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Biochemical thresholds to differentiate mineralizing and stabilizing organic waste amendments for soil carbon management

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Introduction: Identifying organic amendments that can retain stable organic carbon is essential for improving soil health and mitigating greenhouse gas emissions.

Methods: This study assessed the carbon stability of 104 fertilizing residual materials (including manure) using a biochemical fractionation method (Van Soest) and a standardized 91-day incubation protocol. Two kinetic parameters, residual organic carbon and the mineralization rate constant, were derived from first-order kinetic modeling. Eight biochemical indicators were tested for their diagnostic performance using the Cate-Nelson partitioning method to distinguish between amendments with predominantly mineralizing or stabilizing behavior.

Results: Three indicators showed strong discriminatory power and consistent performance: lignin content, the Biological Stability Index, and the stability ratio $\frac{LIC}{SOL+HEM+CEL}$. For each, a critical interval was identified beyond which amendments shifted toward carbon stabilization. These intervals ranged from 27.3 to 32.8 g lignin per 100 g dry matter, 0.77 to 0.99 g stability index per gram, and 0.38 to 0.49 for the lignin-to-polysaccharide ratio. Amendments exceeding these ranges were associated with high residual carbon content (up to 71.8 g per 100 g) and low mineralization rates (as low as 0.067 day^{-1}), indicating enhanced carbon persistence.

Discussion: This work led to the development of a robust diagnostic framework for classifying and recommending organic amendments based on their potential for carbon retention. The approach offers practical value for selecting materials suited to long-term soil improvement and climate mitigation strategies. Further validation under field conditions is recommended to support the implementation of this approach in sustainable agricultural systems.

KEYWORDS
Cate-Nelson partitioning, lignin stability index, mineralization kinetics, organic amendments, recycling waste, soil organic carbon

1 Introduction

Soil is the largest terrestrial carbon reservoir in the biosphere (1). Soil organic carbon (SOC) plays a fundamental role in maintaining and promoting soil health and plant productivity, while contributing to climate change mitigation (2–4). Its stability, regulated by the balance between OC inputs and outputs, makes it vulnerable to intensive farming and land-use changes (5, 6). Residual organic materials, such as livestock effluents and organic urban or industrial wastes, are widely used to enhance SOC content and restore soil fertility (7). However, their behavior varies: while cattle manure promotes carbon stabilization (8), poultry manure may stimulate high carbon mineralization due to its readily degradable organic content and high nitrogen content (9). In this sense, Fertilizing Residual Materials (FRMs), as defined by the two Québec ministries of the Environment and Agriculture, are derived from the recycling of industrial, agrifood, or municipal residues and offer a wide range of organic compositions. As with all organic soil amendments, these FRM_S contain both labile carbon fractions with rapid turnover and more stable, recalcitrant fractions such as humic substances, lignin, or polyphenols (10, 11). These FRMs exhibit a wide range of carbon profiles, which in turn influence their behavior once incorporated into the soil (12, 13). Indeed, some FRMs, which are rich in recalcitrant compounds, may contribute to carbon stabilization and aggregate formation (14). These compounds are likely to integrate into compact soil structures, notably in the form of organo-mineral microaggregates, which limits microbial degradation (15). Bahri et al. (16) emphasized the significance of physical and chemical protection in stabilizing organic carbon by incorporating lignin into protected soil compartments. Among the many biochemical fractionation methods, the Van Soest method is widely used to assess the carbon stability of organic materials. It separates three fractions: soluble (SOL), holocellulosic (HEM + CEL), and lignin/cutin (LIC), which correspond reciprocally to progressively slower rates of biodegradation (17, 18). These fractions underpin several biochemical ratios, such as $\frac{CEL}{LIC}$, $\frac{CEL+HEM}{LIC}$, and $\frac{LIC}{Holocellulose}$ but their predictive performance remains inconsistent across studies (19–21). To overcome these limitations, integrative indices such as the Biological Stability Index (BSI), the Robin's Tr coefficient (22), and the indicator of potential residual organic carbon I_{ROC}, in French called ISMO: indice de stabilité de la matière organique have been developed to estimate the stabilization potential of organic matter in soil (23–25). Among these indicators, I_{ROC} is typically validated through incubation experiments and fitted to kinetic models first-order, exponential, or logarithmic that distinguish degradable and stable carbon pools based on mineralization rates (26, 27). However, no critical threshold values have yet been established to distinguish organic amendments according to their specific agronomic functions, whether the goal is to stimulate microbial activity through readily mineralizable carbon or to enhance soil structure through more stable, persistent forms of carbon. Defining such thresholds would offer a valuable decision-making tool for selecting FRMs based on their suitability for short-term biological activation or carbon stabilization potential.

In this context, the present study aims to identify the most relevant biochemical indicators that can distinguish FRMs with predominantly mineralizing behavior from those with strong stabilization potential. Based on these indicators, we strive to determine critical threshold values that can support a more robust diagnostic framework for assessing organic carbon stability. Ultimately, this approach is intended to improve the prediction of the agronomic value of FRMs and their role in soil carbon persistence potential.

2 Materials and methods

2.1 Collection and physicochemical characterization of fertilizing residual materials

A total of 104 samples, including fertilizing residual materials (FRMs) from various industrial, agrifood, or municipal waste treatment processes, and two farmyard manure samples, used as reference, were collected across the province of Québec, Canada (Table 1), by accredited companies following the sampling protocol (41).

The samples were dried at 38 ± 2 °C and finely ground to 1 mm to obtain a homogeneous powder. The main physicochemical characterization of these samples is presented in Table 2. The dry matter (DM) content was determined for a weighed portion of the prepared sample, which was subjected to a second drying step at 105 °C until constant weight was reached. Organic matter was measured by loss on ignition at 550 °C (42). Total organic carbon (TOC) (43) and total nitrogen (44) were quantified using a dry combustion elemental analyzer (CNSH). pH was measured in aqueous suspensions (1:10 w/v), and electrical conductivity (EC) was determined in water extracts at the same ratio, following the CEAEQ method (2003). Sample digestion was performed using a mixture of nitric acid (HNO_3) and hydrochloric acid (HCl), followed by heavy metal analysis using inductively coupled plasma mass spectrometry (ICP-MS) (45). Mercury (Hg) concentration was determined by thermal decomposition of the FRM samples, followed by UV spectrophotometric analysis at 254 nm (45), with results reported in Table 3.

2.2 Biochemical characterization of fertilizing residual materials

The biochemical characterization of the FRMs was performed using a sequential fractionation process to separate the soluble (SOL), holocellulosic (HEM + CEL), and lignin (LIC) fractions. This procedure was carried out using Filter Bag Technology (FBT), following a modified, more detailed version of the Van Soest method (48) to reflect the actual extraction sequence and fraction yields accurately. Each sample underwent successive extractions to quantify the following components. Each sample was sequentially extracted to determine:

TABLE 1 Typology and agronomic use of fertilizing residual materials (FRMs) and manures based on their carbon mineralization and stabilization potentials.

FRM categories	Descriptions	References
Compost	Mature solid product from aerobic microbial decomposition of organic residues (e.g., biosolids, green waste). The process stabilizes organic matter into humified compounds with low pathogen and labile carbon content. Used to improve soil structure, enhance microbial activity, and support long-term carbon stabilization.	Annabi et al. (28); Amaya-Gómez et al. (29)
De-inking residues	Solid by-products from wastewater treatment during recycled paper production. Contains cellulose fibers and mineral fillers; its high pH and carbon content make it suitable as a liming and organic amendment.	Hébert (30)
Frass	Organic waste excreted by insects (e.g., larvae) comprises feces and residual feed particles. Rich in essential macro- and micronutrients, including nitrogen, phosphorus, and potassium. It contains biostimulant compounds that enhance soil fertility and plant growth.	Poveda et al. (31); Chiam et al. (32); Mostafaie et al. (33)
Municipal solid waste	Solid or semi-solid by-products from municipal wastewater treatment are often dewatered and stabilized. They originate from primary (physico-chemical) and secondary (biological) treatment processes. Rich in organic matter and nutrients, they improve soil fertility and support organic carbon input.	Hébert (30); Malone et al. (34)
Pond biosolids	Dewatered solid residues derived from municipal or industrial wastewater treatment are typically collected from aerated lagoons. These materials are rich in organic matter and nutrients, improving soil fertility and enhancing organic carbon levels.	Hébert (30)
Paper biosolids	Organic residues from primary and secondary wastewater treatment in the pulp and paper industry. Derived from wood fiber processing, they are rich in organic matter and suitable for soil amendment.	Faubert et al. (35); Faubert et al. (36)
Digestates	Solid, dewatered by-products from anaerobic digestion or biomethanation of organic waste (municipal, industrial, or agrifood). Contains partially stabilized organic matter, residual nutrients, and microbial biomass. Used to improve soil fertility and supply organic carbon with moderate mineralization potential.	Hébert (30); Zhen et al. (37)
Animal manures	A mixture of solid and liquid excreta from livestock, combined with bedding materials such as straw. Rich in organic matter and nutrients, particularly nitrogen and phosphorus. Improves soil structure, microbial activity, and long-term carbon inputs.	Hébert (30); Clément (38)
Neutralization sludge	Solid residue generated from wastewater neutralization processes is typically of industrial origin. It may contain carbonates and mineral matter, used primarily for pH adjustment and soil conditioning.	Trpčevská et al. (39)
Wood ash	Wood and lignocellulosic residue combustion by-products, including municipal biosolids and plant biomass. Mainly produced by the forest industry pulp and paper mills, cogeneration plants, and sawmills. Rich in minerals (Ca, K, Mg) and alkaline compounds, it corrects soil acidity and supplies nutrients. It may contain residual carbon from incomplete combustion, contributing marginally to organic carbon inputs.	Hébert (30); Ma and Rosen (40)

- Neutral detergent fiber (NDF) is extracted at 100 °C for 75 minutes using a neutral detergent.
- Acid detergent fiber (ADF) is extracted at 100 °C for 60 minutes using an acid detergent.
- Acid detergent lignin (ADL) is extracted by cold extraction with 72% sulfuric acid for 3 hours following the ADF step.

The dry weight residue from the filter bags, dried at 103 ± 2 °C, was used to calculate the biochemical fractions following the XP U44-162 (25) standard, using Equations (1–4):

$$SOL = 100 - NDF \tag{1}$$

$$HEM = NDF - ADF \tag{2}$$

$$CEL = ADF - ADL \tag{3}$$

$$LIC = ADL \tag{4}$$

2.3 Incubation experiment

An agricultural soil was sampled at a 0–30 cm depth from grassland in the Cap Bon region of Tunisia (36°34’44.3”N; 10° 41’49.0” E). This soil (Table 4, Figure 1) was selected according to the specifications of the French standard FD U44-163 (49), which relates to the mineralization potential of organic products through incubation under controlled conditions.

Collected in autumn 2023, the soil was prepared following the FD U44–163 protocol (49). It was air-dried, sieved to 4 mm, and stored until the start of the incubation experiment. The incubation was carried out at the Agronomic Sciences and Techniques Laboratory (LR16INRAT05) of INRAT (Tunisia). Dried FRMs were thoroughly mixed with 25 g of oven-dry soil (103 ± 2 °C) and placed in airtight glass jars, which were then incubated at 28 ± 1 °C. The amount of FRM applied provided 50 mg, equivalent to 0.2% of organic carbon, following the FD U44–163 protocol (49). Six control samples consisting of soil without any FRM addition and eight empty jars lacking soil or FRM were also included. Soil moisture was adjusted and maintained at a water retention tension of 60 kPa. Carbon mineralization was measured on days 3, 7, 14, 21, 28, 49, 70, and 91 after incubation in FRM-amended soils, control soils, and empty jars. The CO_2 produced was trapped in 10 mL of 0.5 N NaOH and quantified by titration with 1 N HCl in the presence of excess $BaCl_2$ (49). Carbon mineralization was expressed in g of C-CO_2 100 g^{-1} organic C and calculated as the difference between the soil+FRM treatment and the average of the six control replicates. This study did not rely on biological replication of individual FRMs but instead prioritized a broad

TABLE 2 Descriptive statistics of physico-chemical parameters for nine categories of fertilizing residual materials (FRMs) and manure categories.

FRM categories (number of materials)	Descriptive statistics	DM (%)	pH	EC (µs/cm)	OM (g/100g FRM)	TO C (% DM)	Total N (%DM)	N-NH_4 (%DM)	Total P (%DM)	Total K (%DM)	C/N
Compost (13)	Min	41.9	6.8	786	13.8	11.0	0.6	0.4	0.1	0.2	10.1
	Max	88.7	8.4	5660	50.2	25.4	1.9	2.1	0.8	1.6	20.3
	Mean	56.9	7.8	2254	33.0	19.0	1.4	1.3	0.4	0.8	13.2
	SD	18.3	0.5	1428	10.0	4.1	0.3	0.5	0.2	0.5	3.1
De-inking residues (7)	Min	28.0	7.8	515	32.4	23.2	0.1	0.1	0.0	0.0	40.8
	Max	50.2	8.8	985	46.2	26.7	0.5	1.1	0.3	1.4	139.1
	Mean	40.8	8.3	737	42	24.7	0.3	0.6	0.1	0.3	92.8
	SD	10.4	0.3	175	5.5	1.3	0.1	0.4	0.1	0.6	43.1
Frass (7)	Min	82.4	7.5	3730	–	–	–	–	–	–	–
	Max	93.7	7.7	6060	–	–	–	–	–	–	–
	Mean	91.9	7.5	4425	57.7	33.4	2.1	0.2	1.5	1.7	15.7
	SD	4.1	0.0	1096	–	–	–	–	–	–	–
Municipal solid waste (29)	Min	11.8	5.6	76	35.2	19.8	0.7	1.9	0.9	0.1	5.4
	Max	44.5	11.9	7720	82.5	41.0	6.4	9.3	3.9	1.1	27.1
	Mean	21.0	7.1	3354	64.1	31.3	3.7	5.8	2.1	0.6	10.7
	SD	9.3	1.4	2447	10.6	5.4	1.6	1.9	0.7	0.2	6.4
Pond biosolids (4)	Min	9.1	5.5	874	27.2	15.2	1.5	0.7	0.8	0.2	7.8
	Max	86.3	6.5	3390	48.8	22.4	2.7	6.1	7.4	0.7	10.9
	Mean	41.9	6.2	2226	35.8	18.5	2.0	2.5	3.6	0.3	9.3
	SD	32.8	0.4	1055	9.1	3.4	0.4	2.4	2.9	0.2	1.4
Paper biosolids (23)	Min	13.0	6.1	311	37.8	21.2	0.6	0.1	0.1	0.0	9.2
	Max	35.2	9.2	4880	96.8	53.3	4.3	5.4	1.1	0.6	59.8
	Mean	23.1	7.3	1642	72.0	38.7	2.1	2.2	0.4	0.2	25.4
	SD	6.0	0.7	1277	19.8	9.0	1.2	1.7	0.3	0.2	16.2
Digestates (17)	Min	17.9	6.1	1079	28.2	16.0	1.5	4.0	0.8	0.2	6.4
	Max	40.2	9.7	7071	76.5	37.5	4.9	6.1	5.2	3.9	17.7
	Mean	26.3	7.7	3590	63.6	31.2	2.9	4.9	2.6	1.9	11.4
	SD	5.7	0.9	2072	14.0	5.0	0.9	0.8	1.2	1.1	3.1
Animal manures (2)	Min	–	9.4	5300	81.0	40.1	2.0	–	–	–	18.9

(Continued)

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TABLE 2 Continued

FRM categories (number of materials)	Descriptive statistics	DM (%)	pH	EC (µs/cm)	OM (g/100g FRM)	TO C (% DM)	Total N (%DM)	N-NH_4 (%DM)	Total P (%DM)	Total K (%DM)	C/N
	Max	–	9.4	5300	83.1	40.5	2.1	–	–	–	19.7
	Mean	26.2	9.4	5300	82.1	40.3	2.0	–	0.4	1.5	19.3
	SD	–	0.0	0.0	1.5	0.2	0.1	–	–	–	0.5
Neutralization sludge (1)	Min	–	–	–	–	–	–	–	–	–	–
	Max	–	–	–	–	–	–	–	–	–	–
	Mean	90.6	7.5	2196	–	–	0	ND	ND	ND	–
	SD	–	–	–	–	–	–	–	–	–	–
Wood ash (1)	Min	–	–	–	–	–	–	–	–	–	–
	Max	–	–	–	–	–	–	–	–	–	–
	Mean	61.7	11.6	5350	13.8	15.2	0.1	ND	2.1	0.9	218.2
	SD	–	–	–	–	–	–	–	–	–	–

DM, Dry matter; MO, Organic matter; TOC, Total Organic Carbon; Total N, Total nitrogen; Total P, Total Phosphorus; Total K, Total Potassium; C/N, Carbon Nitrogen Ratio; Min, Minimum; Max, Maximum; SD, Standard Deviation.

diversity of FRM sources. This design choice reflects the study's primary objective, not to compare treatments but to identify functional thresholds separating mineralizing and stabilizing behaviors across a wide spectrum of organic materials. For threshold-based approaches such as the Cate–Nelson partitioning method, capturing inter-material variability is statistically more informative than reducing intra-material experimental uncertainty. Expanding the number of FRMs increases coverage of biochemical compositions and strengthens the robustness of detected breakpoints, whereas increasing replication primarily refines parameter estimates for individual materials without substantially affecting threshold positioning.

Carbon mineralization data were fitted using a first-order kinetic model known as the law of diminishing increments. This model, commonly referred to as the Mitscherlich equation (50), is expressed as follows (Equation 5):

$$C_{min} = C_0(1 - e^{-kt}) \quad (5)$$

Where:

- C_{min} is the cumulative mineralization of organic carbon (g C-CO_2–100 g^{-1} organic C) at time t (days),
- C_0 is the potentially mineralizable carbon (g C-CO_2–100 g^{-1} organic C),
- k is the mineralization rate constant (day^{-1}).

Based on this model, residual carbon (RC) was estimated using Equation 6, expressed in g C-CO_2–100 g^{-1} organic C:

$$RC = C_T - C_0 \quad (6)$$

Where:

- C_T is the total amount of carbon added via fertilizing residual materials (FRMs) during the incubation (g C-CO_2–100 g^{-1} organic C),
- C_0 is the potentially mineralizable carbon (g C-CO_2–100 g^{-1} organic C).

2.4 Evaluation of carbon stability indicators

Based on analytical data from 104 FRM samples, several indicators of organic carbon stability were assessed. These indicators were derived either from biochemical fractionation alone or from a combined approach incorporating biochemical data and carbon mineralization measurements. The following indices were evaluated across all samples: BSI (Biological Stability Index), I_{ROC} (an indicator of potential residual organic carbon), Tr coefficient (an indicator of organic matter restitution potential), and a set of biochemical ratios (SOL, HEM, CEL, and LIC fractions). The indicators were calculated using Equations 7 (23), 8 (24), and 9 (22), and biochemical ratios (Equations 10–14):

$$BSI = 2.112 - 2.009 \times SOL - 2.378 \times HEM - 2.216 \times CEL + 0.84 \times LIC \quad (7)$$

TABLE 3 Inorganic contaminants concentrations (mg kg^{-1} in dry matter) for nine categories of fertilizing residual materials (FRMs) and manure categories compared with the standards limits for inorganic contaminants agricultural application.

FRM categories (number of materials)	Al	As	Cd	Co	Cr	Cu	Hg	Mo	Ni	Pb	Se	Zn
Compost (13)	1950-12800	3.7–12.7	<0.9 – 1.3	3-7	11-52	46-249	0.05-0.72	2-6	<10 - 66	16-282	<0.5-2.4	155-599
De-inking residues (7)	1301-5363	0.4-0.7	<0.9-0.9	1-2.57	4.5-8	15-40	0.02-0.07	3.5-5	1-2	3-5.1	0.3-0.5	25-367
Frass (7)	18-595	<1.5	<1.0	<10	<10	13-28	<3.0	2	<10	<10	1.0	128-284
Municipal solid waste (29)	22-171000	2.2-10.6	<0.9-3.5	<2-12	14-113	24-621	0.18-2.1	2-9.4	9.7-165	<10-38	1.3-24.2	28-999
Pond biosolids (4)	4850-62483	2-8	1-2.5	5-14	40-94	248-474	0.8-1.5	3-13	26-91	17-67	1.9-2.6	660-1527
Paper biosolids (23)	225-6160	<0.7-10.4	<0.9-5.6	<2-3	6-18	<10-45	<0.04-0.25	<1.5-6	3-13	<5-46	<0.5	16-294
Digestates (17)	17-26000	1.6-3.8	0.37-0.4	3-10	17-37	50-150	<0.04-0.31	2-9.3	10-25	9-18	<0.5-1.7	167-2337
Animal manures (2)	1095-1181	–	–	–	–	29-48	–	–	–	–	–	107-168
Neutralization sludge (1)	548	17.9	<0.9	<10	910	11	0.2	4.7	37	<10	0.8	15
Wood ash (1)	12100	59.1	6.2	6	63	121	0.1	3	24	181	<0.5	878
Standards limits for inorganic contaminants [mg kg^{-1} in dry matter] agricultural application												
USA[a]		13[a]0	10.0	136.0	-	-	1.00	42.000	250	61	26.0	420
Canada[b]		12[b]0	3.0	25.0	-	-	1.00	3.29	30	82	2.0	305

[a]According to Association of American Plant Food Control Officials (46).
[b]According to the Canadian Food Inspection Agency (47).

$$I_{ROC} = 445 + 0.5\,\text{SOL} - 0.2\,\text{CEL} + 0.7\,\text{LIC} - 2.3\,\text{C}_3\text{d} \quad (8)$$

$$\text{Tr} = 0.3221 \times \text{SOL} - 0.7155 \times \text{HEM} + 0.6717 \times \text{CEL} + 1.8919 \times \text{LIC} + 0.0271 \times \text{MM} \quad (9)$$

$$\text{LIC} \quad (10)$$

$$\frac{\text{LIC}}{\text{SOL} + \text{HEM} + \text{CEL}} \quad (11)$$

$$\frac{\text{LIC}}{\text{HEM} + \text{CEL}} \quad (12)$$

$$\frac{\text{HEM} + \text{CEL}}{\text{LIC}} \quad (13)$$

$$\frac{\text{CEL}}{\text{LIC}} \quad (14)$$

Where: SOL = soluble fraction (g soluble 100g FRM), HEM = hemicellulose fraction (g hemicellulose 100g FRM), CEL = cellulose fraction (g cellulose 100g FRM), LIC = lignin fraction (g lignin 100g FRM), C_3d = carbon mineralized on day 3 of incubation, MM = mineral matter content (%).

This study evaluated these indicators based on their response to two key parameters: the carbon stabilization potential and the carbon mineralization rate constant *k*.

2.5 Statistical analysis

The calculation of statistical descriptors was conducted in Microsoft Excel® (2010) to determine the physicochemical characteristics, heavy metal concentrations, and biochemical fractions of FRMs. Parameter fitting for the Mitscherlich equation was performed in Python 3.11.11 (compiled with GCC 11.4.0), with additional calculations conducted in Microsoft Excel® (2010). Using the Cate-Nelson partitioning method, a binary classification was established to define a critical threshold distinguishing FRMs with stable carbon retention potential from those characterized by readily mineralizable carbon (51). The classification was optimized using the R software (version 4.2.1; 52) and the companion package (53). The Cate-Nelson method, initially developed to interpret crop responses to fertilization, was used here to determine critical thresholds for carbon stabilization or mineralization indicators. This iterative procedure seeks the cutoff value that maximizes the sum of squares along the X-axis (indicator values) while minimizing classification errors along the Y-axis (mineralized or residual carbon) (54). The scatterplot is divided into four quadrants: FP (false positives) in quadrant I, TN (true negatives) in quadrant II, FN (false negatives) in quadrant III, and TP (true positives) in quadrant IV. These counts were used to compute the performance metrics of the partitioning model using Equations 15–19:

$$\text{Robustness } (R^2) = \frac{\text{TP} + \text{TN}}{\text{TP} + \text{TN} + \text{FP} + \text{FN}} \times 100 \quad (15)$$

TABLE 4 **Physicochemical properties of the sampled soil compared to the standard requirements for a reference soil (49).**

Parameter	Value of soil sampled	FD U44–163 standard requirements
Clay (g kg^{-1})	198	100 – 250
pH_{water}	7.9	6.0 – 8.0
Total limestone (g kg^{-1})	< 4	< 5
Mineral nitrogen (mg kg^{-1})	14.3	< 35
Organic carbon (g kg^{-1})	9	5 – 12

$$\text{Specificity}\ (\%) = \frac{TN}{TN + FP} \times 100 \tag{16}$$

$$\text{Sensitivity}\ (\%) = \frac{TP}{TP + FN} \times 100 \tag{17}$$

$$\text{PPV}\ \ (\%) = \frac{TP}{TP + FP} \times 100 \tag{18}$$

$$\text{NPV}\ \ (\%) = \frac{TN}{TN + FN} \times 100 \tag{19}$$

Where PPV is positive predictive value, and NPV is negative predictive value.

These five probability metrics collectively provide a quantitative assessment of the model's ability to accurately differentiate between FRMs with stable carbon retention potential and those dominated by easily mineralizable carbon, thereby ensuring a robust and reliable diagnostic framework for assessing carbon stability.

A sensitivity analysis was conducted to assess the impact of fertilizing residual materials (FRMs) that did not reach a mineralization plateau within the 91-day incubation period. Cate–Nelson partitioning was repeated after excluding these samples, and the resulting cutoff values were compared with those obtained from the full dataset.

3 Results

3.1 Biochemical fractionation of fertilizing residual materials using the Van Soest method

Based on the Van Soest method, the results of the biochemical organic matter fractionation are presented in Table 5 for 104 FRM samples, including two manure samples used as a reference for conventional organic amendment practices.

Table 5 summarizes descriptive statistics by FRM category and reveals distinct distribution patterns for the three main organic fractions: soluble (SOL), holocellulosic (HEM + CEL), and lignin (LIC). Significant variability in the biochemical composition was observed both between and within the FRM categories. Pond biosolids exhibited the highest internal variability, with standard deviations ranging from 37% to 78% of the mean, highlighting firm compositional heterogeneity likely due to inconsistent treatment processes. In contrast, manure samples showed much lower variability (4%-8%), suggesting a more consistent composition across sources.

Distinct biochemical profiles also emerged for certain categories. Neutralization sludges had the lowest lignin (LIC) content, confirming their mineral-rich nature and low recalcitrant organic matter content. Conversely, frass samples were characterized by the highest concentrations of soluble compounds (SOL), which aligns with their insect-based origin and high organic bioavailability. Compost samples stood out for their high lignin content, reflecting their advanced degree of stabilization and humification. Within-category variability was also notable. For instance, pond and paper biosolids exhibited large fluctuations in both SOL and LIC contents, likely due to variability in primary versus secondary sludge composition and treatment processes. Similarly, wide ranges were observed in polysaccharide (HEM + CEL) and lignin contents for digestates and municipal biosolids, reflecting differences in feedstock origin and digestion conditions. Altogether, this biochemical diversity across the 104 FRMs creates a broad gradient of organic matter quality. This gradient is crucial for

FIGURE 1
Location of the reference soil sampling site used for the incubation experiment.

TABLE 5 Descriptive statistics of biochemical fractions (g FRM 100 g^{-1} organic C) for nine categories of fertilizing residual materials and two farmyard manure samples used as a reference.

FRM categories	n	Biochemical fractions	Min	Max	Mean	SD	RSD (%)
Composts	13	SOL	15.8	37.7	28.3	6.9	24
		HOLO	8.3	33.5	17.5	8.7	50
		LIC	39.8	61.5	54.2	6.0	11
De-inking residues	7	SOL	40.5	52.1	45.5	4.3	10
		HOLO	17.9	49.8	37.2	13.3	36
		LIC	4.8	31.2	17.3	11.0	64
Frass	7	SOL	42.4	92.1	53.6	17.6	33
		HOLO	4.1	51.1	40.8	16.8	41
		LIC	3.8	6.5	5.6	0.9	16
Municipal solid waste	29	SOL	37.9	71.4	58.2	7.2	12
		HOLO	10.4	39.5	22.8	7.3	32
		LIC	2.7	29.8	19.0	6.9	37
Pond biosolids	4	SOL	27.7	64.4	48.7	18.2	37
		HOLO	3.0	17.0	9.8	7.7	78
		LIC	19.7	57.6	41.4	18.2	44
Paper biosolids	20	SOL	10.9	84.6	35.8	17.2	48
		HOLO	13.5	57.8	38.6	11.7	30
		LIC	1.9	38.8	25.6	10.0	39
Digestates	17	SOL	24.5	59.7	41.7	12.6	30
		HOLO	3.5	47.6	28.1	13.9	49
		LIC	15.3	44.1	30.2	7.8	26
Animal manures	2	SOL	39.5	42.6	41.0	2.3	6
		HOLO	36.6	41.0	38.8	3.1	8
		LIC	19.6	20.8	20.2	0.9	4
Neutralization sludge	1	SOL	–	–	49.4	–	–
		HOLO	–	–	47.6	–	–
		LIC	–	–	3.0	–	–
Wood ash	1	SOL	–	–	29.0	–	–
		HOLO	–	–	36.5	–	–
		LIC	–	–	34.5	–	–
Total	104						

FRM, fertilizing residual materials; n, number of samples; min, minimal; max, maximal; SD, Standard deviation; RSD, Relative Standard deviation; SOL, soluble fraction; HOLO, holocellulosic (HEM + CEL); LIC, lignin fraction.

establishing a reliable relationship between biochemical composition and the potential for carbon mineralization/stabilization, thereby supporting the development of robust diagnostic tools.

3.2 Carbon mineralization kinetics and stabilization potential of organic amendments

Because the analysis focused on functional partitioning rather than treatment comparison, kinetic parameters (C_0, k, RC) were interpreted at the population level to identify threshold behavior, rather than as replicated estimates for individual FRMs. Figure 2 illustrates the dynamics of two antagonistic processes following the application of sample no. 7 from the category Frass to soil: carbon mineralization, expressed as the cumulative amount of CO_2 released, and residual carbon (RC), representing the remaining non-mineralized carbon, following AFNOR standard (2018). The blue curve represents the mineralization kinetics of organic carbon, modeled using a simple first-order kinetic equation (Equation 5). Three key parameters characterize this curve: the mineralization rate constant (k), the mineralization threshold C_0, corresponding to the plateau, and the temporal range beyond which the mineralization rate becomes negligible. In the illustrative example, cumulative C-CO_2 increased rapidly, indicating an active mineralization phase with a relatively high rate constant (k=0.16 day^{-1}). This exponential phase lasted for approximately 19 days, after which mineralization slowed and stabilized at a plateau of 29.1

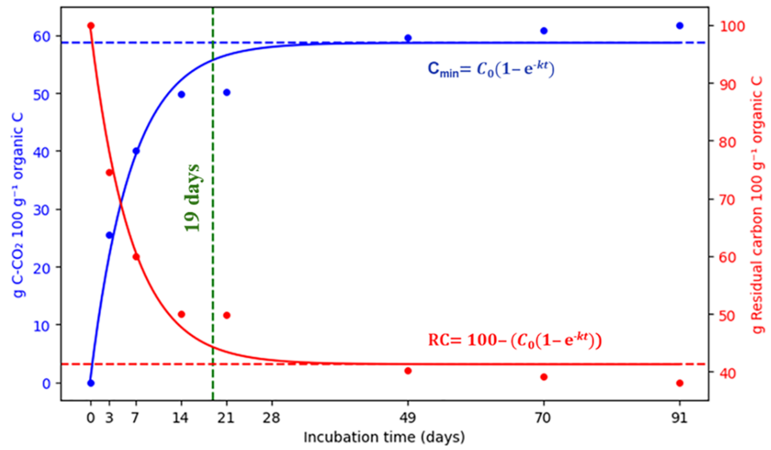

FIGURE 2
Illustrative example of carbon mineralization and stabilization curves for a Frass sample, showing the cumulative mineralized carbon (C_{min}) and residual carbon (RC) over the incubation period.

g $C\text{-}CO_2$ 100 g OC, marking the beginning of the stabilization phase. The red curve, symmetrical to the first, depicts the carbon stabilization process. It reflects the quantity of carbon retained in the soil, i.e., the non-mineralized fraction of the added organic matter. RC is calculated as the difference between the initial amount of carbon applied (50 mg C, as per 49) and the amount mineralized at each time point (Equation 6). In this example, the stabilization curve reaches a plateau of 20.6 mg C, indicating the amount of stabilized carbon at the end of incubation. This stabilization phase followed the same temporal range as the mineralization phase (approximately 19 days), after which residual carbon levels remained stable.

Carbon mineralization curves were generated for all 104 samples, including the manure, using the same modeling approach illustrated in Figure 3. The experimental data were fitted to first-order kinetic models (Equation 5), and most samples exhibited excellent model performance, with coefficients of determination (R^2) ranging from 0.84 to 1.00. As demonstrated in Figure 3, the carbon mineralization rates during soil incubation exhibited significant variability, ranging from 6.7 to 126.7 g $C\text{-}CO_2$ per 100 g of organic C at the end of the incubation period. However, for eight FRM samples, compost (CM3), Paper biosolids (Pb1, Pb18, Pb, Pb15), and digestates (DIG19, DIG21, DIG34), no apparent mineralization plateau (C_0) could be identified. These samples exhibited a continuously increasing mineralization trend that followed a diminishing-returns pattern, without approaching stabilization, suggesting either a predominance of easily degradable organic matter or an incomplete mineralization phase within the incubation timeframe.

The fitting results for all 104 kinetic curve parameters are summarized by category in Table 6.

3.3 Defining biochemical cutoff values for carbon retention and turnover in organic waste amendments

Critical threshold values were determined for each of the eight biochemical indicators (Equations 7–14) that represent the transition point between labile carbon mineralization and stable carbon retention. Given the scope and complexity of the dataset, we chose to illustrate the binary partitioning method (Cate-Nelson) using a single representative biochemical indicator: the lignin fraction (LIC). This example demonstrates how two distinct critical thresholds were determined, one for carbon stabilization potential and the other for the mineralization rate constant *k*, as shown in Figures 4, 5, respectively. While it was not feasible to present this level of detail for all eight indicators, a summary of the critical values and partitioning performance metrics (R^2, specificity, sensitivity, PPV, NPV) is provided in Table 6. To ensure transparency and completeness, we also include two graphical plots for each of the remaining seven indicators in the Supplementary Material. These visualizations allow readers to examine how each indicator differentiates between carbon behaviors dominated by mineralization versus stabilization.

3.3.1 Determination of the critical lignin threshold in relation to carbon stabilization potential

A critical threshold of 32.8 g LIC 100 g^{-1} FRM was identified as the point that best separates the 104 samples into two distinct behavioral groups based on their lignin content. This threshold was determined using the Cate-Nelson partitioning method, which maximizes the between-groups sum of squares (Figure 4c), thereby highlighting a shift in carbon dynamics from rapid mineralization to enhanced stabilization. Samples with LIC content above 32.8 g LIC 100 g^{-1} form a group characterized by significantly higher carbon retention. This group reached a carbon persistence potential of up to 71.8 g RC 100 g^{-1}, indicating a strong tendency toward carbon stabilization. This grouping also minimized the number of misclassified observations in the false-positive (FP) and false-negative (FN) quadrants (Figure 4b), reinforcing the relevance of this threshold. Conversely, samples with LIC content below 32.8 g LIC 100 g^{-1} were associated with lower residual carbon levels and a more mineralizing behavior, confirming their lower capacity for stabilization. These amendments released a larger proportion of the applied carbon,

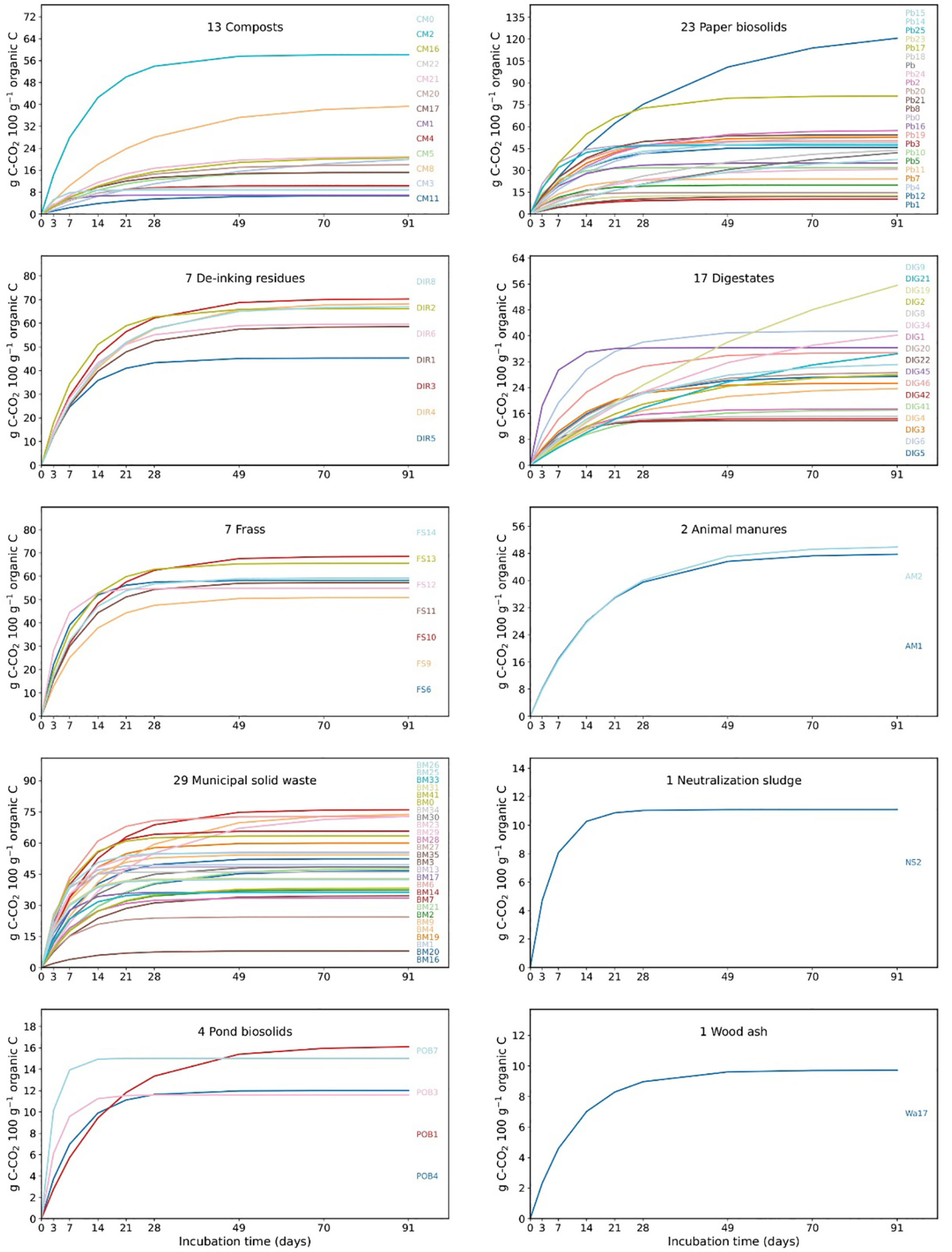

FIGURE 3
Cumulative C mineralization rates of 104 fertilizing residual material (FRM), measured over the incubation period. CM, Compost; Pb, Paper biosolids; DIR, De-inking residues; DIG, Digestates; FS, Frass; AM, Animal manures; BM, Municipal solid waste; NS, Neutralization sludge; POB, Pond biosolids; Wa, Wood ash.

with RC values systematically lower than 71.8 g RC 100 g^{-1}. The classification performance was further evaluated based on the distribution of points across the four quadrants of the partition diagram (Figures 4a, d). Notably, the majority of data points were correctly assigned to the true positive (TP) and true negative (TN) quadrants, with relatively few falling into the error zones (false positives, FP, and false negatives, FN). From this distribution, five performance metrics (Equations 15–19) were calculated, all of which exceeded or were equal to 70%, as shown in Figure 4d. This underscores the robustness and diagnostic reliability of using LIC as a predictive indicator. The clear plateau observed in the TN quadrant, corresponding to amendments with high lignin content and stable carbon, illustrates a meaningful biological transition toward carbon-stabilization systems. This transition validates the

TABLE 6 Summary statistics of first-order kinetic parameters for nine fertilizing residual materials (FRMs) and manure categories.

FRM categories	Descriptive statistics	C_0	*k*	RC	Rg
Unit		$g100g^{-1}$ OC	day^{-1}	$g100g^{-1}$ OC	day^{-1}
Composts (13)	Mean ± SD	19.5 ± 14.9	0.09 ± 0.1	80.4 ± 14.8	47.2 ± 28.8
	Min – Max	6.7 - 59.1	0.02 - 0.32	40.9 - 93.2	10 - 123
De-inking residues (7)	Mean ± SD	62.5 ± 8.8	0.09 ± 0.017	37.4 ± 8.8	35.7 ± 6.9
	Min - Max	45.1 - 71	0.07 - 0.11	29 - 54.9	27 - 45
Frass (7)	Mean ± SD	59.7 ± 6.4	0.13 ± 0.05	40.3 ± 6.4	25.1 ± 7.4
	Min - Max	51.5 - 69.5	0.09 - 0.24	30.4 - 48.4	12 - 34
Paper biosolids (20)	Mean ± SD	43.4 ± 25.4	0.09 ± 0.05	57.7 ± 21.8	48.9 ± 39.4
	Min - Max	10.2 - 126.7	0.02 - 0.2	0 - 89.7	15 - 170
Animal manures (2)	Mean ± SD	48.8 ± 1.8	0.06 ± 0.00	51.1 ± 1.8	50.0 ± 2.8
	Min - Max	47.5 - 50.1	0.06	49.8 - 52.4	48 - 52
Municipal solid waste (29)	Mean ± SD	49.2 ± 15.8	0.14 ± 0.06	50.7 ± 15.8	26.6 ± 12.2
	Min - Max	7.9 - 77.2	0.05 - 0.32	22.7 – 92	9 - 61
Pond biosolids (4)	Mean ± SD	13.6 ± 2.3	0.21 ± 0.14	86.3 ± 2.3	23.0 ± 18.0
	Min - Max	11.5 - 16.1	0.06 - 0.38	83.8 - 88.4	8 - 48
Digestates (17)	Mean ± SD	30.6 ± 15.9	0.07 ± 0.05	69.3 ± 15.9	64.8 ± 53.2
	Min – Max	13.7 - 79.1	0.01 - 0.24	20.8 - 86.2	13 - 221
Neutralization sludge (1)		11.1	0.18	88.8	16
Wood ash (1)		10	0.09	89.9	33

FRM, fertilizing residual materials; OC, Organic Carbon; min, minimal; max, maximal; SD, Standard deviation; C_0, potentially mineralizable carbon (g mg C 100 g^{-1} OC); *k*, mineralization rate constant (day^{-1}); RC, residual carbon (g mg C 100 g^{-1} OC); Rg, range (duration of the active mineralization phase (days).

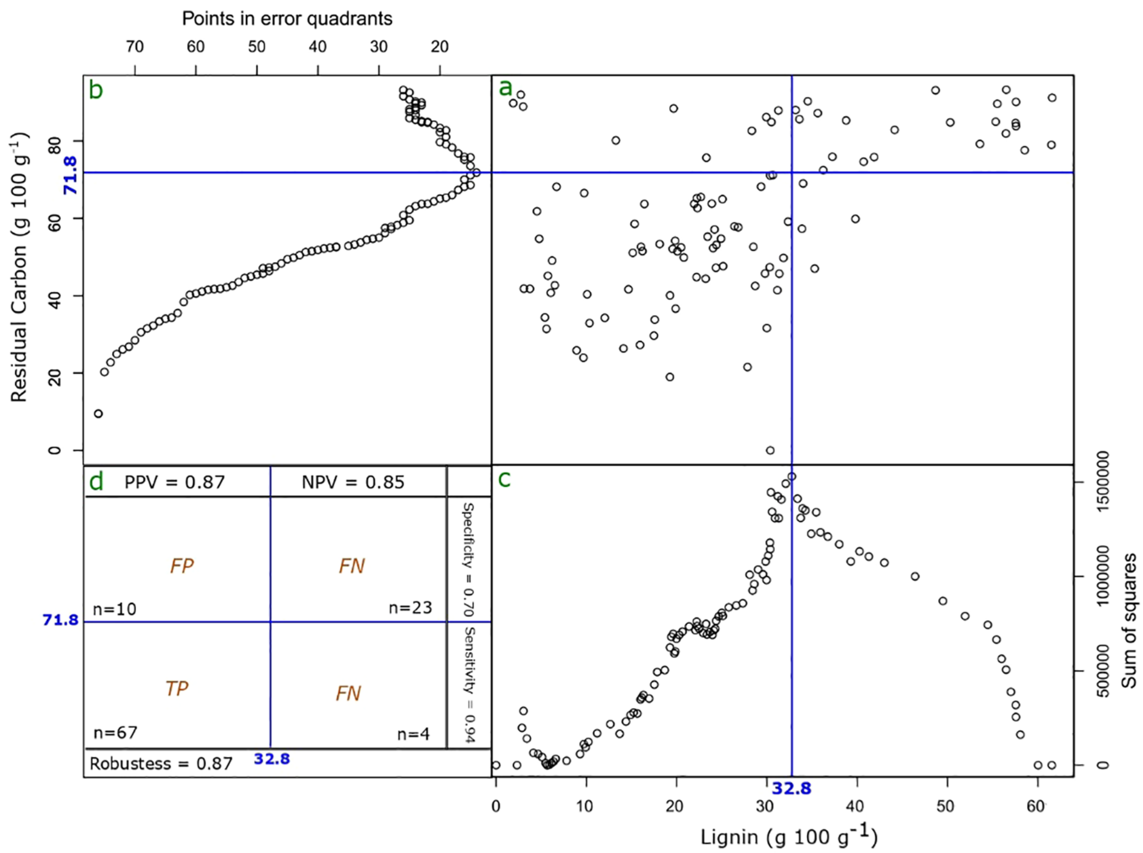

FIGURE 4
Cate-Nelson partitioning of lignin content (LIC) in organic amendments to identify critical thresholds for carbon stabilization. **(a)** Cate-Nelson plot showing the classification of samples based on LIC and RC, **(b)** distribution of misclassified points for determining the RC threshold, **(c)** sum of squares for identifying the optimal LIC cutoff, **(d)** model performance metrics: R^2, specificity, sensitivity, NPV (negative predictive value), and PPV (positive predictive value).

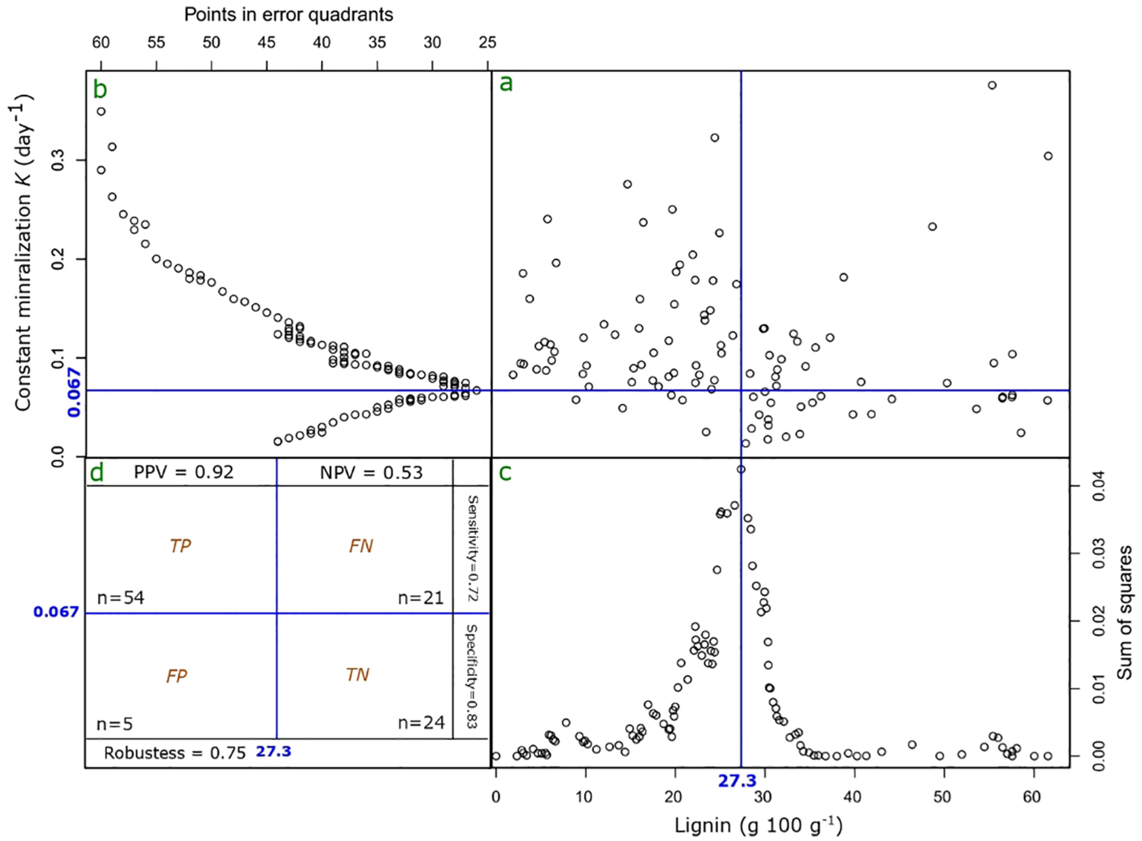

FIGURE 5
Cate-Nelson partitioning of lignin content (LIC) in relation to the carbon mineralization rate constant k for fertilizing residual materials (FRMs) and farmyard manure. **(a)** Cate-Nelson plot showing the classification of samples and the identified thresholds, **(b)** number of misclassified points used to determine the *k* threshold, **(c)** sum of squares used to determine the optimal LIC cutoff, **(d)** performance metrics of the classification model: R^2, specificity, sensitivity, NPV (negative predictive value), and PPV (positive predictive value).

use of LIC as a relevant and effective biochemical index for distinguishing between mineralizing and stabilizing amendment types in fertilizing residual materials and manure.

3.3.2 Determination of the critical lignin threshold in relation to the carbon mineralization rate constant *k*

Unlike the ascending relationship observed between lignin content (LIC) and carbon stabilization (RC), the relationship between LIC and the carbon mineralization rate constant *k* follows a descending pattern. As lignin content increases, the value of *k* systematically decreases until reaching a lower asymptote, indicating a slowdown in the mineralization dynamics (Figure 5a). The descending Cate-Nelson classification identified a critical LIC threshold of 27.3 g LIC 100 g^{-1} (Figure 5c). Below this value, FRMs tend to exhibit higher mineralization rates, with an average *k* value above 0.067 day^{-1}, thus forming the rapid mineralization group. These amendments are characterized by low lignin content and high biodegradability, which accelerate carbon turnover. Above the 27.3 g LIC 100 g^{-1} threshold, however, the mineralization rate stabilizes around or below 0.067 $days^{-1}$, delineating the carbon-sequestering group (Figure 5b). In this group dominated by samples located in the true negative (TN) quadrant, the low *k* values reflect reduced microbial accessibility to organic substrates, consistent with a higher proportion of recalcitrant carbon, primarily lignin. The overall quality of the classification was assessed through performance metrics derived from the distribution of points across the four quadrants (Figure 5d). Although the negative predictive value (NPV) was slightly lower, the other four indicators, R^2, specificity, sensitivity, and positive predictive value (PPV), exceeded the 70% threshold. This confirms the diagnostic relevance of LIC in identifying amendments with slower carbon turnover. Taken together, these findings demonstrate that the mineralization rate constant *k* decreases with increasing lignin content, reaching a plateau beyond the 27.3 g LIC 100 g^{-1} threshold. This plateau marks a transition toward materials with greater carbon stability and a lower tendency to rapidly release CO_2, further reinforcing LIC's role as a robust indicator for distinguishing between mineralizing and sequestering amendment systems.

3.3.3 Comparative diagnostic of eight biochemical indicators to identify the most reliable predictors of carbon stabilization and mineralization in organic amendments

To broaden the scope of the analysis, we applied the same Cate-Nelson partitioning method to eight selected biochemical indicators (Equations 7–14). In contrast, the simpler biochemical fractions,

TABLE 7 Based on Cate-Nelson partitioning, performance metrics of eight biochemical indicators of carbon stability in fertilizing residual materials in relation to carbon stabilization potential (g RC 100 g^{-1} OC) and mineralization rate constant *k* (day^{-1}).

Indicators (unit)	Critical value of indicator	Critical value of RC or *k*	Robustness	PPV	NPV	Sensitivity	Specificity
RC (g 100 g^{-1})							
BSI	**0.99**	**71.8**	**0.88**	**0.87**	**0.92**	**0.97**	**0.70**
LIC (g 100 g^{-1})	**32.8**	**71.8**	**0.87**	**0.87**	**0.85**	**0.94**	**0.70**
Stability ratio	**0.49**	**71.8**	**0.87**	**0.87**	**0.85**	**0.94**	**0.70**
I_{ROC}	861	71.8	0.86	0.85	0.88	0.96	0.64
Lignocellulosic ratio	2.1	71.8	0.84	0.81	0.94	0.99	0.51
Tr (%)	106	89.3	0.86	0.97	0.29	0.88	0.63
Lability ratio	1.4	52.6	0.30	0.24	0.38	0.33	0.27
Decompositional Ratio	1.2	41.7	0.24	0.05	0.56	0.15	0.26
Mineralization constant *k* ($days^{-1}$)							
LIC (g 100 g^{-1})	**27.3**	**0.067**	**0.75**	**0.92**	**0.53**	**0.72**	**0.83**
Stability ratio	**0.38**	**0.067**	**0.75**	**0.92**	**0.53**	**0.72**	**0.83**
BSI	**0.77**	**0.067**	**0.72**	**0.91**	**0.50**	**0.68**	**0.83**
Tr (%)	63	0.2	0.76	0.25	0.95	0.64	0.77
Lability ratio	1.2	0.12	0.59	0.42	0.74	0.60	0.58
Decompositional Ratio	0.85	0.12	0.60	0.40	0.75	0.58	0.61
Lignocellulosic ratio	0.82	0.067	0.53	0.74	0.30	0.53	0.52
I_{ROC}	745	0.075	0.50	0.67	0.34	0.45	0.57

PPV, positive predictive value; NPV, negative predictive value; RC, Residual Carbon; BSI, Biological Stability Index; LIC, lignin fraction; SOL, soluble fraction; HEM, hemicellulose fraction; CEL, cellulose fraction; I_{ROC}, indicator of potential residual organic carbon; Tr, Tr coefficient (organic matter restitution potential), Stability ratio = $\frac{LIC}{(SOL+HEM+CEL)}$, Lability ratio = $\frac{HEM+CEL}{LIC}$, *Ligno cellulosic ratio* = $\frac{LIC}{(HEM+CEL)}$, Decompositional Ratio = $\frac{CEL}{LIC}$.

Bold values indicate biochemical indicators exhibiting the highest diagnostic performance and statistical robustness (Robustness ≥ 0.70) for distinguishing carbon stabilization and mineralization behaviors based on the Cate–Nelson partitioning approach.

SOL, HEM, and CEL, were excluded from this comparative evaluation due to their poor performance. The data associated with these fractions showed high variability and diffuse scatterplots, making it difficult to identify critical thresholds using the Cate-Nelson approach reliably. As a result, they were not considered robust enough to discriminate between carbon-stabilizing and carbon-mineralizing behaviors. The results for the retained indicators are summarized in Table 7, which presents a comparative synthesis of their classification performance based on carbon stability. Indicators are ranked in decreasing order of robustness to highlight the most effective predictors.

The results show that the Biological Stability Index (BSI) is one of the most effective indicators for predicting carbon stabilization potential, with a high robustness score of 0.88. However, its performance decreased slightly when evaluated under the constant carbon mineralization rate (k), with robustness dropping to 0.72. Similarly, both lignin content (LIC) and the $\frac{LIC}{SOL+HEM+CEL}$ ratio demonstrated strong performance, with robustness values above 75% for both carbon stabilization and mineralization. These three indicators also shared consistent and convergent critical values: a carbon stabilization threshold of 71.8 g RC 100 g^{-1}, and a mineralization threshold of 0.067 day^{-1} for *k*, further supporting their relevance for differentiating between carbon-sequestering and carbon-mineralizing systems. In contrast, some indicators exhibited a marked shift in performance between the two evaluation criteria. For example, both I_{ROC} and the $\frac{LIC}{HEM+CEL}$ ratio showed good performance concerning carbon stabilization, with classification probabilities ranging from 51% to 99% and stabilization thresholds similar to those observed for LIC and BSI. However, when these indicators were applied to the mineralization constant (*k*), a significant drop in effectiveness was observed, reflected in lower robustness scores and reduced predictive values. This limits their diagnostic utility for assessing short-term carbon turnover dynamics. Additionally, the critical thresholds identified for mineralization tended to show greater variability across indicators than those for stabilization, underscoring the greater complexity of modeling mineralization behavior. Despite achieving moderate robustness, the Tr coefficient was limited by low positive and negative predictive values (PPV and NPV) for both response variables, reducing its overall diagnostic reliability. Finally, the $\frac{HEM+CEL}{LIC}$ and $\frac{CEL}{LIC}$ ratios emerged as the least effective indicators, with classification probabilities below 50% and inconsistent critical threshold values across stabilization and mineralization analyses. This weakens their relevance as robust diagnostic tools. Excluding the eight FRMs that did not reach an apparent mineralization plateau within 91 days resulted in only marginal changes in the identified critical thresholds across all biochemical indicators (Supplementary Table 8). The positions of the cutoffs and the overall classification of mineralizing versus stabilizing materials remained consistent with the main analysis, indicating that the threshold-based framework is robust to uncertainties arising from incomplete mineralization curves.

In summary, LIC, the $\frac{LIC}{SOL+HEM+CEL}$ ratio and the BSI index can be considered the most reliable and consistent indicators for assessing both carbon stabilization potential and mineralization behavior of organic amendments. These indicators provide a valuable basis for selecting and applying fertilizing residual materials in long-term soil carbon management.

4 Discussion

The results of this study highlight the influence of the biochemical composition of fertilizing residual materials (FRMs) on soil carbon dynamics, including short-term mineralization and short-term residual carbon. The relationships observed between biochemical characteristics (Table 5) and kinetic parameters (Table 6) confirm that the nature of FRMs' organic constituents strongly influences their biodegradability.

Frass and municipal biosolids, which are rich in soluble compounds, showed high mineralization rate constants k, indicating rapid carbon turnover likely driven by enhanced microbial activity. These findings are consistent with those of Francou et al. (20) and Moreno-Cornejo et al. (55), who reported that the soluble fraction directly controls the extent of carbon mineralization. In fact, up to 86.7% of the organic matter in municipal waste has been recovered in this labile fraction, stimulating microbial degradation (56, 57).

In contrast, pulp and paper biosolids, rich in holocellulose (HEM + CEL), displayed lower k values. Previous studies confirm that paper-derived materials typically contain high levels of holocellulose, a moderately degradable fraction associated with microbial biomass production (58–63).

Composts stood out for their high lignin content, which was associated with the lowest mineralization rates consistent with their greater potential for carbon stabilization (17, 24, 64, 65). Lignin is widely recognized as a recalcitrant compound structurally similar to soil humic substances and may account for 45–54% of total organic matter in composts (22, 61, 66, 67). Notably, a high degree of variability was observed within individual FRM categories. For example, some pond biosolids and composts displayed relatively high k values despite their high lignin content. This variability may be attributed to the partial degradation of the lignin fraction or to the heterogeneity of the soluble fraction, which can encompass both labile and recalcitrant organic compounds (24). Similar trends have been reported for food waste-derived materials (19, 68).

Our results confirm that the biochemical composition of FRMs plays a critical role in regulating soil carbon dynamics, both in short-term mineralization and stabilization potential, consistent with the findings of Doblas-Rodrigo et al. (17). While direct correlations between biochemical fractions and kinetic parameters were not always strong, the discriminatory power of biochemical indicators proved to be highly effective. Notably, when these indicators reach their critical threshold values, they consistently exhibit convergent behaviors, either stabilizing or mineralizing, beyond which the system tends to plateau. As with many biological processes, this stabilization beyond the critical value is a recurrent and observable phenomenon, reflecting a shift toward carbon persistence.

The partitioning approach based on the Cate-Nelson method provided more than a statistical classification; it offered a functional interpretation of how FRMs contribute differently to soil carbon dynamics. By distinguishing between amendments that predominantly promote carbon mineralization and those that support carbon stabilization, this framework highlights the dual roles organic amendments can play depending on their biochemical makeup. Such a distinction is not purely descriptive; it directly informs agronomic and environmental decision-making. In this context, the robustness of the identified thresholds arises from the diversity of FRMs analyzed rather than from replication of individual materials, aligning with the study's objective to capture functional transitions rather than estimate treatment-specific means.

The high performance scores obtained for key biochemical indicators, particularly lignin (LIC), the Biological Stability Index (BSI), and the $\frac{LIC}{SOL+HEM+CEL}$ ratio, confirm the potential of these metrics as reliable diagnostic tools. Their ability to delineate meaningful thresholds reflects the biological reality that carbon dynamics tend to shift toward stability once a certain compositional threshold is exceeded. This plateau effect, consistently observed in our results, is emblematic of broader patterns in soil biology, where microbial degradation rates decrease as substrates become more recalcitrant.

From a practical standpoint, these findings suggest that the biochemical profiling of FRMs can support more targeted soil amendment strategies. Materials with high stabilization potential may be prioritized in contexts aiming to build soil organic matter persistence potential, improve structure, or enhance resilience against erosion and drought. Conversely, highly mineralizing FRMs may be better suited for short-term fertility boosts, provided their use is timed to minimize carbon losses and environmental risks. Thus, the proposed classification system offers not only diagnostic clarity but also actionable guidance for sustainable soil and carbon management.

Building on this classification approach, the application of the AFNOR (49) standardized incubation protocol provided a robust means of evaluating the carbon-stabilization and mineralization potential of FRMs. The proposed framework is intended as a screening and comparative tool to assess the intrinsic mineralization and stabilization potential of fertilizing residual materials under standardized soil conditions, rather than as a universal predictor of field-scale carbon dynamics across all soil types. The soil used for the incubation experiments complied with the AFNOR FD U44–163 standard (Table 4), ensuring physico-chemical conditions compatible with functional microbial activity and representative carbon dynamics of non-limiting agricultural soils. Under these standardized conditions, the observed differences among organic amendments can therefore be attributed primarily to their intrinsic biochemical characteristics rather than to constraints imposed by the receiving soil. Although soil physico-structural properties (e.g., aggregate stability and porosity) and enzyme activities involved in carbon and nitrogen cycling (e.g., β-glucosidase, cellulase, and urease) were not directly measured, previous studies have shown that they play a key role in mediating organic matter turnover. Consequently, the biochemical thresholds and ratios identified in this study should be interpreted as integrative indicators of the mineralization or stabilization potential of organic amendments, whose expression at the field scale will depend on interactions among soil properties, environmental conditions, and biological activity. The kinetic modeling applied, particularly the first-order models widely validated in the literature (26, 69–72), provided a solid framework for estimating both the residual carbon fraction and the mineralization rate constant k under controlled laboratory conditions. These parameters allowed for a meaningful characterization of organic matter behavior across a wide range of materials. However, the 91-day incubation period defined by the AFNOR standard may not fully capture the mineralization trajectory of certain FRMs, especially those with more recalcitrant or heterogeneously treated organic components, such as composts, pulp and paper biosolids, and digestates. Residual carbon measured after 91 days should therefore be interpreted as short-term residual C rather than direct evidence of long-term stabilized carbon; nevertheless, it provides a meaningful proxy for stabilization potential, as materials exhibiting low mineralization rates and high residual carbon over this period are more likely to contribute to carbon persistence once incorporated into soil. Incomplete mineralization curves observed for these materials suggest that their carbon stabilization potential dynamics might be underestimated when confined to a short incubation window. This limitation underscores the importance of tailoring incubation durations to the material type, particularly for slowly degrading substrates. As previously reported by Alburquerque et al. (73) and Nyang'au et al. (74), the combination of high labile carbon content and complex biological treatments (e.g., composting, anaerobic digestion) can prolong the mineralization phase, potentially increasing short-term instability but contributing to longer-term stabilization. Although incomplete mineralization curves introduce uncertainty in the estimation of C_0 and RC for a limited number of FRMs, sensitivity analyses showed that excluding these materials had minimal impact on Cate–Nelson cutoffs. This indicates that the proposed classification framework primarily captures intrinsic biochemical contrasts among FRMs rather than being driven by incubation duration constraints. While the incubation-based approach applied in this study provides a controlled, mechanistic framework for discriminating the mineralization and stabilization potential of fertilizing residual materials, the transferability of these thresholds to field conditions should be approached with caution. At the field scale, soil carbon dynamics are influenced by a broader range of interacting factors, including climatic variability, soil moisture and temperature fluctuations, and plant-driven processes such as rhizodeposition and root-mediated priming effects. These interactions may modulate microbial activity and alter the temporal expression of carbon mineralization and stabilization processes compared to laboratory conditions. Consequently, the biochemical thresholds identified here should be viewed as reference indicators of intrinsic amendment behavior rather than absolute predictors of field-scale carbon sequestration. Future validation under field conditions, integrating soil/plant/climate interactions, will be essential to fully assess the robustness and applicability of this diagnostic framework across contrasting agroecosystems.

Beyond the minority of samples (n = 8) for which stabilization or mineralization plateaus were not reached within the 91-day incubation period, the proposed threshold-based diagnostic approach demonstrated strong overall robustness. For these specific cases, primarily involving complex or slowly degrading materials, future research should consider extending the incubation window to fully capture carbon persistence potential and ensure accurate classification of stabilization potential.

A key outcome of this study lies in the identification of a first critical threshold at 32.8 g LIC 100 g^{-1}, which represents a pivotal boundary in the functional differentiation of FRMs. Amendments falling below this threshold exhibit a low lignin content and thus tend to be highly mineralizable. These materials are rapidly decomposed by soil microbial communities, promoting immediate CO_2 release and elevated oxygen demand in the rhizosphere conditions that may induce short-term aerobic stress (56, 75). Such behavior, while potentially beneficial for microbial activation, may compromise longer-term soil health and carbon retention.

Conversely, FRMs with lignin contents above the 32.8 g 100 g^{-1} threshold exhibited markedly greater carbon stabilization capacity, with residual carbon concentrations reaching up to 71.8 g RC 100 g^{-1}. This accumulation reflects a shift toward more recalcitrant organic matter fractions, indicating a clear transition from a mineralization-dominated process to one favoring carbon sequestration. These findings support the value of using lignin as a key indicator in carbon-based FRM classification frameworks.

This functional shift beyond the lignin threshold signifies a transition toward amendments with highly persistent carbon dynamics, reinforcing the diagnostic value of this indicator. Our findings are consistent with earlier studies by Andriulo (76) and Eklind and Kirchmann (58), who demonstrated that lignin content is a strong predictor of residual carbon accumulation in soils. Elevated lignin levels enhance organic matter's resistance to microbial degradation, thereby reducing short-term CO_2 release (77, 78).

Beyond its role in carbon persistence, lignin also contributes indirectly to atmospheric carbon retention by transforming into humic substances. Several studies have shown that the microbial oxidation of lignin leads to the formation of humic acids (HA), which possess a notable capacity to capture and retain atmospheric CO_2. For instance, Carpanez et al. (79) and Spietz et al. (80) reported that humic acids can absorb up to 10.9 g of CO_2 per kg of material, highlighting the dual role of lignin-rich FRMs in both enhancing soil carbon retention and mitigating short-term carbon emissions.

When FRMs were classified based on the mineralization rate constant *k*, a second critical threshold of lignin content emerged, slightly lower at 27.3 g LIC 100 g^{-1}. This value, while close to the previously identified threshold of 32.8 g LIC 100 g^{-1} derived from residual carbon accumulation, reflects the fact that carbon mineralization and stabilization are related but distinct processes. The first threshold (32.8) represents the point at which carbon begins to accumulate as persistent residual matter, whereas the second (27.3) marks the point beyond which carbon mineralization becomes substantially slowed.

This distinction may be interpreted as a functional transition zone: FRMs with lignin contents between 27.3 and 32.8% represent intermediate materials that do not mineralize rapidly but have not yet reached the full persistence associated with high residual carbon accumulation. Below this range, carbon turnover is more rapid ($k >$ 0.067 day^{-1}), as confirmed in earlier studies (81). Above it, organic matter becomes increasingly recalcitrant and resistant to microbial degradation. Numerous studies have consistently reported that lignin concentrations between 20 and 30% constitute a critical range beyond which microbial accessibility to organic substrates declines markedly (61, 82, 83).

Therefore, lignin content stands out as a robust predictor of both carbon mineralization rate and persistence in soil. For instance, Ribeiro et al. (57) demonstrated that lignin-rich amendments like compost lead to significantly lower CO_2 losses (< 159 g kg^{-1} of applied C) compared to poultry manure, where losses may exceed 648 g kg^{-1} due to the abundance of labile compounds.

Comparable diagnostic performance was achieved with two other integrative biochemical indicators, the Biological Stability Index (BSI) and the $\frac{LIC}{SOL+HEM+CEL}$ ratio. Both indicators exhibited the same discriminative capacity as lignin content (LIC) in distinguishing carbon-mineralizing from carbon-stabilizing FRMs. They also led to identical critical thresholds for residual carbon (71.8 g RC 100 g^{-1}) and for the mineralization constant (k = 0.067 day^{-1}), reinforcing the robustness of this dual diagnostic framework. These findings align with earlier studies by Morvan et al. (84) and Marcato et al. (64), which underscore the value of biochemical composition in assessing organic matter stability and predicting its decomposition dynamics. This convergence of results across three independent indicators suggests that FRMs exhibit a consistent functional response once a biochemical stability threshold is surpassed, regardless of the specific metric used. It highlights a universal behavior of organic amendments: beyond a certain compositional threshold, the organic matter behaves more conservatively, contributing less to immediate microbial respiration and more carbon persistence potential.

While all three indicators offer comparable diagnostic power, LIC stands out for its conceptual clarity, analytical simplicity, and suitability for rapid, cost-effective assessment using spectroscopy-based methods (85). This makes lignin content a highly accessible and scalable tool for routine classification and recommendation of carbon-stabilizing organic amendments in soil management strategies.

From a management perspective, translating biochemical thresholds into practical guidance allows fertilizing residual materials to be aligned with specific soil management objectives. FRMs characterized by lignin contents, BSI values, or LIC/(SOL+HEM+CEL) ratios below the identified critical thresholds exhibit high mineralization rates and low short-term residual carbon, making them more suitable for situations where rapid nutrient release and short-term fertility enhancement are desired. In contrast, FRMs exceeding these thresholds exhibit lower mineralization rates and higher short-term residual carbon, indicating greater stabilization potential and making them more appropriate for strategies aimed at building soil organic carbon persistence, improving soil structure, and enhancing resilience to degradation processes. Materials falling within intermediate threshold ranges may serve dual functions, providing moderate nutrient availability while contributing to carbon persistence. This threshold-based classification framework therefore offers a flexible decision-support tool that can be adapted to diverse agronomic contexts depending on management priorities.

5 Conclusion

This study demonstrated that the biochemical characterization of fertilizing residual materials (FRMs), including manures, is a relevant and effective approach for predicting their behavior in soil carbon dynamics. By applying the Cate-Nelson partitioning method to eight biochemical indicators, we identified three reliable indicators capable of distinguishing between carbon-mineralizing and carbon-stabilizing FRMs: lignin content (LIC), the Biological Stability Index (BSI), and the $\frac{LIC}{SOL+HEM+CEL}$ ratio. Among these, LIC stands out for its simplicity as it is based on a single recalcitrant fraction, whereas BSI and the composite ratio offer a more integrative perspective on organic matter stability. All three indicators revealed consistent critical intervals that define a transition from mineralization-dominated to stabilization-dominated carbon behavior. These intervals ranged from 27.3 to 32.8 g LIC 100 g^{-1}, 0.77 to 0.99 g BSI g^{-1}, and 0.38 to 0.49 for the $\frac{LIC}{SOL+HEM+CEL}$ ratio. Above these intervals, FRMs were associated with high residual carbon contents (up to 71.8 g RC 100 g^{-1}) and lower mineralization rates (as low as 0.067 day^{-1}), reflecting more stable carbon forms.

These findings support the development of a robust diagnostic and recommendation system to identify FRMs with greater potential for carbon persistence, soil health preservation, and climate change mitigation. Field validation under real-world conditions is now needed to confirm the operational applicability of these indicators in sustainable agricultural and environmental management.

Data availability statement

The dataset is not publicly available due to institutional or legal restrictions. Requests to access the datasets should be directed to lotfi.khiari@fsaa.ulaval.ca.

Author contributions

SR: Writing – original draft, Methodology, Investigation, Conceptualization, Software. LK: Visualization, Project administration, Validation, Data curation, Resources, Funding acquisition, Supervision, Methodology, Conceptualization, Software, Investigation, Writing – review & editing, Formal analysis. HB: Writing – review & editing, Methodology. MA: Methodology, Writing – review & editing. SM: Writing – review & editing, Validation, Supervision, Methodology. RB: Writing – review & editing, Project administration. CA: Writing – review & editing, Supervision.

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Conflict of interest

The author(s) declared that this work 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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