and PEs, while those adopting the self-breeding and self-rearing model are less affected. Generally, self-breeding and self-rearing farms are small and medium-sized farms, while piglet-finishing farms are larger (Li et al., 2023), which means that piglet-finishing farmers purchase more materials during production than self-breeding and self-rearing farmers, and their total value of equipment assets is greater. This situation may expose piglet-finishing farms to greater risks, resulting in this group of farmers being more sensitive to transaction costs than other groups of farmers are. Therefore, as the transaction frequency, asset specificity and uncertainty increase, the probability of piglet-finishing farmers participating in the PMOTP decreases.

4.3.3 Role of the rearing scale in farmers' participation in the PMOTP

Referring to Zhou et al. (2023), we defined farmers with fewer than 50 pigs per year as free-range (small-scale) farmers, farmers with 50 to 500 pigs per year as medium-scale farmers, and farmers with more than 500 pigs per year as large-scale farmers.

Table 8 shows that the impact of transaction frequency on small-scale farmers' participation in the PMOTP is not significant and that the effect on medium-and large-scale farmers is more pronounced. Asset specificity has a negative effect on medium-and large-scale farmers but a positive effect on small-scale farmers. Uncertainty has a disincentive effect on the participation of small-and medium-scale farmers in the PMOTP, but the effect is not significant for large-scale farmers. PEs have a positive effect on small-and medium-scale farmers' participation in the PMOTP, but the effect of PEs on large-scale farmers is not significant.

Depending on the actual situation, small-scale farmers generally follow self-breeding and self-rearing models; therefore,

TABLE 7 Impact of different farming modes on farmers' willingness to participate in the PMOTP.

Variable	(1) WTPAR	(2) WTPAR
	Self-breeding and self-rearing	Piglet-finishing
TF	−0.249** (0.109)	−0.567* (0.299)
AS	−0.042*** (0.012)	−0.098*** (0.031)
UNC	−0.301*** (0.076)	−0.439** (0.188)
PEs	0.577*** (0.106)	0.875*** (0.236)
OR	Control	Control
Counties	Control	Control
Towns	Control	Control
Control variables	Yes	Yes
Constant	1.208 (1.301)	4.398 (3.643)
Pseudo R ²	0.408	0.450
Observations	383	121

Robust standard errors are in parentheses; *** $p < 0.01$, ** $p < 0.05$, and * $p < 0.1$.

WTPAR, willingness to participate; AS, Asset specificity; UNC, Uncertainty; TF, transaction frequency; PEs, peer effects; RM, rearing model; OR, organizational style.

TABLE 8 Role of the rearing scale in farmers' willingness to participate in manure recycling projects.

Variables	(1) WTPAR	(2) WTPAR	(3) WTPAR
	Small Scale	Mediate Scale	Large Scale
TF	0.119 (0.202)	−0.385** (0.169)	−4.858* (2.669)
AS	0.307*** (0.110)	−0.040** (0.020)	−0.320** (0.160)
UNC	−0.345** (0.145)	−0.440*** (0.112)	−0.477 (0.518)
PEs	1.342*** (0.247)	0.979*** (0.129)	1.645 (1.050)
PS	Control	Control	Control
OR	Control	Control	Control
Counties	Control	Control	Control
Towns	Control	Control	Control
Control variables	Yes	Yes	Yes
Constant	7.973*** (2.532)	4.609** (2.071)	4.095 (10.082)
Pseudo R ²	0.546	0.387	0.767
Observations	143	265	111

Robust standard errors are in parentheses; *** $p < 0.01$, ** $p < 0.05$, and * $p < 0.1$.

WTPAR, willingness to participate; AS, Asset specificity; UNC, Uncertainty; TF, transaction frequency; PEs, peer effects; RM, rearing model; OR, organizational style.

these farmers have less frequent transactions than other farmers. As the scale of rearing increases, the purchase of feed, drugs, and disinfectants for epidemic prevention becomes more frequent, thus increasing transaction costs. Large-scale farming can reduce production costs and allow farmers to resist risks through the advantages of modern operation and involves optimal manure treatment equipment, which leads to higher transaction costs, such that the behaviors of their peers have little impact on these farmers.

4.4 Further analysis: impact of transaction costs and PEs on farmers' payment levels

After exploring the influence of farmers' willingness to participate in the PMOTP, we further examined farmers'

willingness to pay for such participation, which is helpful for establishing an effective environmental payment service mechanism for livestock (Ren, 2022). The Heckman probit model was used to analyze the effects of transaction costs and PEs on farmers' payment levels. In Column (1) of Table 9, asset specificity, transaction frequent, and risk are shown to have a significantly negative effect on farmers' willingness to participate, and PEs are positive. As Column (2) of Table 9 shows, the higher the transaction frequency and risk levels are, the less the farmers are willing to pay to participate in the PMOTP; when PEs are more significant, farmers are willing to pay more to participate in the PMOTP. Moreover, farmers with more manure treatment equipment assets tend to be willing to pay more to participate in such projects than those with fewer such assets.

TABLE 9 Results of the Heckman probit model.

Variables	(1) WTPAR	(2) WTPAY
TF	−0.311* (0.166)	−0.141* (0.075)
AS	−0.050*** (0.016)	0.002 (0.002)
UNC	−0.271*** (0.089)	−0.195*** (0.054)
PEs	0.569*** (0.101)	0.544*** (0.064)
Athrho	1.870*** (0.848)	
Control variables	Yes	Yes
Constant	2.340** (0.989)	1.161*** (0.300)
Observations	519	519

Standard errors are in parentheses; *** $p < 0.01$, ** $p < 0.05$, and * $p < 0.1$.

WTPAR, willingness to participate; AS, Asset specificity; UNC, Uncertainty; TF, transaction frequency; PEs, peer effects; RM, rearing model; OR, organizational style.

5 Discussion

This study aimed to assess the effectiveness of the current PMOTP in China from the perspective of farmers' willingness to participate. Therefore, we established an analytical framework of farmers' willingness to participate in the PMOTP based on transaction cost theory and PE theory and conduct an empirical analysis using survey data from 519 pig farmers. In addition, to better understand the factors influencing farmers' willingness to participate in the PMOTP and their level of participation so that the government can better establish an environmental payment service project, we explored the influence of group heterogeneity and the level of payment on farmers' participation in the PMOTP. The results indicate that farmers are strongly willing to participate, which is somewhat different from the findings of Zhao et al. (2019). While Zhao et al.'s study revealed that farmers' willingness to participate in the PMOTP (41%) and level of payment (4.6 yuan, on average) were low, our study obtained somewhat different results (52.6% and 7.2 yuan, respectively). There are several reasons for this result. (1) As the Chinese government has strengthened its efforts to control the environment, environmental protection has become a bottom line for farmers (Bai et al., 2022). The government inspects farms for environmental problems at least 3–5 times per year, which raises the levels of environmental awareness and places higher pressure on farmers (Li et al., 2023). If a farm is found to have failed to comply with the standards of manure treatment or even to have discharged manure clandestinely, the farmer is fined (0–50,000 yuan) and may even face farm closure or jail time due to environmental concerns (Pan, 2023). Farmers participate in the PMOTP as long as they can guarantee the good environmental status of their farms. (2) The cost of PMOTP participation can vary significantly depending on external factors, such as transport distance. In addition, the survey used in this study took place in 2023, and compared with the data of Zhao et al.'s survey in 2019, price increases are an important factor that must be considered. All of the above factors can lead to differences in farmers' willingness to participate and their payment levels.

Our study also further confirms the role of transaction cost theory and peer effects in PMOTP participation. Although farmers are strongly willing to participate in the PMOTP, transaction costs

are an important factor inhibiting them. In fact, the Chinese government emphasizes that farmers should invest in environmental treatment equipment (Bai et al., 2019), which can increase the transaction costs of farmers participating in the PMOTP and create a conflict in the promotion of these projects. Therefore, how to reduce the transaction costs of farmers adopting PMOTP is the key to promote environmental protection projects (Coggan et al., 2010; Palm, 2017). Peer participation and support are important factors that motivate farmers to participate in the PMOTP. Furthermore, PEs reduce the inhibitory effect of transaction costs on farmers' PMOTP participation, and the "information exchange," "behavior imitation," and "consumption cluster" are verified. A large amount of sociological literature also supports this assertion, and the role of PEs have been verified in schools, enterprises and factories (Ali-Rind et al., 2023).

Heterogeneity analyses show that large-scale farmers are more significantly affected by transaction costs and PEs than are other types of farmers. In China, the number of large-scale farms continues to increase, which has also led to stricter government regulation of these farms. To ease the pressure of environmental regulation, large-scale farms tend to pay more attention to market information and methods of handling livestock manure, and they are willing to adopt new methods suggested by their peers (Zhou et al., 2024). Our further analyses reveal that although a higher degree of asset specificity is associated with a lower willingness of farmers to participate in the PMOTP, it is conducive to increasing the level of farmer payments, although this phenomenon still deserves further investigation. This result shows that transaction costs have two effects on farmers' adoption of new technologies: on the one hand, transaction costs will reduce the willingness and behavior of some farmers to adopt new methods; on the other hand, farmers with high asset specificity have better capital to adopt new technologies than those with low asset specificity.

6 Research conclusions and policy recommendations

This paper adopts the traditional probit model and Heckman probit model and uses research data from 519 pig farmers in Sichuan Province to analyze the impact of transaction costs and peer effects

on farmers' participation in the PMOTP. The main conclusions are as follows. (1) Farmers' willingness to participate in the PMOTP decreases with asset specificity, transaction frequency and uncertainty but increases with PEs. (2) The inhibitory effect of transaction costs on farmers' willingness to participate in the PMOTP is weakened by PEs. (3) Heterogeneity analyses show that nonparticipating farmers, self-breeding and self-rearing farmers, and large-scale farmers are more significantly affected by transaction costs and PEs. (4) Increased transaction frequency and uncertainty can reduce farmers' willingness to pay for PMOTP participation, but asset specificity and PEs are likely to facilitate increased farmer investment in the PMOTP.

Based on the above conclusions, this paper offers the following policy implications. First, transaction costs have a significant inhibitory effect on farmers' participation in the PMOTP. Therefore, the transaction time, transaction frequency and transaction method should be reasonably arranged to reduce farmers' information search costs and transaction costs, the transaction contract should be standardized, and farmers should cooperate with the government as a guarantor to reduce the degree of transaction risk. Second, by exploiting collective consumption, cooperatives, farming associations or village collective organizations can unify their negotiations with PMOTP providers and reduce transaction costs by leveraging scale advantages and competitiveness. Finally, large-scale farmers have shown sufficient willingness to adopt PMOTP, so the government should also subsidize this project and encourage large-scale farmers to adopt PMOTP. The number of large-scale farmers is increasing in China, and these farmers can play a better demonstration role and peer effects.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: the data that support the findings of this study are available from the corresponding author [JL], upon reasonable request. Requests to access these datasets should be directed to Ljq9801@126.com.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the

[patients/participants OR patients/participants legal guardian/next of kin] was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

KZ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft, Writing – review & editing. HW: Funding acquisition, Writing – review & editing, Supervision. ZZ: Conceptualization, Data curation, Methodology, Writing – review & editing. JL: Conceptualization, Funding acquisition, Writing – review & editing, Supervision.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by the National Nature Science Foundation of China [72103152] and Sichuan Provincial Social Science Planning Project [SC19B084].

Acknowledgments

Thanks to the editors and reviewers for their suggestions.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

EDITED BY

Maria Pilar Bernal,
Spanish National Research Council
(CSIC), Spain

REVIEWED BY

Marco E. Mng'ong'o,
Mbeya University of Science and
Technology, Tanzania
Eric C. Peterson,
INRS Eau Terre Environnement, Canada

*CORRESPONDENCE

Keiji Jindo
✉ keiji.jindo@wur.nl

RECEIVED 30 March 2024

ACCEPTED 13 January 2025

PUBLISHED 07 February 2025

CITATION

Jindo K, Ghaffari G, Lamichhane M, Lazarus A,
Sawada Y and Langeveld H (2025) Assessment
of trade-off balance of maize stover use for
bioenergy and soil erosion mitigation in
Western Kenya.
Front. Sustain. Food Syst. 9:1409457.
doi: 10.3389/fsufs.2025.1409457

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Assessment of trade-off balance of maize stover use for bioenergy and soil erosion mitigation in Western Kenya

Keiji Jindo^{1,2*}, Golaleh Ghaffari³, Manisha Lamichhane³,
Asher Lazarus³, Yoshito Sawada⁴ and Hans Langeveld³

¹Agrosystems Research Group, Wageningen University and Research, Wageningen, Netherlands, ²Plant Production Systems Group, Wageningen University and Research, Wageningen, Netherlands, ³Biomass Research, Wageningen, Netherlands, ⁴Graduate School of Horticulture, Chiba University, Chiba, Japan

Introduction: Kakamega Forest, Kenya's last tropical rainforest, faces threats from escalating demands for firewood, charcoal, and agricultural expansion driven by population growth. Sustainable resource management is critical, with maize stover—a common lignocellulosic crop residue—proposed as a source for biogas and bioslurry production. However, its removal raises concerns about soil fertility and erosion control. This study evaluates the trade-off between maize stover's use for energy generation and its role in soil erosion mitigation in Western Kenya, considering biophysical conditions and households' socio-economic status.

Methods: Socio-economic data were collected from 91 households to assess energy usage. Soil erosion was quantified using the Revised Universal Soil Loss Equation (RUSLE). High-resolution remote sensing, self-organizing maps (SOM), and Hidden Markov Models (HMM) were integrated for annual field condition monitoring. A decision-tree machine learning model identified farm characteristics favorable for maize stover use in biogas production.

Results: Larger households were found to consume more energy per capita, while proximity to forests did not significantly influence firewood or charcoal consumption. Maize yields were significantly associated with land preparation methods (tractor, oxcart, or manual plowing; $p < 0.001$) and field size ($p < 0.05$). Remote-sensing data indicated that the distance between homesteads and fields impacted crop growth status. RUSLE analysis revealed that soil erosion was more strongly influenced by landscape features than by soil properties or farming practices.

Discussion: The integration of SOM-HMM and microsatellite data improved field monitoring and data accuracy, providing valuable insights for sustainable agricultural practices in Kakamega. These findings highlight the complex trade-offs between maize stover utilization for energy production and its role in maintaining soil health, emphasizing the need for balanced resource management strategies.

KEYWORDS

bioslurry, crop residues, deforestation, energy, remote-sensing, smallholder farmers

1 Introduction

Western Kenya, home to the Kakamega Forest—the nation's last tropical rainforest covering 238 square kilometers—faces significant environmental challenges due to rapid population growth and urbanization (Obonyo et al., 2023; Mitchell et al., 2009). Sustainable resource utilization is critical to balance conservation efforts and livelihood needs (Reetsch et al., 2020). Maize

stover, the residue from maize harvesting, offers a dual benefit: it can serve as a renewable energy source through biogas production and as crop residue mulch to reduce soil erosion and enhance soil fertility. These practices are particularly vital in fragile ecosystems like Kakamega Forest and Mt. Elgon, where traditional fuel demands drive deforestation (Tumwesige et al., 2011; Smith et al., 2015).

Anaerobic digesters using maize stover for biogas production not only provide energy but also generate nutrient-rich effluent that improves soil fertility, reducing the need for chemical fertilizers (Kinyua et al., 2016). However, maize stover collection presents trade-offs. It may mitigate nitrate leaching but increase sediment runoff or greenhouse gas emissions due to additional fertilizer use (Gramig et al., 2013; Mann et al., 2002). Studies suggest that sustainable management practices, such as no-till farming, can minimize these adverse effects (Moebius-Clune et al., 2011, 2008).

In Sub-Saharan Africa (SSA), competing demands for maize stover—primarily as livestock feed—limit its use for soil health management (Hellin et al., 2013; Erenstein et al., 2011). Addressing these challenges requires context-specific strategies incorporating socio-economic and environmental factors (Jaleta et al., 2015; Orskov et al., 2014). Promoting anaerobic digesters, advancing maize stover research, and aligning practices with local farmer needs are essential steps forward.

Kenya has initiated significant efforts to reduce greenhouse gas (GHG) emissions by 30% (Dalla Longa and van der Zwaan, 2017), prioritizing expanded access to renewable energy. The Draft Strategy and Action Plan for Bioenergy and LPG Development in Kenya (2015–2020) highlights advancements in modern bioenergy technologies (Ministry of Energy Pe, 2015) and the sustainable utilization of agricultural crops and residues for biogas and biomass fuel production. Progress in renewable energy adoption is evident, with the number of installed biogas systems increasing from 6,749 in 2012 (Patinvoh and Taherzadeh, 2019) to ~20,000 by 2015 (Ministry of Energy Pe, 2015).

This study investigates maize stover utilization in Western Kenya, examining its dual potential for energy generation and soil erosion mitigation. Using the Revised Universal Soil Loss Equation (RUSLE) model, we estimate soil loss at the farm level while machine learning techniques evaluate the feasibility of maize stover-based biogas production. To further refine these analyses, we integrate high-resolution remote sensing data with hybrid classification methods, offering a comprehensive perspective on year-round farm conditions (Kim et al., 2012). This approach aims to contribute to sustainable resource management and energy solutions.

2 Material and methods

This section outlines the research area (Section 2.1), data collection of socio-economic information (Section 2.2), soil loss estimation using RUSLE (Section 2.3), application of remote sensing (Section 2.4), and machine learning-based estimation of partial energy replacement by maize stover-based methane production (Section 2.5). Statistical methods are described in Section 2.6.

2.1 Study area

The study included 96.7% of farmers from Busia, Bungoma, Kakamega, and Vihiga counties (Western Kenya) and 3.3% from Siaya County (Nyanza Province). The region experiences long and short rainy seasons, with an average maize yield of 1.9 tons/ha (van Ittersum et al., 2016). Fertilization practices include DAP at planting and CAN for top-dressing, with a density of 53,333 plants/ha. Detailed descriptions of soil types, agro-climatic zones, and farm systems are in Jindo et al. (2020). Farmers were supported by Agrics, a Kakamega-based social enterprise owned by ICS, which provides input packages and services, as previously detailed (Duflo et al., 2008).

2.2 Socio-economic information

Socio-economic data were collected from 91 farmers in 2016 using 33-question digital surveys via ODK on Android devices (FAO, 1983). Interviews lasted ~30 min per farmer and captured household status, agronomic practices, and energy use. GPS coordinates of farmer fields and nearby forests were recorded (Kenya Forest Service), and maize yield (reported as 90-kg bags) was converted to dry weight using a 0.9 ratio. Distance to forests was measured using QGIS. Data were validated with local collaborators.

2.3 Soil loss estimation using Revised Universal Soil Loss Equation (RUSLE)

We employed the Revised Universal Soil Loss Equation (RUSLE), a widely used empirical model, to assess soil erosion risks in maize fields under varying stover management practices (Benavidez et al., 2018). RUSLE estimates soil loss (A , $t\ ha^{-1}\ year^{-1}$) as the product of five factors:

$$A = R \times K \times LS \times C \times P \quad (1)$$

where A is the annual soil loss ($t\ ha^{-1}\ year^{-1}$), R is the rainfall erosivity ($MJ\ mm\ t\ ha^{-1}\ h^{-1}\ year^{-1}$), K is the soil erodibility ($t\ ha^{-1}\ MJ\ mm^{-1}$), LS is the length-slope factor, C is the cover management factor, and P is the conservation practice factor (Wischmeier and Smith, 1978). Data sources for RUSLE factors are detailed in Supplementary Table 1. The R factor was derived using WorldClim's precipitation data (<http://www.worldclim.org/current>). For the K factor, soil properties (texture, organic carbon, bulk density) were extracted from SoilGrids ISRIC (250 m resolution) and calculated using Williams's (1995) approach, commonly applied in models like EPIC and SWAT (Arnold et al., 1998). The LS factor was computed using Moore and Burch's (1986) method with a 30 m resolution DEM from the Shuttle Radar Topography Mission (SRTM). Flow accumulation and slope data were processed in ArcGIS, combining unit stream length (L) and slope (S) values (details in Supplemental material and Figure 1). The CCC factor, based on vegetation cover, was estimated using biannual NDVI data from PlanetScope (Karmage

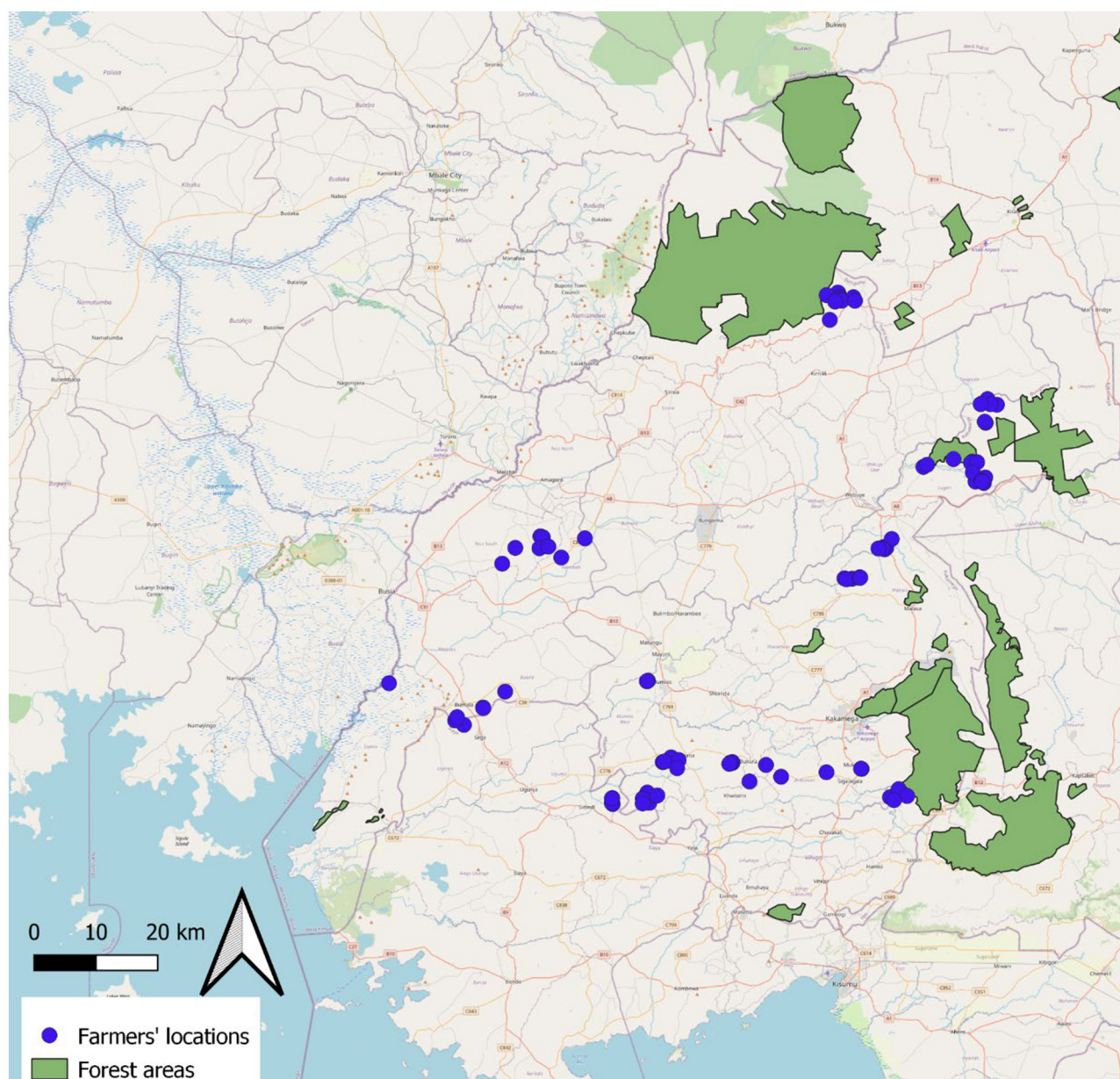


FIGURE 1

Location of farmers' fields (blue circle) and Kakamega forest areas (green polygons) in western Kenya.

et al., 2017). Finally, the P factor was calculated using slope data, following Kogo et al. (2020).

This approach integrates terrain, soil, and vegetation dynamics to assess erosion risks comprehensively.

2.4 Remote-sensing data and the Self-Organizing Maps–Hidden Markov Models (SOM-HMM) approach

The PlanetScope constellation comprises ~130 CubeSats, offering near-daily global coverage at a 3-meter resolution across

four spectral bands (red, green, blue, and near-infrared) (Planet, 2020). This high-resolution imagery supports applications such as disaster monitoring (Ganci et al., 2020), detecting fine-scale land cover changes (Halls and Magolan, 2019), precision agriculture (Breunig et al., 2020), and tree-crown phenology analysis (Wu et al., 2021). However, like other satellite data, PlanetScope imagery is prone to artifacts from BRDF effects and cloud contamination, necessitating preprocessing to reduce these errors (Wang et al., 2021; Valman et al., 2024).

Despite selecting cloud-free images, some contamination remained in our dataset (Figure 2). To address this, we applied a time-series modeling approach using SOM-HMM (Sawada, 2010). Self-Organizing Maps (SOM) cluster and visualize

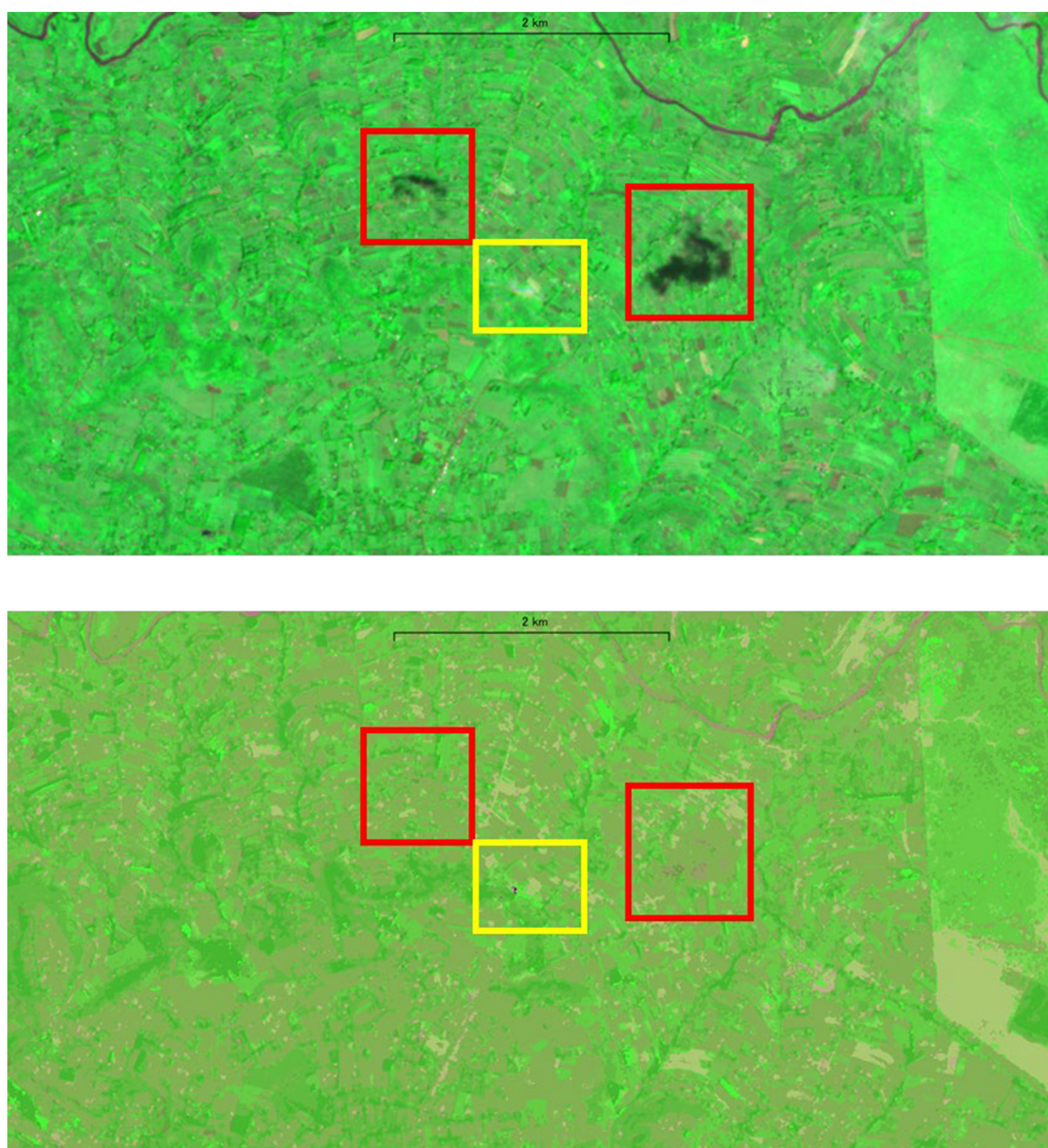


FIGURE 2

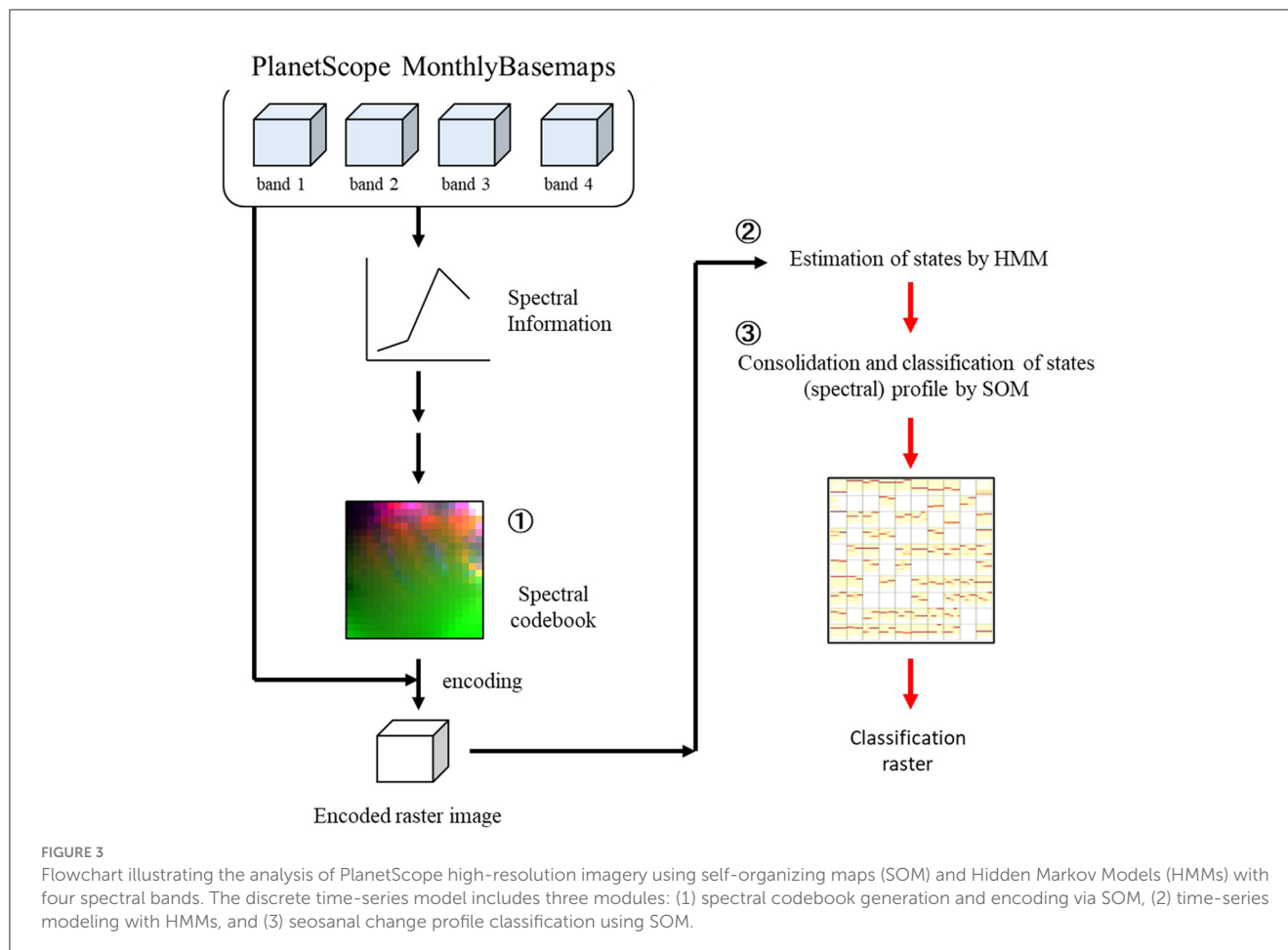
Change in the PlanetScope basemap satellite imagery (monthly, Nov. 2020). **(Upper image)** Original imagery before processing; **(lower image)** processed using the Hidden Markov Models (spectral cluster). Red boxes indicate cloud shadows, while the yellow box highlights the clouds.

high-dimensional data, while Hidden Markov Models (HMM) identify sequences of underlying states, enabling monthly and annual field state classifications based on spectral analysis (Figure 3).

In addition to optical data, radar remote sensing data from PALSAR (Phased Array type L-band Synthetic Aperture Radar) was used to monitor field and soil conditions. As an active microwave sensor, PALSAR provides cloud-free, day-and-night observations (JAXA). Backscatter coefficients (HV and HH) were retrieved from the “Global PALSAR-2/PALSAR Yearly Mosaic” on Google Earth Engine, and additional metrics, such as HV/HH and HH+VV, were derived (Shimada et al., 2014). The Radar Vegetative Index (RVI) was calculated as per Yadav et al. (2022).

2.5 Learning for estimating partial energy replacement via maize stover-based methane production

Decision tree learning uses a structured model to analyze observations and predict target variables, applied widely in statistics, data mining, and machine learning. Classification trees predict discrete variables, with class labels at the leaves and feature combinations on branches, while regression trees estimate continuous variables. Decision trees aid decision analysis and summarize complex datasets for actionable insights. Random forests extend decision trees by constructing multiple trees during



training. For classification, they select the majority class; for regression, they compute the average prediction (Breiman, 2001). The predictor variable in this study is the “replacement of energy consumption by maize stover-based biogas,” derived from three variables: (1) household energy consumption per capita, (2) household size, and (3) maize stover yield (calculated using a harvest index ratio of 0.5 based on other works (Lal, 2004; Karlen et al., 2015; Obrycki et al., 2018; Paul et al., 2019). Methane yield from maize stover is estimated at 288 m³ CH₄/ton (Total Solid) based on Langeveld and Peterson (2018), and converted to calorific value (MJ) following Suhartini et al. (2019). Potential methane energy is then compared to household energy needs. The households currently derive energy from some combination of firewood, charcoal and/or LPG gas, so conversion efficiency of methane to electricity is not considered. Farmers experiencing soil erosion (RUSLE > 0) are assumed to retain maize stover in the field, prioritizing soil conservation.

2.6 Statistical analysis

Statistical analyses included *t*-tests, Mann–Whitney tests, one-way ANOVA, Kruskal–Wallis tests, and correlation analyses. *Post-hoc* Tukey and Games–Howell tests were applied for group comparisons. Normality was assessed using Anderson–Darling tests, with $p \leq 0.05$ considered significant. R packages such as “agricolae” and “randomForest” were utilized (R Core Team,

2020). ChatGPT (OpenAI model GPT-4) was utilized to assist in the correction of our R script, ensuring accuracy and optimization for the analyses conducted in this study. The input prompts and outputs related to this use are provided in the [Supplementary material](#) for transparency.

3 Results

3.1 Household dataset (Linear regression and *t*-test)

Field management practices, such as type of land preparation, significantly impact maize yield ($p < 0.001$; Figure 4A). The average yields in maize fields prepared by tractor, oxcart, and farmer’s hand are 317.0, 251.3, and 152.8 kg/ha respectively (Figure 4A). Maize yield was notably influenced by field size ($p < 0.01$; Figure 4B) and mechanization level. Farmers with larger fields tend to utilize tractors for land preparation, whereas those with smaller fields cultivate manually. The amount of weeding and manure applications also weakly affects maize yield ($0.1 < p < 0.05$). The number of hired laborers had no statistically significant effect on yield. Duration of family food self-sufficiency is notably influenced by the household size ($p < 0.05$) and, to some extent, field size ($0.1 < p < 0.05$). Interestingly, farmers without off-farm income exhibit a superior duration of self-sufficiency (7.9 months compared to 6.6 months, $p < 0.05$). Energy consumption expressed per individual increases with family size ($p < 0.05$).

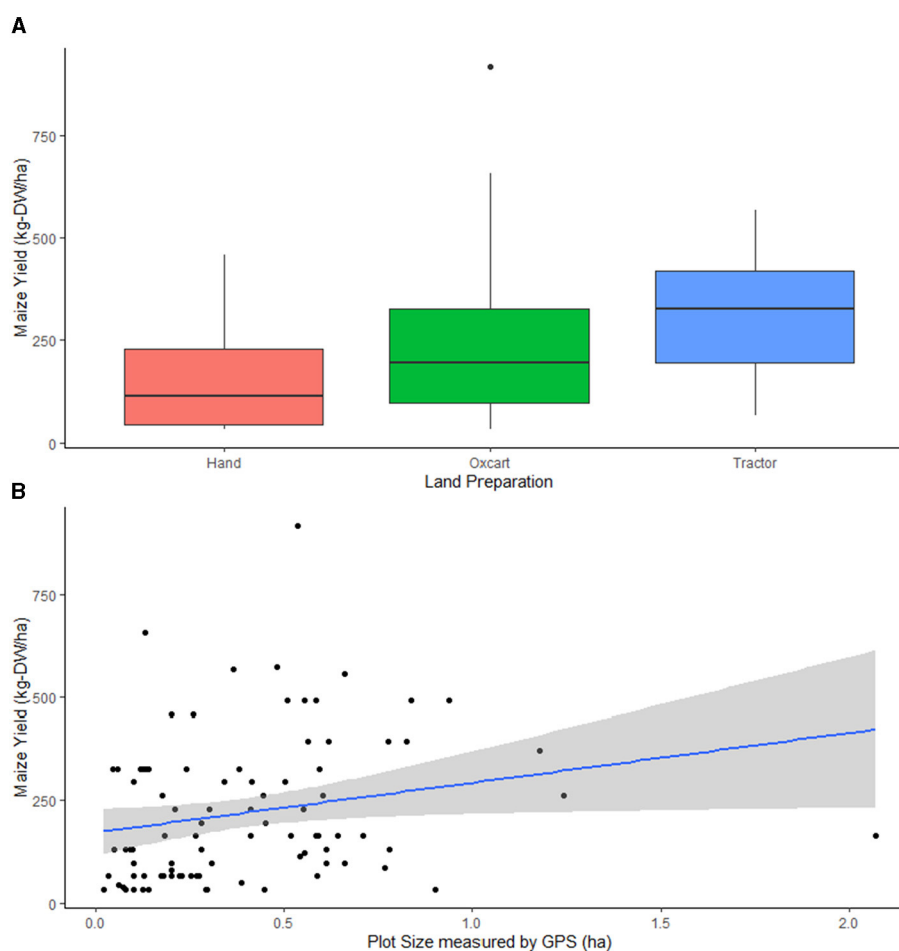


FIGURE 4

(A) Maize yield (kg dry weight/ha) based on the land preparation methods: Hand (red), Oxcart (green), tractor (blue). (B) Maize yield (kg dry weight/ha) in relation to plot size (ha), measured using GPS. The blue line represents the linear regression line.

While not achieving statistical significance, farmers with off-farm income allocate a greater proportion of maize for sale compared to those without such income ($0.1 < p < 0.05$). No gender disparities were found in maize yield, land size, or livestock stock units (LSU). Widows and single mothers do not exhibit inferior performance or asset levels in relation to crop yield, land size, or livestock. No significant difference was shown in the relationship between distance to the homestead and maize yield. The mean maize stover value is 540.4 kg per household, with a standard deviation of 430.66 kg per household, indicating considerable diversity among the smallholder farmers in our dataset.

3.2 Soil erosion

The mean value of the potential soil erosion rate of the farmers' fields is 0.01 ton/ha per year. The maximum and minimum values of the potential soil erosion rate are 0.19 and 0.003 ton/ha per year, respectively, but 80.3% of farmers show no soil loss according to RUSLE ($A = 0$) as is explained by their fields' low LS factors. The mean values of factors K, R, LS, C, and P are 0.0267, 6,529.4, 0.028, 0.0184 and 0.1063, respectively.

3.3 Self-Organizing Maps–Hidden Markov Models (SOM-HMM)

A spectral codebook for PlanetScope's multi-band images was created using SOM, and it contains 400 codes. Next, images of the target area were encoded using the obtained spectral codebook. The time series modeling software using SOM-HMM includes a feature for automatically determining the number of HMM states, and the optimal number of states was found to be 20. The mean spectral reflectance spectra of the obtained states corresponded to "water," "bare soil/artificial features," and "vegetation" land cover categories for each of the 20 states. States 1 to 6 represent "water bodies," state 7 corresponds to "bare soil," and states 8 to 20 correspond to "vegetation." Additionally, the temporal changes in states at the pixel level were found to represent the phenology of that location. The time series profiles of states for each pixel were classified into 67 categories. As seen in Figure 2, the noise of the cloud and cloud shadow in optical remote-sensing data is removed by this approach.

In our study, there are substantial relationships between the field states variables from SOM-HMM and other variables from the field survey. The distance to the homestead field significantly affected the monthly state of May and September in

the SOM-HMM ($p < 0.05$). The monthly state of March and April was weakly affected to a lesser degree ($0.05 < p < 0.1$). The distance to the nearest forest significantly affected the annual Cluster ID of SOM-HMM ($p < 0.05$). The distance to the nearest forest from the farmer's field significantly affected the monthly state of May, June, October and November in the SOM-HMM ($p < 0.05$), and these months were at the peak of the two cropping seasons (long-rain and short-rain seasons) where plant growth increases strongly. Other months, such as July, August, September and December, which were toward the end of the cropping seasons, have been weakly affected ($0.05 < p < 0.1$). No significant difference is shown in the relationship between annual cluster-ID and maize yield.

3.4 Synthetic Aperture Radar (SAR) remote sensing data

Linear regression analysis was conducted to find relationship with SAR derived variables and others. HH has significantly relationship with monthly state over the year, especially, dry season such as November ($p < 0.05$), December ($p < 0.05$), January ($p < 0.05$), and February ($p < 0.01$), respectively. RVI also has significantly relationship with the monthly state of November ($p < 0.05$) and February ($p < 0.05$). No significant relationship is seen between maize yield and the all SAR-derived variables. Interestingly, manure amount has significant relationship with HV ($p < 0.05$) and HH+HV ($p < 0.05$).

3.5 Correlation matrix

Figure 5 represents the correlation matrix between variables. The order of the variables in the correlation matrix is arranged according to the outcomes of the hierarchical clustering conducted to identify cluster groups across different variables. The correlation analysis shows that elevation positively correlates with distance to the forest, crop yield, land preparation type, field size, and monthly field states from optical remote-sensing data (May, June, Augustus, September, and October). At the same time, it negatively correlates with manure application levels. No clear link was found between the proximity to the nearest forest and energy consumption. Per capita energy consumption was found to have a slightly positive correlation with the number of household members but a negative correlation with self-sufficiency duration. The proportion of the maize that is sold was correlated with plot size. The level of energy consumed per person is positively correlated with household size and negatively with the duration of self-sufficiency. Maize field states derived from remote sensing during the May 2021 to October 2021 period have a significant negative correlation with the distance to the field. Further, annual Cluster ID has a weak negative correlation with distance to the nearest forest, household number and soil loss rate. Maize yield has negatively correlated with the nearest forest, positively with a type of preparation, field size, and elevation. Daily income is highly correlated with the number of hired laborers, the number of household members, and LSU. Variables from SAR-remote sensing (e.g. RVI, HH/HV and HH+HV) have positive correlations with

monthly field states measured by optical imagery from October to February. Interestingly, the variables of HH+HV and HV are positively correlated with the amount of manure application.

Based on the result of the clustering method, the number of clusters was chosen to 2, as seen in the [Supplementary Figure 2](#). Ten variables are selected in the small clustering group, including LSU, Age of the household-head, Off-farm income, and Daily income. Other large cluster group covers other variables.

3.6 Decision tree random forest

Figure 6 represents the result of the decision tree, which is one of machine learning methods. The different ratios (0.7, 0.75, and 0.8) between the train data and the test data were tested. And 0.75:0.25 was chosen due to the lowest value of RMSE. The number of splits is two according to the "Complexity Parameter Method" result for the decision tree machine learning method (Venkatasubramaniam et al., 2017). The mean value of the percentage of replacing the energy consumption per household by the maize stover-based biogas generation is 11% of the total energy composition per household (Figure 6). The largest group with 63% of the households in this study ($n = 57$), could replace 3.4% of the energy consumption by the maize stover biogas. They are categorized as the group which has higher energy consumption than 5.7 GJ/capita and lower maize yield than 279 kg-DW/ha. The second and the third largest groups, including 18% and 13% of the households in our study, respectively, could replace 7.3 and 20% of the energy by the maize stover. Conversely, the maximum benefit is achieved in households with fewer members, primarily relying on LPG gas and producing maize at a rate of 229.46 kg per hectare. This household, representing 1% of our dataset, can make 245% of the replacement. Secondly, farmers who produce higher yields than 197 kg-DW/ha, representing 3% of our dataset, can replace 52% of the energy consumption with maize stover-based biogas.

Energy consumption associated with maize stover-based biogas production is examined in our study. Key variables for assessing this method include energy consumption per capita, household size, crop yield, field size, and duration of self-sufficiency. Additionally, variables such as "Annual cluster ID" and "Distance to the nearest forest," while not directly linked to household energy consumption or maize yield, are deemed relatively important (Supplementary Figure 3). The diversity among farming households is evident in our dataset, as seen in the variability of these variables, thereby resulting in different groups for energy substitution with maize stover.

4 Discussion

Biogas production in Sub-Saharan Africa (SSA) has considerable potential due to favorable climate, feedstock availability and a large part of the workforce involved in agriculture (Timothy et al., 2022). Despite this potential, the uptake of biogas systems remains slow and sporadic, primarily due to high installation costs and limited awareness (Rupf et al., 2015). Biogas also comes with barriers to electrification, as many

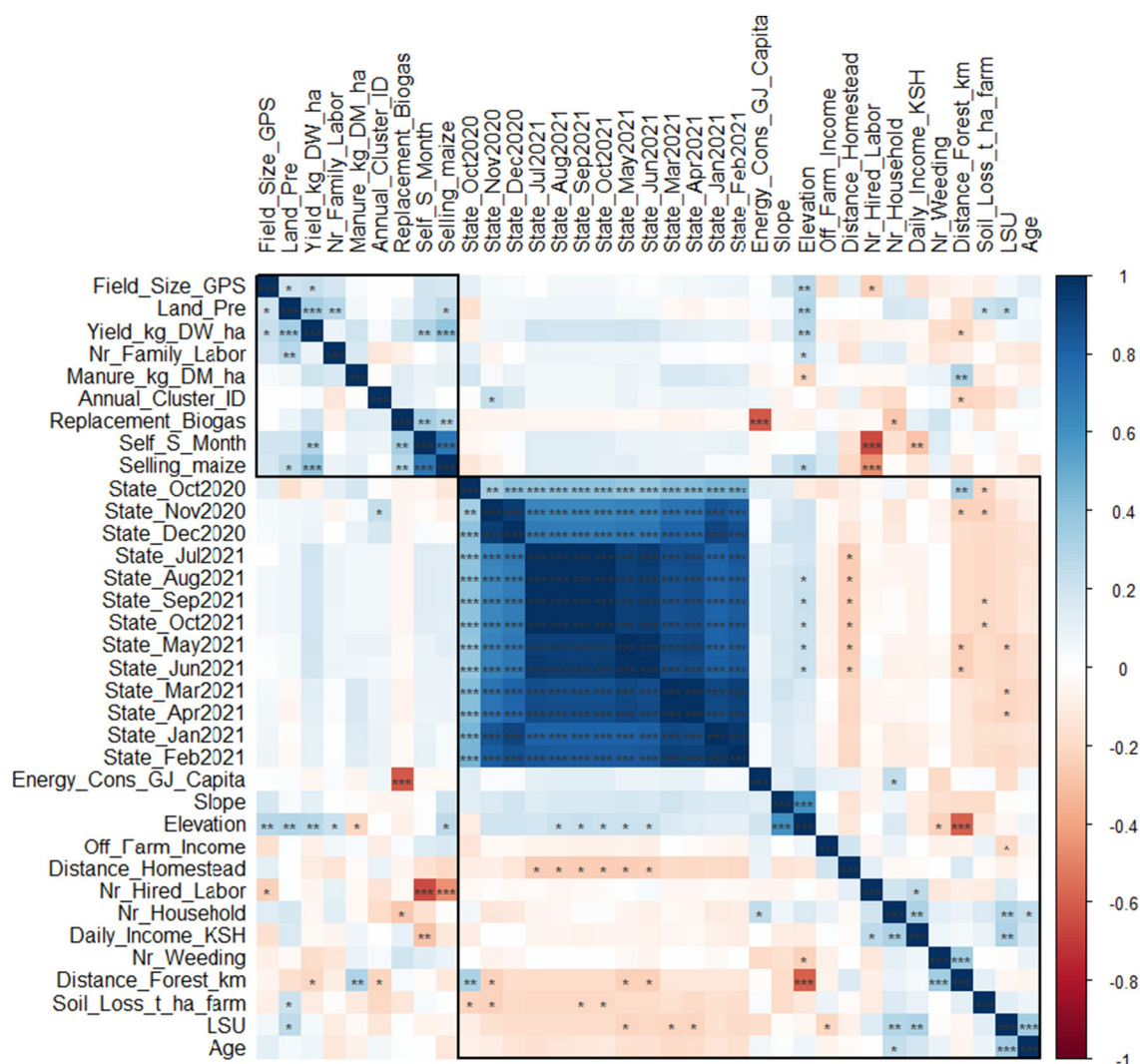


FIGURE 5

Correlation matrix plots using variables from field surveys and remote-sensing data. The correlations are represented with a color scale: red indicates a negative correlation, and blue indicates a positive correlation. Asterisks denote statistically significant correlations, highlighting stronger relationships.

available conversion technologies are expensive, inefficient, and may require costly intermediate steps (Kabeyi and Olanrewaju, 2022). Collaboration among various stakeholders, including government, financial institutions, NGOs, research entities, and entrepreneurs, is deemed essential for successful implementation (Roopnarain et al., 2021). Socio-economic constraints hindering biogas adoption need to be addressed, with proposed solutions emphasizing standardization, quality control, and integrated farming (Mwirigi et al., 2014).

Maize stover, residual biomass left in the field after maize grain harvest, holds promise as a feedstock for biogas production (Mazurkiewicz et al., 2019). Maize stover is more abundant feedstock in smallholder households in Western Kenya than other feedstocks (Torres-Rojas et al., 2011). This carbon rich material, holds cellulose, hemicellulose and lignin with the proportion of 34:19:19 (Langeveld and Peterson, 2018), and contains the range of 0.6%–0.75% of Nitrogen (Karlen et al., 2015; Ludemann et al.,

2022; Meya et al., 2023) and 1.2%–1.7% of Potassium (Islam et al., 2018; Meya et al., 2023). Its high methane yield potential, valuable digestate (suited for nutrient recovery), and low hydrogen levels exceed those of alternative feedstocks such as coffee pulp, cotton waste, sugarcane leaves, and banana stalks (Nzila et al., 2015). Converting maize stover to biogas circumvents the conflict between food and biofuel production, and it contributes to sustainable agricultural practices by returning nutrients to the soil through digestate application (Mazurkiewicz et al., 2019). However, in practice, variations in lignin content across maize stover fractions can impact methane yield, which necessitates further research (Wozniak et al., 2021). Also, harvesting corn stover for energy production can lead to increased erosion, reduced soil quality, and impacts on soil organic carbon dynamics (Mann et al., 2002).

In practice, most farmers included in our dataset had an extremely low risk of soil erosion, confirming other soil erosion estimates in Western Kenya (Kogo et al., 2020). The average loss

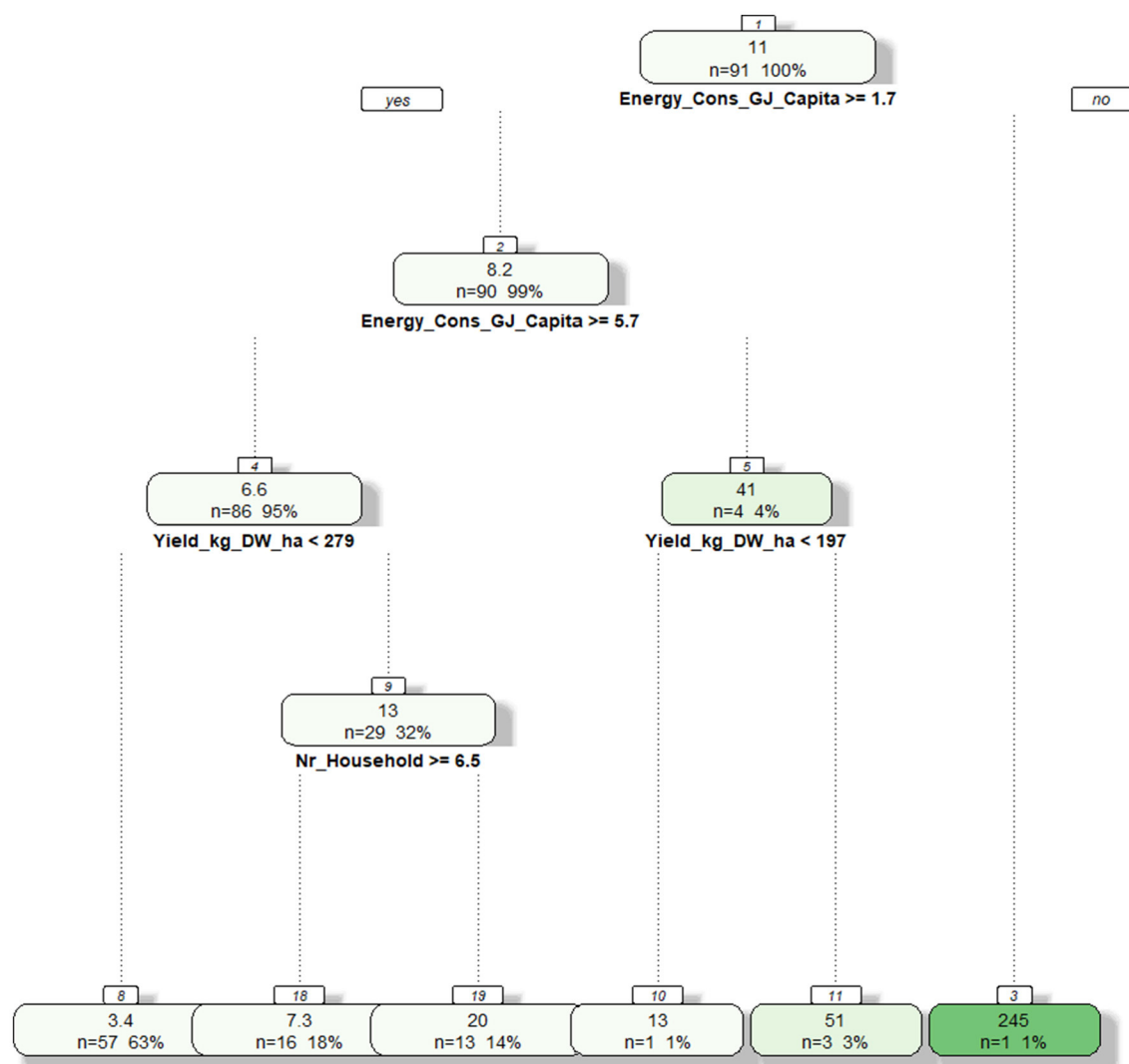


FIGURE 6

Decision tree chart from machine learning analysis for substituting energy consumption with maize stover-based methane generation. The key variables in this models are energy consumption per capita ("Energy_Cons_GJ_Capita"), maize yield ("Yield_kg_DW_ha"), and number of household members ("Nr_Household").

is 0.4 t/ha/y. The study area's most dominant severity class has low erosion rates (<5 ton/ha/y), which are explained by low LS factors as supported by other studies (Kogo et al., 2020; Schürz et al., 2020). Future studies could address this limitation by incorporating field measurements of erosion rates under varying crop residue and soil management practices, providing empirical evidence to complement the modeling results presented in this study.

Several agro-ecological zones exist in Western Kenya, given its topographic diversity. The positive correlations between elevation and monthly field states during the cropping seasons could reflect this difference (Figure 5). As an example, the fields located at higher elevations close to Mt. Elgon encounter favorable climate and soil conditions for maize growth which allow higher yield levels. On the contrary, fields located in lowland areas require higher manure applications, as reflected in the negative correlation between elevation and manure application.

Apart from the varied landscape and agro-ecological zones, the location of the farmer's field in relation to the farmer's home

is important, given that different field management (e.g. fertilizer, manure, weeding, pest management) depending on the distance to the home creates a fertility gradient within the farm (Vanlauwe et al., 2006; Titttonell et al., 2007; Kihara et al., 2015; Assefa et al., 2020). Generally, soils located close to homesteads show higher fertility including soil organic carbon and cation exchange capacity due to organic amendments of crop residues and animal manure compared to those located far from the home (Yakob et al., 2023). Farmers utilize decomposed plant material, kitchen refuse, and household wastes, often leaving them in heaps around their households for decomposition (Nkamleu, 2007). The decomposed material is applied differentially across fields, with homestead fields receiving higher application rates.

Our study found that the SOM-HMM approach, utilizing remote-sensing data, correlates significantly with the distance between homes and homestead fields. Specifically, we observed significantly higher above-biomass status during the cropping season, as reflected by the variables of remote-sensing data,

in fields closer to the home. High-resolution remote sensing data has been instrumental in monitoring smallholder farmers' fields globally (Burke and Lobell, 2017). Additionally, innovative approaches such as the insurance index and platform-based advice to farmers have been developed based on vegetative indices like NDVI. Furthermore, radar sensor measurements can provide supplementary monitoring of soil moisture and vegetative water content (Narvekar et al., 2015), as demonstrated in our study, where positive correlations were observed with variables of the SOM-HMM during the dry season (January and February).

The effectiveness of these technologies is further enhanced when combined with freely available soil digital maps, providing insights into various soil properties. While the use of soil digital maps at the regional level is often criticized for lacking precision in capturing spatial heterogeneity at the smallholder farming level (Jemo et al., 2014; Maynard et al., 2023), the HMM-SOM model integrated with microsatellite data serves as a valuable complementary tool for enhancing the monitoring of field status over the years and improving data quality.

It should be noted that in our study, the variables derived from the SOM-HMM model have no significant correlation with farmer self-report yield. The mean value of maize yield self-reported by farmers, 0.22 ton/ha, is much lower than values reported elsewhere for small-scale farms in Kenya in the same cropping season (2016 long-rain; around 0.80–1.0 ton/ha; Marinus et al., 2021; Kätterer et al., 2022). The difference is probably attributed to the different measurement methods between self-report and physical crop-cutting. Earlier studies found the inaccuracy of farmer self-reported yields being a strong constraint for the understanding of farming systems in SSA (Carletto et al., 2015; Burke and Lobell, 2017) while the gaps with field monitoring based on remote sensing approach have been often reported (Burke and Lobell, 2017; Paliwal and Jain, 2020). It is important for future work to identify ways to increase the accuracy of self-reported yield estimates at the plot-level and find an alternative method (e.g. integration between self-reported yield and physical crop-cutting measurements).

In Kenya, unsustainable logging for wood fuel, particularly charcoal production, is a primary cause of deforestation (Watson and Diaz-Chavez, 2011), while also causing land degradation and destruction of water catchment areas (Nunes et al., 2021). Promoting biogas usage can reduce fuelwood and charcoal demand, contributing to deforestation mitigation efforts. By shifting from biomass to biogas as an energy source, the demand for non-renewable biomass decreases, addressing a major driver of deforestation (Piadeh et al., 2024). In addition, it should be noted that biogas systems constitute important mid-term household cooking transition alternatives not only for energy savings but also for atmospheric emission reductions (e.g. PM_{2.5}, NO_x, and NMVOCs) from firewood fuel, which is a major social issue in Western Kenya (Carvalho et al., 2019, 2020). Furthermore, biodigestate from biogas production, known as “bioslurry,” enhances soil fertility, particularly over the long term, and helps reduce the need for harmful land-use practices while supporting forest conservation efforts (Piadeh et al., 2024). Other studies (Musse et al., 2020; Yadav et al., 2023) have reported that liquid bioslurry contains 1.5%–2% total nitrogen, 300 mg/kg of available phosphorus, and 715 mg/kg of available potassium.

This nutrient management cycle, which includes the application of bioslurry, not only helps maintain soil fertility but also has the potential to reduce dependence on chemical fertilizers, thus benefiting smallholder farmers by lowering input costs.

Replacing traditional energy sources and chemical fertilizers with biogas in Kenya is estimated to save households between \$21 and \$25 per month (Kimutai et al., 2024). However, the high cost of installing biogas systems continues to pose a significant challenge, particularly in resource-limited settings (Naik et al., 2014). The cost of a small-scale plant in Kenya was estimated at \$1,500 due to high construction material costs (Roopnarain et al., 2021). For example, construction materials in Kenya are not only expensive but also difficult to access, as transportation to and from villages adds to the expense. The Kenyan government has facilitated the implementation of ~20,000 biogas units nationwide (Kimutai et al., 2024). Also, it is reported that there exist several companies in Kenya that have reduced construction cost up to 20% by modifying design and materials (Clemens et al., 2018). Despite this effort, these systems account for only 11.4%–14.6% of the household energy mix in peri-urban areas, reflecting a limited adoption rate (Kimutai et al., 2024). While these figures show progress, they highlight barriers to wider adoption. Financial, cultural, and technical factors likely limit uptake. Future research should address these to promote biogas use among smallholder farmers.

Our study revealed significant variability in the potential for maize stover-based biogas to substitute household energy consumption (Figure 5). This variation reflects the heterogeneous nature of smallholder farming in Western Kenya, influenced by factors such as family size, energy consumption levels, and maize yields. Farms with fewer household members and higher maize yields are particularly well-positioned for maize stover biogas generation. Notably, one subgroup, which accounted for 14% of our observations, demonstrated the capacity to replace up to 20% of their energy consumption with biogas. However, the economic feasibility of this solution hinges on addressing the high installation costs of anaerobic digestion systems. These findings suggest that while biogas from maize stover offers promising potential for partial energy substitution in certain contexts, its broader adoption will require targeted interventions to make the technology economically viable and accessible for smallholder farmers.

5 Conclusion

While there is significant potential for biogas production from crop residues in Sub-Saharan Africa (SSA), including maize stover, the economic feasibility of adopting anaerobic digestion systems remains a key challenge. The high installation costs of efficient biogas systems may limit their accessibility, particularly for smallholder farmers. Addressing this economic barrier is crucial for realizing the full potential of biogas as a sustainable energy source in the region.

This study highlights the dual challenges of utilizing maize stover for biogas production and managing its removal to mitigate soil erosion risks. By analyzing geography, household

characteristics, and field management practices, we propose strategies that support sustainable development, productivity, and resource conservation for smallholder farmers in Western Kenya. Our findings reveal that biogas production from maize stover can partially substitute conventional energy sources for specific household profiles, particularly larger families with higher per capita energy demands. However, this potential remains constrained by the economic cost of biogas systems and the variability in maize stover availability across farms.

Integrating bioslurry, a by-product of biogas production, as an organic fertilizer further underscores the importance of a holistic approach to stover management. Practices such as proper land preparation, controlled manure application, and timely weeding are essential for enhancing maize yields and stover availability. Additionally, advanced monitoring techniques like the integration of SOM-HMM and remote-sensing data enable more precise field-level assessments, improving our understanding of household energy needs, crop yields, and soil erosion risks. Further research is needed to explore the financial and technical constraints on biogas adoption, including the economic viability of bioslurry as a partial substitute for chemical fertilizers. Future studies should also integrate farm-level soil loss assessments and refine yield estimates using physical crop-cutting data combined with remote-sensing technology.

In conclusion, this study provides valuable insights into the trade-offs associated with maize stover use for biogas production in Western Kenya. By addressing both the technical and economic dimensions of biogas adoption, it contributes to the broader discourse on sustainable agriculture and energy production in SSA. These findings emphasize the importance of targeted interventions to reduce the cost of biogas systems, making this technology more accessible to smallholder farmers and enhancing its role in improving livelihoods and promoting environmental sustainability.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

Author contributions

KJ: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. GG: Data curation, Writing – review & editing. ML: Data curation, Formal analysis, Visualization, Writing – review & editing. AL: Writing – review & editing. YS: Data curation, Formal analysis, Visualization, Writing – review & editing. HL: Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The

authors thank “MINERVA: Dutch network on MICrowaves for a New Era of Remote sensing of Vegetation for Agricultural monitoring” by NWO (NSOKNW.2019.001), “Geodata for Agriculture and Water (G4AW) project” financed by space agency of the Dutch government, Organic Fertiliser Valorisation Implementer (OFVI) African Biodigester Component (ABC), and the Agrosystems Research Group of Wageningen University and Research (3710473400).

Acknowledgments

Keiji Jindo thanks the Plant Production System Group for hosting him and providing his postdoc position. The authors also acknowledge the farmers whose activities were the basis and inspiration of this research. ChatGPT (OpenAI model GPT-4) was utilized to assist in the correction of our R script, ensuring accuracy and optimization for the analyses conducted in this study. The input prompts and outputs related to this use are provided in the Supplementary material for transparency.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare Gen AI was used in the creation of this manuscript. ChatGPT (OpenAI model GPT-4) was utilized to assist in the correction of our R script, ensuring accuracy and optimization for the analyses conducted in this study. The input prompts and outputs related to this use are provided in the [Supplementary material](#) for transparency.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2025.1409457/full#supplementary-material>

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OPEN ACCESS

EDITED BY

Fiona Nicholson,
Agricultural Development Advisory Service,
United Kingdom

REVIEWED BY

Biswarup Sen,
Independent Researcher, Bengaluru, India
Victoria Anthony Uyanga,
Lincoln University, United States

*CORRESPONDENCE

Gabriela Gregová
✉ gabriela.gregova@uvlf.sk

RECEIVED 02 February 2024

ACCEPTED 24 February 2025

PUBLISHED 10 March 2025

CITATION

Dančová N, Gregová G, Szabóová T and
Marcinčák S (2025) The effects of natural
additives on litter condition, microclimate
environment and antimicrobial resistance in
the broiler chickens rearing.
Front. Sustain. Food Syst. 9:1380876.
doi: 10.3389/fsufs.2025.1380876

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The effects of natural additives on litter condition, microclimate environment and antimicrobial resistance in the broiler chickens rearing

Nikola Dančová¹, Gabriela Gregová^{1*}, Tatiana Szabóová¹ and
Slavomír Marcinčák²

¹Department of Public Veterinary Medicine and Animal Welfare, The University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia, ²Department of Food Hygiene, Technology and Safety, The University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia

Introduction: Feed additives like humic substances (HS) and probiotics (P) can enhance broiler health, production, welfare, and environmental conditions.

Methods: This study involved 120 one-day-old ROSS 308 broiler chicks divided into three groups for a 39-day fattening period. The first experimental group (HS) received a feed mixture with 0.6% HS; the second group (HS + P) got the same HS concentration along with a probiotic strain (*Limosilactobacillus fermentum*) in water. The control group (C) was fed a basal diet without additives. The HS mixture was also added to the litter in both the HS and HS + P groups, whereas the C group did not receive any HS. The study examined the effects of HS as litter additives on the physical– chemical properties of poultry litter and its capacity to emit fewer harmful gases. Gas emissions were measured using a plastic chamber connected to a uniTOX instrument, and litter moisture was assessed using AOAC methods.

Results: By days 21, 28, and 35, the moisture levels in the HS and HS + P groups were significantly lower ($p < 0.001$) compared to the C group. HS maintained a slightly acidic pH in the experimental groups, whereas the C group's pH became slightly alkaline. The absorption properties of HS and pH stabilization contributed to the rise in NH_3 and CO_2 emissions to the environment at a lower rate than in the C group ($p > 0.05$). The second aim was to monitor the influence of HS and P as feed and litter additives on bacterial counts and the antimicrobial resistance of *E. coli* isolates by the microdilution method. Coliform and fecal coliform counts were significantly lower ($p < 0.001$) in the litter of the experimental groups on day 35. Minimum inhibitory concentration revealed resistance to ciprofloxacin, tetracycline, ampicillin + sulbactam, and cotrimoxazole among *E. coli* isolates, with resistance ranging from 5 to 15% across all groups.

Discussion: It is concluded that the effect of the additives used increased litter quality. These findings underscore the importance of incorporating additives into intensive poultry systems, where maintaining litter quality is crucial to reducing disease transmission, improving animal welfare, and increasing production efficiency.

KEYWORDS

feed additives, humic substances, broilers, *Escherichia coli*, gases

1 Introduction

The poultry industry is one of the largest, fastest-growing, and most prosperous industries in the world (Muhammad et al., 2020). However, intensive poultry farming is associated with many adverse environmental impacts, such as the production of harmful gases (carbon dioxide–CO₂ and ammonia–NH₃) or poultry waste (Kousar et al., 2021). Also, due to the EU ban on the routine farm use of antibiotics as growth promoters and the limited use of prophylaxis and metaphylaxis in EU countries, the interest in alternative additives for animal production has increased. When utilized appropriately, they can improve feed quality and nutrient availability, promote animal health and growth, increase productivity, or improve breeding condition (Ayalew et al., 2022).

Humic substances are complex heterogeneous organic compounds that are naturally formed by the decomposition and transformation of plant and animal matter in the soil, as well as microbial activity. They occur in soil, water, sediment, coal, peat, and other sources. Their composition consists of carbon, hydrogen, oxygen, and nitrogen, with a small proportion of phosphorus and sulfur (Goel and Dhingra, 2021). Based on their solubility differences, HS are divided into three main fractions: humic acid, fulvic acid, and humin. Humin has a high molecular weight. It is insoluble in acids, bases, or water, and its high resistance to biodegradation is an important characteristic. Humin is able to retain water, improve the structure and stability of the material, and is also involved in cation exchange (Gautam et al., 2021). In general, humic acids are polymers of medium molecular weight, with an aromatic core and aliphatic chains with multiple functional groups. Humic acids are insoluble in an acidic environment (pH lower than 2), but they become soluble in an alkaline environment. They also have numerous different mineral elements bound to their molecules, thus playing an important role in ion exchange and metal complexing (Alomar et al., 2023). Fulvic acids are soluble at all pH values. Of all the fractions, they have the lowest molecular weight (Gautam et al., 2021).

The use of HS in the breeding of certain animals, such as broiler chickens, appears more than promising. HS have been shown to have anti-inflammatory, antimicrobial, and anticarcinogenic potential in human and animal populations (Hriciková et al., 2023). Their ability to activate the metabolism of nutrients and water, including their bactericidal action, makes them valuable tools in the treatment of several diseases (Khil'ko et al., 2011). They are also used in the veterinary sector and animal husbandry for their antidiarrheal, analgesic, immunostimulating, and adsorptive properties. Currently, HS are used in the rearing of various types of animals, including poultry, pigs, cattle, goats, rabbits, fish, and others (Domínguez-Negrete et al., 2019).

A large number of experiments have been carried out in order to demonstrate the effect of the use of HS in poultry farming, including broiler chickens, laying hens, quails, and other types of poultry (Bezuglova and Klimenkom, 2022). The addition of HS to feed or water stimulated the growth of broiler chickens and laying hens (Ozturk et al., 2010). Broiler chicken feed containing HS resulted in a significant improvement in the growth rate and viability of chickens exposed to high environmental temperatures (Edmonds et al., 2014). Jađutová et al. (2019) observed that supplementation of broiler feed with HS improved feed conversion and resulted in an increased final weight of broilers. The digestibility and usability of feed are improved

by humic acids, which also enhance the environment in the gastrointestinal tract of poultry. The number of enterobacteria, including *Escherichia coli*, decreased (de Lourdes Angeles et al., 2022). HS are responsible for stabilizing the intestinal microbiome and destroying pathogenic bacteria, viruses, and fungi. At the same time, they are capable of buffering and modulating the pH in the intestine (Arif et al., 2019). The addition of HS to the feed for layers had a positive effect on laying performance, egg weight, feed conversion, and eggshell quality. An immunostimulatory effect was also confirmed (Mudroňová et al., 2021). The beneficial effect of administration of HS to broiler feed was observed in the study by Kocabağlı et al. (2002). Marcinčáková et al. (2015) concluded that the addition of HS to the feed of the experimental group significantly increased the carcass weight and carcass yield of experimental broilers. Lower consumption of feed was an additional positive. The composition, quality, and sensory properties of the broiler meat were significantly affected by the supplementation of feed with HS. Although there was a decrease in fat content and pH detected, it increased the oxidative stability of the meat during storage (Hudák et al., 2021).

The popularity of probiotics has surged in recent years, particularly for enhancing the growth performance of broilers. Additionally, probiotics help sustain advantageous gastrointestinal microflora and prevent the proliferation of harmful bacteria. They also affect metabolism by boosting the activity of digestive enzymes while diminishing bacterial enzyme activity and ammonia generation, improving digestion and nutrient absorption, as well as stimulating the immune system (Ahfeethah et al., 2023).

There are numerous studies describing the beneficial, neutral, or even detrimental effects of additives on the broiler organism itself (Ahfeethah et al., 2023). However, there is a lack of information and studies conducted that address the issue of the impact of natural additives–humic substances and probiotics–on environmental conditions in general in animal husbandry. Consequently, the first aim of our research was to determine if incorporating HS into poultry litter alters its physical–chemical characteristics or the levels of harmful gases emitted during broiler chicken rearing. The secondary aim was to examine whether HS alone and in conjunction with probiotics (when used together, humic substances and probiotics can have synergistic effects) can influence the survival and quantities of fecal bacteria as well as the antimicrobial resistance of *E. coli* isolates.

2 Materials and methods

2.1 Experimental design

The experiment was carried out on 120 one-day-old ROSS 308 broiler chickens from a commercial hatchery. Two experimental groups and a control group were randomly formed. Each group consisted of 40 broiler chickens, with three replications (13, 13, and 14 broiler chickens per pen). The nutrition, zootechnical measures, environmental conditions, and welfare standards complied with the criteria for chicken fattening. The broilers were reared for 39 days in pens with a concrete floor on which there was a litter of wood shavings. The groups were kept in separate rooms with artificial lighting and ventilation. Up until the seventh day of age, the light intensity remained between 30 and 40 lux. After that, it was lowered to 5–10 lux until the fattening period was over. The air exchange rate

in the rooms ranged from 2 to 2.5 m³/h/kg, which meets the requirements for broiler chickens. A negative-pressure ventilation system ensured the ventilation of the chicken house. This system pushed air out of the building to create a pressure differential that pulled fresh air into the chicken house through the inlets spaced evenly around the perimeter of the building. To ensure fresh air was evenly distributed and directed throughout the chicken house, the air velocity in the inlets was maintained at a level of 3.0 m/s. The room temperature was regulated from 32 ± 1.3°C at the beginning to 22 ± 1.5°C at the end of chicken rearing. In the initial week, heat lamps were utilized to provide increased warmth for the chicks. The humidity was maintained at around 65–70%.

Ad libitum access to feed and water was ensured. The chickens were fed conventional mixed feeds: BR1 (since day 1); BR2 (since day 11); and BR3 (since day 28), according to Jačuttová et al. (2019). The control group (C) was fed a compound regular feed without supplementation during the experiment. The feed mixture given to the first experimental group (HS) included HS at a concentration of 0.6%. In the second experimental group (HS + P), the chickens received a HS together with the feed and a probiotic strain (*Limosilactobacillus fermentum*; 1 mL/chicken/day) in water. In Table 1, there are listed the components that make up the HS. HS were added to the feed in powder form. Every day, feed and water were prepared separately for each group, and all containers were labeled to prevent confusion. Throughout the experiment, each pen contained a plastic non-mechanical circular fountain along with a non-mechanical feeder. The fountains ensured a steady supply of clean and fresh drinking water for the broilers. The chickens involved in the experiment were not vaccinated or treated with any medication. The health status of broiler chickens was checked every day.

Granular HS was applied (in three doses) by sprinkling on the wood shavings litter used in the pens in which both experimental groups (HS and HS + P) were housed at a concentration of 0.6% and a final dose of 900 g/m². The rooms with pens were isolated from each other. No humic material was added to the bedding in the control pen. The litter depth was approximately 7 cm in all pens. We used the 0.6% concentration of the specific HS based on results from previous studies where the effects of HS as feed additives were

controlled in different concentrations (Hriciková et al., 2024; Hudák et al., 2021; Mudroňová et al., 2021; Bartkovský et al., 2021; Mudroňová et al., 2020; Marcinčáková et al., 2015). In addition, the study examined the impact of HS on reducing emissions in the agricultural environment and mitigating the occurrence of antibiotic resistance in *E. coli*. The probiotic strain (*Limosilactobacillus fermentum*) was administered (HS + P) by oral supplementation using a dose of 1 mL/chicken/day in water, following a pilot experiment. The effect of HS and probiotic strain on production parameters and immune system of broilers is reported by Hudec et al. (2024).

2.2 Samples and physical–chemical analysis of litter

Mixed samples of litter (i.e., a combination of bedding materials, excreta, feathers, spilled feed, and water) were collected weekly from each group (days 7, 14, 21, 28, and 35). According to STN ISO 2859-1 (STN EN 2859-1, 1999), litter samples were collected from all four corners and the central area of pens, and thoroughly mixed to obtain a representative material (subsample). A total of five representative samples were obtained from each pen.

Litter moisture content was determined using the AOAC (2000) methodology. Samples were dried at 105°C to a constant weight in an oven. After drying, the samples were cooled in a desiccator and weighed. The following formula was used to calculate the moisture content:

$$\text{Moisture} = \frac{\left(\frac{\text{weight of sample before drying} - \text{weight of sample after drying}}{\text{weight of sample before drying}} \right) \times 100}$$

According to the instructions of ISO standard 10390 (STN EN 10390 (838445), 2021), the pH was measured using a digital pH meter (Hach, Loveland, USA) with a glass electrode. Samples of litter were mineralized in a Digesdahl apparatus (Hach, Loveland, USA) to determine the total nitrogen (N) content according to ISO standard 25663 (STN EN 25663, 2000). Steam distillation with NaOH (for NH₄-N distillation with modified pH 7.4) and H₂SO₄ was used for the digestate. The determination of nitrogen (N and NH₄-N) included titration with NaOH (Mulvaney, 1996).

2.3 Detection of greenhouse gas emission

Throughout the rearing period, several sensors were placed on the premises to measure and automatically record the measured data at regular intervals. Temperature and relative humidity of the environment were measured and recorded by using a thermo-hygrometer (Testo, Schwarzwalde, Germany) throughout the day. Litter gas emissions were measured using a plastic chamber connected to a measuring instrument with a uniTOX. The measuring sensors were placed during 1 h of gas measurement in a plastic measuring chamber (volume of approximately 1 m³) equipped with a forced fan, according to Anderson et al. (2021). The measuring instruments used detected the emission of CO₂ and NH₃ in the ambient air in the rooms. UniTOX.CO2 G infrared carbon dioxide detector (Pro-Service,

TABLE 1 Composition of humic substances.

Components	Quantity	Unit
Humic acids in dry matter	min. 65.0	%
Free humic acids in dry matter	min. 60.0	%
Fulvic acids	min. 5.0	%
Calcium	42,278.0	mg/kg
Magnesium	5,111.0	mg/kg
Ferrum	19,046.0	mg/kg
Cuprum	15.0	mg/kg
Zinc	37.0	mg/kg
Manganese	142.0	mg/kg
Cobalt	1.2	mg/kg
Selenium	1.7	mg/kg
Vanadium	42.1	mg/kg
Molybdenum	2.7	mg/kg

Kraków, Poland), a uniTOX G electrochemical toxic gas detector (Pro-Service, Kraków, Poland), and a portable Multirae instrument (RAE System by Honeywell, CA, USA) were used.

2.4 Bacterial concentrations and antimicrobial resistance

Litter samples from each group were weighed and diluted 1:10 with distilled water. The resulting mixture was then homogenized. A series of 10-fold dilutions were made from the basic suspension, and 1 μ L was inoculated onto the surface of Petri dishes containing the following agars: Meat Pepton Agar (HiMedia, Mumbai, India); Endo Agar (HiMedia, Mumbai, India); Slanetz-Bartley Agar (Merck, Darmstadt, Germany) using the streaking technique. Table 2 is a list of the incubation conditions.

Typical *E. coli* colonies (pink to rose red with metallic sheen) were inoculated from the Endo agar surface onto the Nutrient agar surface and incubated for 24 h at 37°C. The overnight bacterial culture was used to determine phenotypic antibiotic resistance. The minimum inhibitory concentration (MIC) for selected antibiotics for *E. coli* isolates (30 isolates from each group) was determined by a microdilution colorimetric plate method according to Gattringer et al. (2002) and by the automated diagnostic system Bel-MIDITECH (Bratislava, Slovakia). This diagnostic system consists of the following antibiotics: ampicillin (AMP); ampicillin + sulbactam (SAM); piperacillin + tazobactam (TZP); cefuroxime (CXM); cefotaxime (CTX); ceftazidime (CAZ); cefoperazone + sulbactam (SPZ); cefepime (FEP); ertapenem (ETP); meropenem (MEM); gentamicin (GEN); tobramycin (TOB); amikacin (AMI); tigecycline (TGC); ciprofloxacin (CIP); tetracycline (TET); colistin (COL); cotrimoxazole (COT). The results of the MIC values for each antibiotic were interpreted according to the clinical breakpoints described by The European Committee on Antimicrobial Susceptibility Testing, version 7.0 (EUCAST, 2017).

2.5 Statistical analysis

The obtained data were recorded in Microsoft Excel 2016 (Microsoft Corporation, WA, USA). The results obtained in this experiment were expressed as means of the appropriate units \pm standard deviations (SD). Differences in individual physical-chemical and microbiological parameters were analyzed by a Two-Way ANOVA with a post-hoc Tukey test using time and treatment as the main effects. Values of $p < 0.05$, $p < 0.01$, and $p < 0.001$ indicated significant levels of differences between the control and experimental groups. The results were analyzed and evaluated by the software GraphPad Prism 8.3.0 (GraphPad Software Inc., San Diego, CA, USA).

Statistical analysis using the Miditech program generated the percentage of antibiotic-resistant *E. coli* isolates automatically.

3 Results

3.1 Effect of humic substances on litter condition

Figure 1 displays the litter moisture content during the experiment. At the time of spreading the bedding on the floor of the experimental pens (day 0), the moisture of the bedding was $6.83 \pm 1.9\%$ (this value is our control). After the addition of humic substances, the moisture content in the litter increased to the values of $17.80\% \pm 1.5$ (HS) and $17.54\% \pm 1.3$ (HS + P), while most of the moisture came from HS. On day 14, the moisture level in the experimental groups decreased to $12.67\% \pm 0.9$ ($p < 0.05$) in group HS and to $14.36\% \pm 0.5$ ($p < 0.01$) in group HS + P. There was only a slight trend toward increasing the moisture content at the end of the experimental period. During the experiment, we detected a gradual rise in moisture content in the control group. On days 21, 28, and 35, we recorded a significant difference ($p < 0.001$) in both experimental groups in comparison with the control group. The results also showed that the determined litter moisture contents did not exceed the critical range of 35–40%.

Additional essential physical-chemical parameters of litter are summarized in Table 3.

Before the arrival of the flocks (day 0), the studied litters had a slightly acidic pH reaching the following levels: 5.68 ± 0.09 for HS, 5.78 ± 0.06 for HS + P, and 5.84 ± 0.14 for the control group. The pH value of the litter gradually increased in the control group during the experiment. From an initial value of 6.46 ± 0.15 , it increased to 8.11 ± 0.24 on day 35, representing a shift from the slightly acidic to the weakly alkaline region. The pH levels in the litters of the experimental groups with the added HS changed relatively slowly to slightly acidic values. Comparing the experimental groups to the control group revealed no significant differences ($p > 0.05$) between the impact of time and treatment on pH values. According to our findings, HS could regulate and keep the pH of the litter between the acidic and the neutral level.

We also determined the levels of total N and $\text{NH}_4\text{-N}$ in litter samples. In the control group, total N levels ranged from an initial value of $11.71 \text{ g/kg} \pm 0.08$ to $31.87 \text{ g/kg} \pm 0.07$ at the end of the experiment. The levels in experimental group HS ranged from $10.13 \text{ g/kg} \pm 0.13$ to $30.88 \text{ g/kg} \pm 0.11 \text{ g/kg}$ and in the group HS + P from $11.05 \text{ g/kg} \pm 0.04$ to $31.12 \text{ g/kg} \pm 0.24$. During the fattening period, total N levels increased gradually in all groups, but no statistically significant differences were found between time and treatments ($p > 0.05$) compared to the control group.

TABLE 2 Incubation conditions for selected bacterial species.

Group of microorganisms	Microbiological medium	Incubation temperature [°C]	Incubation time [h]
Total count of bacteria	Meat Pepton Agar	37	24
Coliform bacteria	Endo Agar	37	24
Fecal coliform bacteria	Endo Agar	43	24
Fecal enterococci	Slanetz-Bartley Agar	37	48

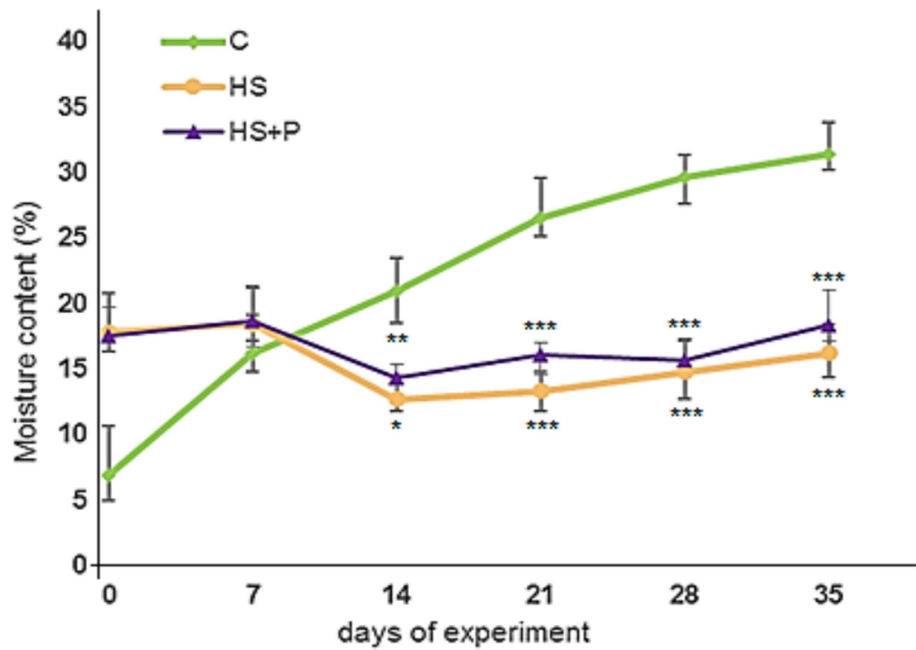


FIGURE 1 Changes in moisture content in the investigated litters during the experiment including the significance of differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). C—control group of broilers fed with a diet without supplementation and without litter additives; HS—experimental group of broilers fed with a diet supplemented with humic substances and with litter additives; HS + P—experimental group of broilers fed with a diet supplemented with humic substances and a probiotic strain and with litter additives. An asterisk symbol indicates that the difference between the control and experimental groups (HS and HS + P) is significant at the confidence levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Error bars show the SD.

TABLE 3 Physical–chemical properties of broiler litters.

Day	Group	pH	N [g/kg]	NH ₄ –N [g/kg]
7	C	6.46 ± 0.15	11.71 ± 0.08	3.58 ± 0.03
	HS	5.99 ± 0.35	10.13 ± 0.13	3.14 ± 0.06
	HS + P	6.09 ± 0.12	11.05 ± 0.04	3.36 ± 0.11
14	C	6.63 ± 0.06	17.29 ± 0.05	4.97 ± 0.10
	HS	6.12 ± 0.11	15.86 ± 0.07	6.04 ± 0.07
	HS + P	6.13 ± 0.18	16.17 ± 0.18	5.98 ± 0.10
21	C	6.90 ± 0.42	19.97 ± 0.14	4.24 ± 0.26
	HS	6.21 ± 0.58	18.18 ± 0.10	7.28 ± 0.09
	HS + P	6.17 ± 0.31	19.64 ± 0.29	7.11 ± 0.19
28	C	7.64 ± 0.21	25.49 ± 0.08	3.22 ± 0.08
	HS	6.58 ± 0.18	23.83 ± 0.03	8.52 ± 0.10
	HS + P	6.63 ± 0.11	23.14 ± 0.17	8.36 ± 0.23
35	C	8.11 ± 0.24	31.87 ± 0.07	2.21 ± 0.09
	HS	6.90 ± 0.13	30.88 ± 0.11	10.04 ± 0.25
	HS + P	6.88 ± 0.17	31.12 ± 0.24	9.99 ± 0.18
Statistics factors	Time	$p > 0.05$	$p > 0.05$	$p > 0.05$
	Treatment	$p > 0.05$	$p > 0.05$	$p > 0.05$
	Interaction	$p > 0.05$	$p > 0.05$	$p > 0.05$

C—control group of broilers fed with a diet without supplementation and without litter additives; HS—experimental group of broilers fed with a diet supplemented with humic substances and with litter additives; HS + P—experimental group of broilers fed with a diet supplemented with humic substances and a probiotic strain and with litter additives. No statistically significant differences were found between time and treatments ($p > 0.05$) compared to the control group.

Compared to total N, the levels of NH₄–N differed between the groups. The initial concentrations were 3.58 ± 0.03 g/kg in the control group, 3.14 g/kg ± 0.06 in HS and 3.36 g/kg ± 0.11 in HS + P. From day 14, we recorded changes in concentrations between the groups. On day 35, the NH₄–N levels in the litter of the control group decreased (2.21 g/kg ± 0.09) when ideal conditions were reached for NH₄ conversion to gaseous NH₃. In contrast, the levels in the experimental groups were higher (10.04 g/kg ± 0.25 for HS and 9.99 g/kg ± 0.18 for HS + P at the end of the experiment) but without significant effect of time and treatments ($p > 0.05$).

3.2 Effect of humic substances on production of harmful gases

In general, the measured gas emissions (CO₂ and NH₃) did not exceed the acceptable recommended limits, which ensured that the animals had suitable living conditions throughout the experiment. The emissions of CO₂ and NH₃ showed no significant differences between the groups (Figures 2, 3). It is evident that as the broilers raised the concentrations of both gases increased. The highest concentrations were detected on day 35 of the experiment. The gas concentrations in the experimental groups of broilers housed on litter amended with HS increased gradually but at a lower rate than those in the control group. The measured CO₂ concentrations ranged from 850 ppm ± 30 to 2,210 ppm ± 115 in the HS group and from 855 ppm ± 50 to 2,300 ppm ± 130 in the HS + P group, in contrast with the control group, where they ranged from 975 ppm ± 45 to 2,630 ppm ± 155. NH₃ emissions

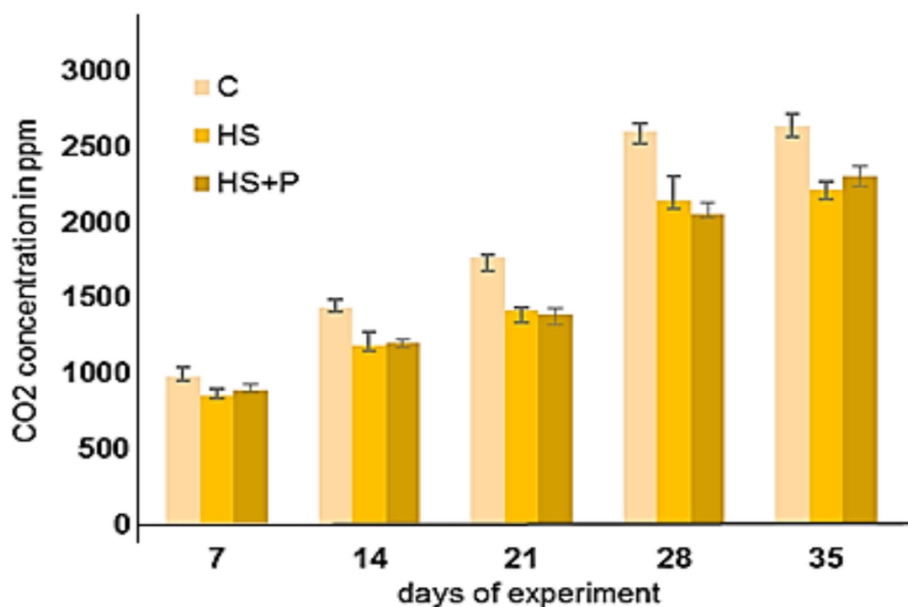


FIGURE 2

Concentrations of CO₂ in the control and experimental groups. C—control group of broilers fed with a diet without supplementation and without litter additives; HS—experimental group of broilers fed with a diet supplemented with humic substances and with litter additives; HS + P—experimental group of broilers fed with a diet supplemented with humic substances and a probiotic strain and with litter additives. Error bars show the SD. No statistically significant differences were found between time and treatments ($p > 0.05$) compared to the control group.

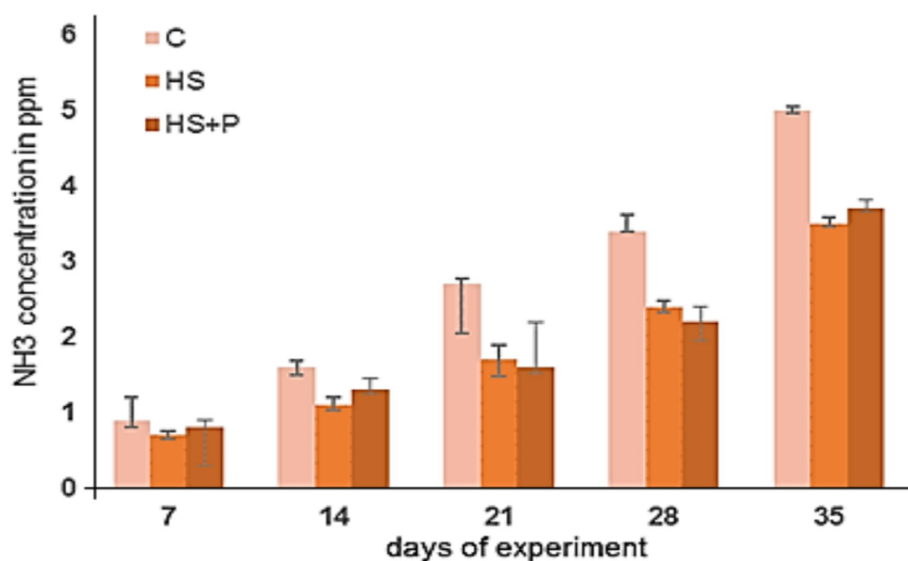


FIGURE 3

Concentrations of NH₃ in the control and experimental groups. C—control group of broilers fed with a diet without supplementation and without litter additives; HS—experimental group of broilers fed with a diet supplemented with humic substances and with litter additives; HS + P—experimental group of broilers fed with a diet supplemented with humic substances and a probiotic strain and with litter additives. Error bars show the SD. No statistically significant differences were found between time and treatments ($p > 0.05$) compared to the control group.

from experimental groups ranged from $0.7 \text{ ppm} \pm 0.1$ to $3.5 \text{ ppm} \pm 0.12$ (HS) and from $0.8 \text{ ppm} \pm 0.6$ to $3.7 \text{ ppm} \pm 0.16$ (HS + P) compared to $0.9 \text{ ppm} \pm 0.4$ – $5.0 \text{ ppm} \pm 0.34$ in the control group. We did not observe a statistically significant effect of time and effect of treatment on CO₂ and NH₃ concentrations

($p > 0.05$). No presence of hydrogen sulfide (H₂S) in the environment (0 ppm) of all three groups was detected during the experiment. HS added to the bedding materials during the experiment could help absorb the unwanted gases, thus reducing their concentration in the air in the broiler house and creating a

more optimal environment for the fattening of broiler chickens. Our findings indicated that HS added to the litter participated in reduced moisture content, ensured a pH lower than 7, and also could reduce the release of harmful gases in experimental groups compared to the control group (although without statistically significant differences).

3.3 Effect of additives on bacterial counts and *Escherichia coli* resistance

Microbial contamination of the litter during the fattening of broilers was checked every 7 days. On day 7, total bacterial counts in the litter of evaluated groups ranged from 7.00 to 7.36 log₁₀ CFU/g. In all groups, we observed a no significant increase in total bacterial counts by 2 logs by the end of the experiment. On day 35, we observed that the effect of HS and the combination of HS and the probiotic strain significantly reduced the counts of coliform bacteria ($p < 0.01$) and fecal coliform bacteria ($p < 0.05$). There was no statistically significant effect of time and treatment on the total count of bacteria and fecal enterococci ($p > 0.05$) compared to the control group (Figures 4–6).

Ninety *E. coli* strains isolated from the litter were subjected to antibiotic susceptibility tests (30 isolates from each group). The tests revealed resistance to ciprofloxacin, tetracycline, ampicillin + sulbactam, and cotrimoxazole. *Escherichia coli* strains were susceptible to TZP, CXM, CTX, CAZ, SPZ, FEP, ETP, MEM, GEN, TOB, AMI, TGC, and COL, and therefore these ATBs are not shown in Figure 7.

There were not many noticeable differences in ATB resistance between the experimental groups. The level of resistance ranged from 3.3 to 10.0% of *E. coli* resistant to ATB. Comparison with the control group allowed us to conclude that the addition of HS and the combination of HS and probiotic strains had no or little effect on the level of antibiotic resistance of *E. coli* isolates.

4 Discussion

4.1 Evaluation of the impact of additives on the litter condition

The physical–chemical properties of the bedding material can influence the activity of microorganisms and the production of gases. The ideal bedding material should be able to not only absorb moisture but also release moisture quickly. An essential characteristic for evaluation of the litter quality is its capacity to retain and discharge water (Garcês et al., 2013).

Rico-Contreras et al. (2017) classified poultry litter according to moisture content into three groups: 1. dry litter (25%), 2. semi-moist litter (25–35%), and 3. wet litter (35%).

During the experiment, we detected a gradual rise in moisture content in the control group. On days 21, 28, and 35, we recorded a significant decrease ($p < 0.001$) in both experimental groups (dry litters) in comparison with the control group (semi-moist litter). At the end of the experiment, the control group reached the highest value, which, however, did not exceed the critical threshold of 35%.

When HS are added to poultry litter, they act as a natural material that can retain moisture through adsorption, binding properties, and aeration. HS incorporated into litter can absorb excess moisture and reduce the overall moisture content of the litter. They can bind with molecules of water, hindering their evaporation and thus leading to drier bedding material. By reducing litter moisture, HS can also help improve the airflow and aeration of the poultry house. These factors can contribute to a healthier environment for poultry and reduce the potential for NH₃ emissions (Vikram et al., 2022; Fragouli et al., 2023; Bay, 2021; Sharma and Anthal, 2016).

In our experiment, we also found an increase in pH, which represents a shift from the slightly acidic to the weakly alkaline region. On the contrary, in the experimental groups, the pH during rearing was maintained in only a slightly acidic area. Due to the low pH, the growth of microbes responsible for the production of NH₃ was

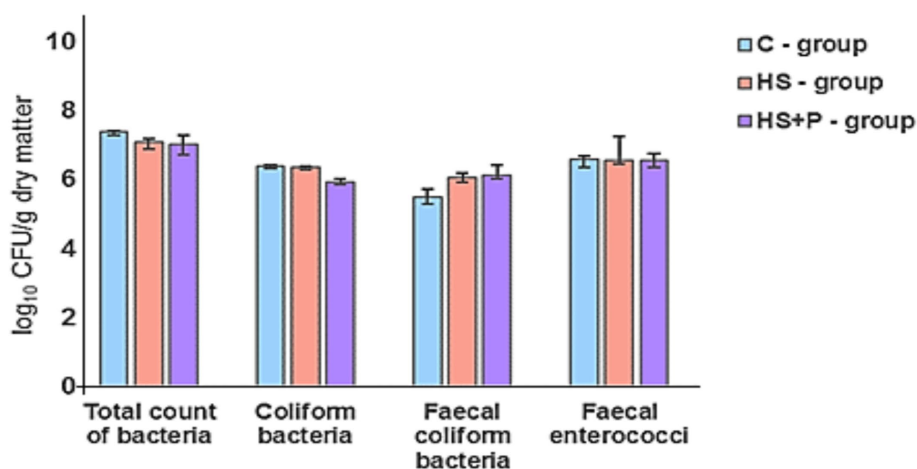


FIGURE 4

Bacterial contamination (log₁₀) in litter (1 g) detected on day 7. C—control group of broilers fed with a diet without supplementation and without litter additives; HS—experimental group of broilers fed with a diet supplemented with humic substances and with litter additives; HS + P—experimental group of broilers fed with a diet supplemented with humic substances and a probiotic strain and with litter additives. Error bars show the SD. No statistically significant differences were found between time and treatments ($p > 0.05$) compared to the control group.

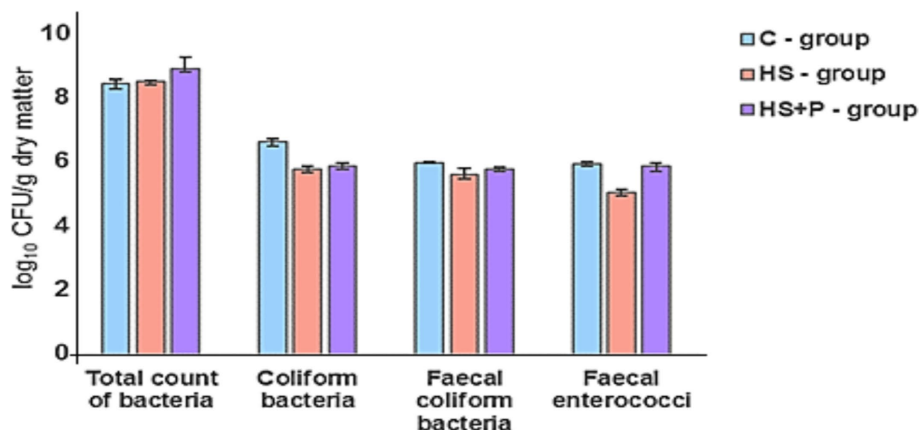


FIGURE 5

Bacterial contamination (\log_{10}) in litter (1 g) detected on day 21. C—control group of broilers fed with a diet without supplementation and without litter additives; HS—experimental group of broilers fed with a diet supplemented with humic substances and with litter additives; HS + P—experimental group of broilers fed with a diet supplemented with humic substances and a probiotic strain and with litter additives. Error bars show the SD. No statistically significant differences were found between time and treatments ($p > 0.05$) compared to the control group.

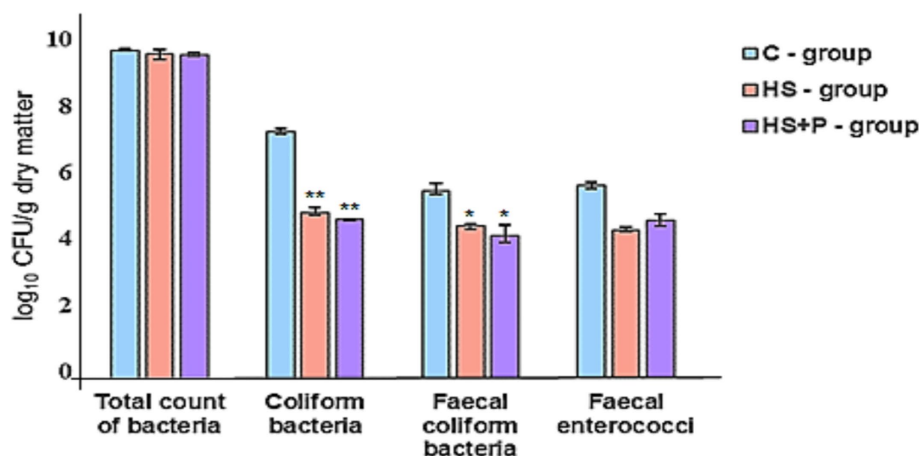


FIGURE 6

Bacterial contamination (\log_{10}) in litter (1 g) detected on day 35. C—control group of broilers fed with a diet without supplementation and without litter additives; HS—experimental group of broilers fed with a diet supplemented with humic substances and with litter additives; HS + P—experimental group of broilers fed with a diet supplemented with humic substances and a probiotic strain and with litter additives. An asterisk symbol indicates that the difference between the control and experimental groups (HS and HS + P) is significant at the confidence levels: * $p < 0.05$; ** $p < 0.01$. Error bars show the SD.

inhibited, the activity of decomposition of nitrogenous substances slowed down, and the ammonium ion (NH_4^+) remained stable, which reduced the concentration of harmful gases in the environment.

The accumulation of chicken feces increases the pH of the litter (Munir et al., 2019). Garcés et al. (2013) found an increase in the pH of wood shaving litter from 6.3 to 8.9 after 35 days of chicken rearing. In other experiments with the addition of peat to the litter for chickens, the pH value increased from 6.53 to 8.13 (Živkov-Baloš et al., 2020) or from 4.0 to 8.1 (Kaukonen et al., 2017).

The pH value of the litter is also an important factor affecting the growth and survival of pathogenic microorganisms (Jie et al., 2022).

Poultry manure is a rich source of nitrogen for the increased intake of amino acids and proteins by feed. Chicken litter also contains a high proportion of many other minerals (C, P, K, Ca, S, Mg, Cu, B,

Fe, Zn, Mo, and Mn) and organic matter, which makes it one of the most valuable livestock wastes (Chen and Jiang, 2014; Živkov-Baloš et al., 2020). Nitrogen occurs in chicken litter in several forms, with uric acid being the predominant form (Murakami et al., 2011). The combination of increased litter moisture and high environmental temperatures favors the growth and activity of bacteria that rapidly convert uric acid into NH_3 gas (Garcés et al., 2013). The total N content of the litter immediately after the hen has defecated is approximately 60 g/kg (Nakatani, 2002). In general, poultry feces generally contain higher amounts of N than other types of animal manure (Murakami et al., 2011).

Although there was an increasing trend in the total N detected values from day 7 to day 35, there were no significant differences between the control and experimental groups according to the effect

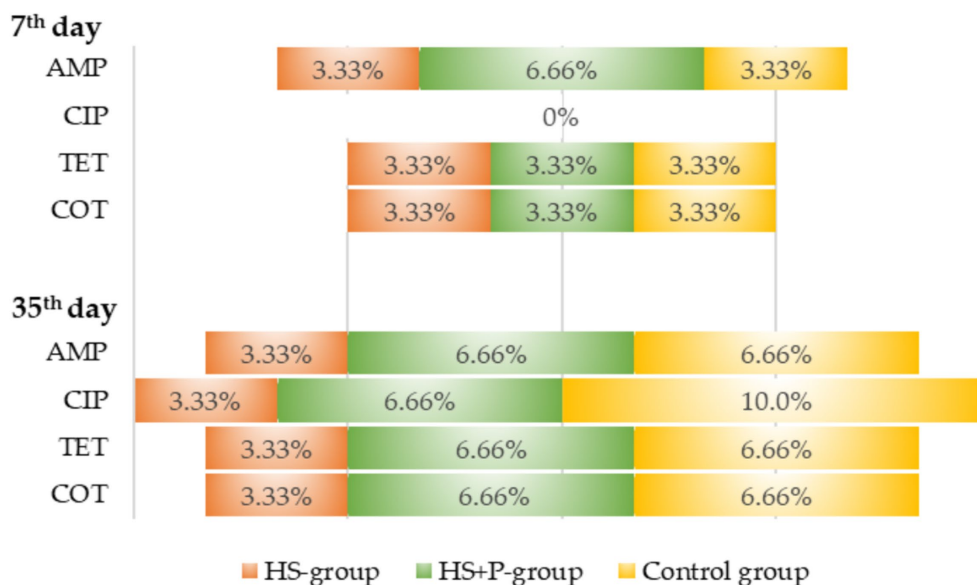


FIGURE 7

Percentage of antibiotic resistance of *E. coli* isolates from individual groups. C—control group of broilers fed with a diet without supplementation and without litter additives; HS—experimental group of broilers fed with a diet supplemented with humic substances and with litter additives; HS + P—experimental group of broilers fed with a diet supplemented with humic substances and a probiotic strain and with litter additives.

of time and treatment. According to the results, HS had no effect on total N concentrations. We believe that it could be explained hypothetically, that litter in the control experiment contains other types of N compounds such as ammonium, nitrite, and nitrate.

The measured NH_3 emissions and the calculated $\text{NH}_4\text{-N}$ values were related. In the control group, we confirmed lower values of $\text{NH}_4\text{-N}$, but high concentrations of gaseous NH_3 . On the contrary, HS in the litter absorbed and accumulated $\text{NH}_4\text{-N}$, which resulted in higher values of $\text{NH}_4\text{-N}$, but we detected lower NH_3 emissions in the air of the chicken house. However, no significant differences were found between the control and experimental groups during the fattening period.

The HS in the litter affected the absorption of NH_4^+ due to environmental factors (humidity, temperature, moisture content, and pH level). NH_4^+ was not converted into NH_3 and released into the atmosphere; instead, it remained as an ionized form of NH_3 in the litter.

In poultry litter, the addition of HS led to a decrease in moisture level ($p < 0.001$). Although statistical significance was not found, HS in the experimental groups appeared to influence pH stabilization (below 7), inhibited NH_4^+ absorption, and retained NH_4^+ as a stable ammonium ion compared to the control group, which also contributed to a reduced release of harmful gases into the environment.

4.2 Evaluation of the impact of additives on the gases production

The quality of the indoor air in the chicken house largely depends on the litter quality (Živkov-Baloš et al., 2020). Volatile particles also originate from NH_3 , which arises mainly from the degradation of nitrogenous substances in feces and urine by the activity of microorganisms present in the litter and negatively affects poultry

performance (Al-Kerwi et al., 2022). One chicken during fattening is capable of producing approximately 1.05 kg of litter and manure (Moore et al., 2011).

The handling, storage, and processing of poultry manure on poultry farms contribute significantly to the production of greenhouse gas emissions. Environmental temperature ($>32^\circ\text{C}$) and humidity ($>70\%$), litter's pH (>9), and litter's moisture ($>40\%$) are the factors that result in increased conversion of NH_4^+ ions to NH_3 gas (Fogaça et al., 2022). In order to reduce and control the emission of harmful gases in the chicken house and to ensure optimum living conditions for the poultry, additives with suitable adsorption properties can be added to the litter (Jie et al., 2022). The addition of HS into bedding material for broilers is able to bind harmful gases (such as NH_3 , CO_2 , CH_4 , or H_2S), as well as other compounds dangerous for the organism (Hriciková et al., 2023).

Concentrations of NH_3 and CO_2 in the air were not exceeded in our experiment with broiler chickens in either group, as stated in Council Directive 2007/43/EC (2007). The emissions of CO_2 and NH_3 showed no significant differences between the experimental groups by the effect of time and treatment. However, the gas concentrations in both experimental groups (HS and HS + P) increased gradually but at a lower rate than in the control group. HS added to the bedding materials during the experiment probably absorbed the investigated gases (CO_2 , NH_3), thus reducing their concentration in the air of the broiler house (2 ppm difference in NH_3).

Adsorption of NH_3 by HS is a complex process that involves a combination of physical and chemical interactions. This process is facilitated by the structure and properties of HS, such as their high surface area, porosity, and functional groups. The combination of these factors allows HS to effectively adsorb NH_3 molecules, thereby helping to reduce NH_3 emissions from broiler litter and improving air quality in the poultry environment. HS have a complex and diverse molecular structure that provides a large surface area for interactions

with other molecules. The large surface area allows more opportunities for NH_3 molecules to come into contact with HS and bind to them (Fragouli et al., 2023; Song et al., 2019). HS contain functional groups such as carboxyl ($-\text{COOH}$), phenolic ($-\text{OH}$), and amino groups ($-\text{NH}_2$), which can attract and bind NH_3 molecules through electrostatic interactions and hydrogen bonding, which allows them to act as effective NH_3 adsorbents, especially in soil and water systems (Mahler et al., 2021). In the case of NH_3 , the positively charged ammonium ion (NH_4^+) can be attracted to the negatively charged functional groups of HS (Zhang et al., 2022). The porous character of HS allows NH_3 molecules to diffuse into their internal structure, where they can be temporarily immobilized. This helps to reduce the immediate release of NH_3 gas into the environment (Alvarez-Puebla et al., 2005). When the NH_3 molecule comes into contact with the surface of the humic substance, it can form chemical bonds with the functional groups present. This adsorption process involves the transfer of electrons between the NH_3 molecule and the humic substance, which leads to the binding of the NH_3 molecule to the surface of the material (Xi et al., 2018).

Vučemilo et al. (2007) found a significant relationship between increasing air NH_3 concentration and the age of animals and humidity. The recorded CO_2 emissions were lower in the experimental groups compared to the control group. Miles et al. (2006) reported that CO_2 levels in broiler houses increased over time as the broiler chickens grew and breathed. As stated in the Council Directive 2007/43/EC (2007), laying down minimum rules for the protection of chickens kept for meat production, as amended, that in poultry farming, it is imperative to guarantee that air NH_3 concentrations of 20 ppm and CO_2 concentrations of 3,000 ppm are not exceeded.

The most reliable strategies to reduce the concentration of harmful gases involve regular examination of the conditions of the internal environment in poultry housings, ensuring sufficient air circulation, monitoring the moisture of the litter, and using additives (e.g., humic substances) to prevent the release of gases (Kilic and Yaslioglu, 2014; Bailey et al., 2021).

4.3 Evaluation of the impact of additives on the microbial concentrations

In general, there is a complex relationship between HS and bacterial populations that can vary depending on the environment and conditions. In addition, HS can positively affect the microflora in the gastrointestinal tract of broilers and in litter. They can act as a source of nutrients and energy, supporting the growth and activity of beneficial bacteria (such as *Lactobacillus* and *Bifidobacterium*) and thus improving gut health and overall broiler performance. They can also help to maintain a balanced gut microbiome and reduce the number of pathogens, including *Salmonella typhimurium*, *Enterobacter cloacae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Staphylococcus* spp., by inhibiting adherence in the avian organism (Korsakov et al., 2019; Domínguez-Negrete et al., 2019; Arif et al., 2019). Overall, the use of HS can support healthy intestinal microflora in broilers and contribute to better litter management. In addition, HS can serve as a surface for some bacteria to attach to and help them colonize and form biofilms. These biofilms can protect these bacteria from environmental factors and facilitate communication with other bacterial species (Bogdanov et al., 2022; Rodrigues et al., 2008). HS can stabilize pH

levels at either acidic or neutral levels, absorb adverse gases, and significantly lower the moisture content of litter.

Apart from the previously mentioned benefits, probiotics used in chicken farming can also replace harmful bacteria and eliminate them from the digestive system (Bhogoju and Nahashon, 2022). According to Ouwehand et al. (2016), probiotics do not contribute to the spread of antimicrobial resistance and may even reduce it. To what extent this is possible is still under investigation. Coliforms, fecal coliform bacteria and fecal enterococci are bacteria naturally occurring in the gastrointestinal tract of broilers. During the experiment, we observed a positive effect of the additives on the counts of the investigated bacteria. HS and combinations of HS with probiotics suppressed the growth of coliform bacteria, fecal coliform bacteria, and fecal enterococci.

The administered additives had a beneficial effect on the adjustment of microflora in the gastrointestinal tract of broilers and also in litter, compared to the control group. Our findings are consistent with those of Thaxton et al. (2003), who discovered that despite the unsuitable conditions, the mentioned bacteria were reduced but not eliminated. They concluded that a defined population of bacteria formed over time. The bacteria concentration remained stable regardless of the number of housed chickens. Winkler et al. (2017) found an average of less than 7.1 \log_{10} CFU/g of coliform bacteria in litter from 12 poultry farms. They did not record any significant differences in counts between the individual sampling sites. According to Barker et al. (2010), the average coliform counts ranged from 6.37 to 7.17 \log_{10} CFU/g on three broiler farms. Likewise, Terzich et al. (2000) detected average counts of coliform bacteria in litter samples from several countries, ranging from 6.42 to 8.77 \log_{10} CFU/g. The counts of fecal enterococci in the control group (6.85 \log_{10} CFU/g) were similar to the findings by Winkler et al. (2017) and Diarra et al. (2007), who reported a mean value of 6.4 and 7.86 \log_{10} CFU/g. The average counts of fecal enterococci in the litter samples in the experimental groups were lower. Our results are consistent with other studies that observed an increase in the total count of bacteria by at least one or two orders of magnitude during the first 3 weeks of broiler fattening (Gontar et al., 2022; Milanov et al., 2019).

Even though the broiler chickens were not given any antibiotics during the fattening period, we discovered that antibiotic-resistant *E. coli* isolates were present in their litter. ATB-resistant *E. coli* isolates in the litter from hatched chicks could originate from the vertical transmission of genetic information from the parents as well as from the environment of the hatchery itself. ATB resistant genes can be transmitted by horizontal gene transfer within flocks and farm staff (Khong et al., 2023). We confirmed resistance to AMP, SAM, CIP, TET, and COT in the *E. coli* isolates from all investigated groups, with a low difference in results. The percentage of resistance ranged from 3.3 to 10.0%. This suggested that even if the broilers did not receive any drugs during fattening, antibiotic-resistant strains of *E. coli* may have been spread in the environment.

Another study conducted on a commercial Slovak broiler farm reported similar or higher results concerning the litter of one-week old broilers. The *E. coli* isolates were resistant to ampicillin (85.3%), enrofloxacin (83.8%), and cephalosporins (ceftiofur – 36.8% and cefquinome – 19.1%) which gradually decreased during their growth (Gregová et al., 2013).

Although there were not evident differences in ATB-resistance of *E. coli* isolates, HS and probiotics can have a positive effect on gut

microflora. The concentrations of coliform bacteria, fecal coliform bacteria, and fecal enterococci were lower in the litter of the HS and HS + P groups.

Another study conducted by our team revealed that 0.8% HS addition to broiler feed had an immunostimulatory effect on phagocytic activity and gut protection (Mudroňová et al., 2020). The 0.6% supplementation of feed with HS positively affected the quality of the produced breast meat (pH and lipid oxidation) (Hudák et al., 2021). The same beneficial effect of humic substance administration during broiler fattening was observed in the study by Kocabağlı et al. (2002).

5 Conclusion

In conclusion, adding humic substances to bedding materials effectively absorbs excess moisture, resulting in a lower overall moisture level in the litter. Although the statistical significance remains unconfirmed, we noticed enhanced physical–chemical properties in the litter, with reduced gas concentrations in groups that received humic substances compared to the control group. Furthermore, the use of humic substances, whether used alone or in conjunction with a probiotic strain, led to a considerable decrease in the counts of coliform and fecal coliform bacteria in the litter. Given these results, further investigation is necessary to examine the potential advantages of these feed additives in intensive farming systems.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by the Ethical Committee for Animal Care and Use of University of Veterinary Medicine and Pharmacy in Košice (Slovakia) and the State Veterinary and Food Administration of the Slovak Republic under the protocol no. 3040-14-221. The “European Directive on the protection of vertebrate animals used for experimental and other scientific purposes” (EC, 2010) was followed in all aspects of the current study’s procedures. The

study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

ND: Conceptualization, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. GG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. TS: Data curation, Methodology, Supervision, Writing – review & editing. SM: Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This publication was supported by the Operational program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund. Also, it was supported by Cultural and educational grant agency by Ministry of Education, Science, Research and Sport of the Slovak Republic, project KEGA 001UVLF-4/2022.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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