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Effect of hydroponic cultivation using MPM-processed methane fermentation digestate on tomato growth and yield

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Introduction: Liquid digestate produced from manure-based methane fermentation contains organic and inorganic forms of nitrogen, phosphorus, potassium, and other micronutrients that have potential as plant nutrients, but its use in soilless horticulture requires appropriate microbial processing due to high ammonium concentrations and possible salinity-related constraints. Ammonium (NH_4^+), the predominant nitrogen form in methane fermentation digestate, must be converted to nitrate (NO_3^-) by nitrifying microorganisms before application in hydroponic systems. The multiple parallel mineralization (MPM) method enables controlled microbial nitrification of digestate nitrogen.

Methods: In this study, MPM-processed methane fermentation digestate (MPM digestate) generated nitrate through microbial nitrification, maintaining an average nitrate–nitrogen concentration of approximately 226 mg L^{-1} in the hydroponic nutrient solution throughout the cultivation period. Yield components of the tomato cultivar ‘Momotaro Hope’ grown in an open hydroponic system were compared between MPM-digestate and chemical fertilizer (CF) treatments for up to 124 days after transplanting (DAT), corresponding to the harvest of the seventh fruit truss.

Results: While total season yield and fruit quality parameters, including total soluble solids (TSS), lycopene, and γ -aminobutyric acid (GABA), did not differ significantly between treatments, plant growth responses varied temporally. The cultivation period was divided into three growth stages (Stages I–III) based on key growth indicators. During Stage III (88–124 DAT), the MPM-digestate treatment exhibited significantly lower total dry matter (TDM), light use efficiency (LUE), and stage-specific cumulative yield than the CF treatment, although the proportion of dry matter allocated to fruit was 18% higher under MPM digestate during this stage.

Discussion: These late-stage reductions may be associated with elevated electrical conductivity (EC) in the drainage solution observed during Stage II (34–87 DAT) under the MPM-digestate treatment. Increased EC values approaching 5 mS cm^{-1} , likely influenced by elevated Na^+ and Cl^- concentrations, may have induced osmotic stress or ion-specific nutrient imbalances that affected subsequent plant performance.

Conclusion: MPM-processed methane fermentation digestate can be utilized in hydroponic tomato cultivation when drainage EC is carefully managed, achieving fruit quality comparable to conventional fertilization, although late-stage biomass accumulation and yield may be reduced under elevated salinity conditions.

KEYWORDS

cow manure, electric conductance, hydroponic cultivation, leaf area index, methane fermentation digestate, multiple parallel mineralization, organic fertilizer, tomato

1 Introduction

The increasing global demand for food, together with mounting environmental challenges associated with conventional agriculture, necessitates the development of sustainable and resource-efficient food production systems. Hydroponics, a soilless cultivation technique, offers several advantages over soil-based agriculture, including efficient water and nutrient use, increased yield per unit area, and reduced reliance on herbicides (Khatri et al., 2024). By supplying nutrients directly to the root zone through aqueous solutions, hydroponic systems allow precise control of plant nutrition. The incorporation of organic nutrient sources into hydroponic cultivation has therefore attracted increasing attention as a potential strategy to improve the sustainability of agricultural production, while contributing to economic and environmental objectives (Chowdhury et al., 2024).

In Japan, livestock farming generates substantial quantities of manure, which can pose significant environmental risks if not properly managed. These risks include nutrient runoff, greenhouse gas emissions, air pollution, pathogen contamination, and, in rare cases, heavy metal accumulation when feed or bedding materials are contaminated (Ijaz et al., 2025; Kılıç and Sönmez, 2024; Czatkowska et al., 2025). Approximately 80 million t of livestock manure are produced annually in Japan (Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan 2018; Sasaki et al., 2024), corresponding to about 0.65 t per capita or approximately 17 t per hectare of arable land per year, based on a population of 123.8 million and 4.66 million hectares of arable land (Statistics Bureau of Japan, 2024). Dairy cows, beef cattle, and pigs each account for approximately 30% of total manure production nationwide (Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan 2018). Although manure generation per capita and per hectare is lower than that in the Netherlands (4.12 t per capita and 40.88 t per hectare), the absolute volume of manure in Japan remains substantial, and its associated environmental risks are considerable (Osada et al., 2019).

Anaerobic digestion (AD) of organic waste, which refers to the methane fermentation of liquid waste from livestock, is widely adopted as a renewable energy technology that produces biogas and a nutrient-rich digestate as a by-product (Mata-Alvarez et al., 2014). Digestate contains organic and inorganic nutrient components that

may be transformed into plant-available forms through subsequent processing. In alignment with Japan's Green Food System Strategy (MIDORI), national targets have been established to reduce the use of chemical fertilizers (CFs) derived from imported raw materials and fossil fuels by 20% by 2030 and 30% by 2050, with a reduction of 6% already achieved by 2021 (Ministry of Environment (MOE) Japan, 2024). These policy initiatives emphasize the substitution of CFs with organic nutrient sources and the development of integrated nutrient management approaches, particularly in high-input protected cultivation systems such as hydroponics. Accordingly, the development of technologies enabling the safe and effective use of livestock-derived digestate in hydroponic systems is directly relevant to national sustainability objectives.

Anaerobic digestion of livestock manure offers two principal benefits: renewable energy production in the form of biogas and the generation of digestate that may serve as a fertilizer resource. Cow manure, a major feedstock for methane fermentation in Japan, yields digestate containing essential macronutrients such as nitrogen (N), phosphorus (P), and potassium (K), as well as trace (Alfa et al., 2014). However, digestate is a chemically and physically complex material that may contain suspended solids, organic acids, pathogens, variable micronutrients, and unstable nitrogen forms, which limit its direct application in hydroponic systems (MartínSanzGarrido et al., 2025). In particular, nitrogen in digestate is predominantly present as ammonium (NH_4^+), which can induce phytotoxic effects when supplied at high concentrations in hydroponic nutrient solutions (Savvas, 2003; Ehret et al., 2005). Consequently, microbial preprocessing to convert NH_4^+ into nitrate (NO_3^-) is required prior to the use of methane fermentation digestate as a hydroponic fertilizer (Shinohara et al., 2011).

The multiple parallel mineralization (MPM) method, originally proposed by Shinohara et al. (2011), is a biological treatment approach designed to promote controlled nitrification of ammonium-rich organic liquid fertilizers prior to hydroponic application. In the case of methane fermentation digestate, ammonification occurs during the AD, which converts organic nitrogen to NH_4^+ (Felton et al., 2014). Subsequent treatment in MPM reactors, operated under aerated conditions, facilitates microbial nitrification, converting NH_4^+ into NO_3^- before the nutrient solution is supplied to hydroponic systems. Thus, the

MPM method functions as a preprocessing step that stabilizes nitrogen form and reduces the risk of ammonium-related phytotoxicity, while dissolved oxygen levels are maintained through system aeration rather than by the method itself.

Tomato (*Solanum lycopersicum*) is one of the most economically important fruit vegetables globally and is valued for its nutritional content and versatility in culinary applications (Kumar et al., 2022). In particular, tomato is widely cultivated in hydroponics, which is frequently grown throughout the year, enabling growers to achieve high yields and consistent quality under controlled environments, regardless of soil conditions or seasonal limitations (Rajaseger et al., 2023). In Japan, tomato production reached 662.6 kt in 2024, representing the highest production volume among fruit vegetables (Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan 2025). Tomatoes are also frequently used as a model crop in hydroponic research because of their sensitivity to nutrient composition and salinity, as well as their importance in commercial greenhouse production (Ezura, 2009). These characteristics make the tomato an appropriate crop for evaluating the performance and limitations of digestate-derived nutrient solutions in hydroponic systems.

Kechasov et al. (2021) reported that hydroponic tomato plants grown using the organic pig manure digestion-based fertilizer exhibited significantly lower fruit quality than those cultivated with CFs containing high mineral concentrations; however, the use of the organic fertilizer treatment increased fruit sizes. Nevertheless, research on the application of cow manure methane fermentation digestate for similar purposes is scarce. Given the widespread availability of cow manure in livestock-intensive regions, its effective valorization represents an important opportunity for sustainable agriculture. Cow manure digestate differs from pig manure digestate in its higher fibrous content and potential impurities, which can adversely affect nutrient stability and system performance without appropriate processing (Risberg et al., 2016). Therefore, further investigation is required to evaluate treatment strategies that enable the controlled nitrification of cow manure digestate prior to its use in soilless cultivation systems.

In this study, we aimed to evaluate the practical application of MPM-processed methane fermentation digestate derived from cow manure in hydroponic tomato cultivation. Specifically, tomato growth and yield components were assessed and compared with those obtained under conventional CF management. This study sought to explore the potential of MPM-processed digestate as a conditional or partial alternative to synthetic fertilizers, while identifying limitations associated with nutrient balance and salinity in hydroponic systems.

2 Materials and methods

2.1 Plant materials and seedling preparation

This study used the Japanese tomato cultivar 'Momotaro Hope' (Takii Seeds Co., Ltd., Kyoto, Japan). The seeds were sown in seed

trays filled with nursery soil and germinated in the dark at 28°C for 3 days. They were then transferred to a seedling growth chamber with a fluorescent light (Nae Terrace; Mitsubishi Chemical Agri Dream Co., Ltd., Tokyo, Japan) with a light period of 14 h per day, air temperature settings of 23°C (daytime) and 16°C (nighttime), and 1000 $\mu\text{mol mol}^{-1}$ CO₂. Seedlings were irrigated once every 2 days using a chemical nutrient solution (High-Tempo; Sumitomo Chemicals, Tokyo, Japan); the solution comprised 663.5 mg L⁻¹ NO₃⁻, 246.3 mg L⁻¹ K⁺, 432.8 mg L⁻¹ Ca²⁺, 92.4 mg L⁻¹ Mg²⁺, 698.3 mg L⁻¹ H₂PO₄⁻, 212.2 mg L⁻¹ Fe, 0.38 mg L⁻¹ Mn, 0.26 mg L⁻¹ B, 0.15 mg L⁻¹ Zn, 0.05 mg L⁻¹ Cu, and 0.07 mg L⁻¹ Mo, adjusted to an EC of 1.5 dS m⁻¹.

2.2 Growth conditions in the greenhouse

The experiment was conducted from August 2024 to January 2025 in a greenhouse 21 × 9 × 6 m (L × W × H, north-south oriented) with an environmental control system facility at the National Agriculture and Food Research Organization, Tsukuba, Ibaraki Prefecture, Japan (36°1' N, 140°6' E). After 3 weeks in the growth chamber, the seedlings were transplanted into a 7.5 × 7.5 × 7.5 cm cube of rockwool (The Grodan Delta NG 2.0 block; Grodan, Roermond, Netherlands), then moved to the greenhouse. After 2 weeks, the seedlings were transplanted into 100 × 15 × 7.5 cm (L × W × H) rockwool slabs (Grodan Vital NG 2.0; Grodan, Roermond, Netherlands) in the greenhouse compartment, where plants were grown in an open hydroponic system with a plant density of 2.5 plants m⁻², and the CO₂ level was set at 400 ppm before initiating the treatments. Tomato plants were pinched at three leaves above the 7th truss; old leaves were removed periodically. Flowers were pollinated using 4-chlorophenoxyacetic acid (Ishihara Tomato tone, Ishihara Biosciences, Ltd., Tokyo, Japan) and gibberellin (Gibberellin Meiji, Meiji Seika Pharma Co., Ltd., Tokyo, Japan), which were sprayed after four flowers bloomed in each truss according to the manufacturer's protocol. The number of fruits per truss was adjusted by pruning four fruits per truss. The cultivation period lasted for approximately 124 days after transplanting.

2.3 Treatments

The treatments included a CF nutrient solution formulated using Otsuka SA (Otsuka Agrio, Tokyo, Japan) and an organic nutrient solution produced from methane fermentation digestate processed using the MPM method. The methane fermentation digestate was obtained from a biogas facility that treats a mixture of cow manure and urine in Miyazaki, Japan. Additionally, OAT Formula No. 5 (Otsuka Agrio) was added to the MPM-digestate nutrient solution for supplementation of micronutrients, including manganese (Mn), boron (B), iron (Fe), copper (Cu), zinc (Zn), and molybdenum (Mo). The required amount of OAT Formula No. 5 was calculated based on the initial Fe concentration of the MPM-digestate nutrient solution so that the Fe level matched that of the CF treatment. This calculation accounted for the daily production

volume of the MPM-digestate nutrient solution (150 L day^{-1}), resulting in an estimated application rate of approximately 2 g day^{-1} of OAT Formula No. 5.

Methane fermentation digestate was mineralized into inorganic nutrients, such as nitrate, using the MPM method described by Shinohara et al. (2011). A 1% (v/v) methane fermentation digestate solution, supplemented with 0.5% (w/v) bark compost (Sanyo Bark; Sanyou Chip Kogyo, Yamaguchi, Japan) and 0.5% (v/v) culture solution, was added to a 2-t reactor tank as a microbial inoculum. The mixture was continuously aerated using an air pump (AP 120N; Yasunaga Air Pump Inc., Tokyo, Japan) until ammonium nitrogen was fully converted to nitrate. This process was continued while gradually adding methane fermentation digestate until the final concentration reached 10%. The solution was aerated for approximately two months, during which dissolved oxygen was maintained at $\geq 6 \text{ mg L}^{-1}$ and the temperature was controlled at approximately $32 \text{ }^\circ\text{C}$ to ensure effective nitrification of ammonium nitrogen. After nitrification was completed, the culture solution was transferred to a storage tank and used as the nutrient solution for direct irrigation. Adjustment of the water-to-digestate ratio required approximately an additional month to achieve the target nitrate–nitrogen concentration. Consequently, the total processing period prior to use was approximately three months. The nitrate–nitrogen concentrations in both the MPM-digestate and CF treatments were standardized to approximately 226 mg L^{-1} . The initial ionic compositions of the raw digestate, MPM-processed digestate, and CF nutrient solutions are presented in Table 1.

The greenhouse contained five rows, each consisting of 14 rockwool slabs arranged over a length of 14 m, with 0.8 m spacing between rows. The first and fifth rows were designated as guard rows, and in the second, third, and fourth rows, the front and back slabs were also used as guard plants. Treatments were arranged

in a randomized complete block design (RCBD) with three blocks. Within each block, plants were assigned alternately to the MPM-digestate and CF treatments. Each treatment comprised eight plants per block, resulting in 24 plants per treatment across the three blocks. For destructive sampling, four plants per treatment were randomly selected at each sampling point (0, 33, and 87 days after transplanting [DAT]) to measure leaf area and dry weight of individual organs (stem, leaf, and fruit). At the final harvest (124 DAT), nine plants per treatment were destructively sampled for leaf area index (LAI) and total dry matter (TDM) measurements. The remaining nine plants per treatment were used for monitoring cumulative yield (CY) throughout the cultivation period.

2.4 Data collection

This study was divided into three stages of cultivation based on the three destructive measurements performed. Stage I covered the early period of cultivation until before the harvest period (0–33 DAT). Stage II was the harvest period from the first to the third fruit truss (34–87 DAT), while Stage III covered the harvest period of the fourth to the seventh fruit truss (88–124 DAT), marking the end of the cultivation period. The yield components (LAI, LUE, cumulative IL, TDM, total dry fruit yield, number of fruits, dry matter (DM) distribution to fruit, and fruit DM) in tomatoes were analyzed at each stage and compared between treatments based on the hierarchy of yield components, growth, and fruit characteristics of tomato (Heuvelink and Dorais, 2005; Higashide and Heuvelink, 2009; Higashide, 2022).

Leaf area was measured to calculate LAI using an LI-3100C leaf area meter (Li-Cor Inc., Lincoln, NE, USA). Dry weight was determined by oven-drying (JMB-28DPN-S, Kato Co., Ltd.,

TABLE 1 Electric conductance and ion compositions of raw methane digestate and irrigation solution on day 0 after transplanting.

Composition	Unit	Chemical fertilizer	MPM-digestate	Raw methane digestate	Recommended range for hydroponic tomato
EC	mS cm^{-1}	1.33	1.5	20.9	
NO_3^- -N	mg L^{-1}	110	110	<2.2	38-100 (Kageyama, 1991)
NH_4^+ -N		0.8	<0.8	1900	$\leq 10\%$ Total-N (Akl et al., 2003)
K^+		170	260	3900	200-400 (Sonneveld and Voogt, 2009)
Na^+		18	100	1200	< 50 (Savvas and Gruda, 2018)
Cl^-		14	100	1400	< 100 (Savvas and Gruda, 2018)
Ca^{2+}		120	44	120	150-200 (Sonneveld and Voogt, 2009)
Mg^{2+}		25	12	25	40-60 (Sonneveld and Voogt, 2009)
Fe		1.1	1.0	16	
Mn		0.37	0.20	0.71	
Mo		<0.05	<0.05	0.13	
Zn		0.07	0.31	5.8	
Cu		<0.05	0.07	0.84	
B		0.30	0.27	1.7	

Fujimi, Saitama, Japan) samples at 100.5 °C for approximately 72 h until a constant weight was achieved. In this study, the total aboveground dry matter (TDM) of each plant was calculated as the sum of the dry weights of the stem, leaf, and fruit. The light extinction coefficient (k) was obtained in separate experiments conducted in 2021 in the same greenhouse as described by Higashide et al. (2017). The cumulative interception of photosynthetically active radiation (cumulative IL) by individual plants at each growth stage was also assessed based on the methods described by Higashide et al. (2012, Higashide et al., 2015). In this study, the photosynthetically active radiation (PAR) fraction was assumed to represent 50% of the global radiation, as reported previously (Ohtani, 1997). LUE was calculated as the slope of the linear regression between TDM accumulation and cumulative intercepted PAR at each respective stage. The greenhouse environment and the integration of environmental data were managed using a ubiquitous environmental control system (Yasuba et al., 2012).

2.5 Nutrient solution analyses

To monitor the decomposition process and track changes in the drainage fluid during cultivation, the daily concentrations of ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) ions, as well as the pH and electrical conductivity (EC) of the irrigation and drainage solution from both treatments, were measured three times per week with three replications. The inorganic nitrogen concentrations were measured using an RQ-Flex Plus Analyzer (Merck, Frankfurt, Germany), pH levels were determined using a pH meter (HM-42X; TOA DKK Co., Ltd., Tokyo, Japan), and EC was measured with a conductivity meter (DS-72; HORIBA, Ltd., Kyoto, Japan).

In addition, to investigate the components of the raw materials, irrigation, and drainage solutions, (1) raw material of methane fermentation digestate, (2) irrigation solutions on 0 DAT for both MPM-digestate and CF treatments, and (3) drainage solutions on 84 DAT, when the drainage EC of the MPM-digestate treatment peaked were collected, and ion analysis was performed. The following analytical methods were used to assess ion concentrations in the irrigation and drainage solutions: Ion chromatography (Dionex Integriion; Thermo Fisher Scientific, Waltham, MA, USA) was used to measure concentrations of NH_4^+ -N, NO_3^- -N, Cl^- , SO_4^{2-} -S, and PO_4^{3-} -P. Additionally, concentrations of K, Na, Ca, Mg, Fe, Mn, Mo, Zn, Cu, and B were determined using ICP-OES (Agilent 5110; Agilent Technologies, Santa Clara, CA, USA).

2.6 Fruit quality analysis

Fruits were harvested at the orange to red stage. Twenty samples were harvested in each treatment across two periods (10 samples between 45 and 52 DAT and 10 samples between 73 and 80 DAT).

Tomato fruit samples were analyzed for various quality parameters, including individual fruit weight, TSS, lycopene, GABA, and citric acid, as these parameters are representative of key sensory attributes (sweetness and acidity), nutritional value, and functional compounds relevant to consumer health and market preference. Fruit weight and lycopene content were measured using a visible-near-infrared (VIS-NIR) spectrometer (K-SS900LC, Kubota, Osaka, Japan), following the method described by Ito (2014). For sample preparation, tomatoes were vertically sliced into eight pieces, and two diagonal segments were selected. These were combined with an equal volume of ultrapure water and heated in a microwave at 600 W until the internal temperature reached 80°C to deactivate endogenous enzymes. The mixture was then homogenized using a mixer (IFM-800DGM, Iwatani, Osaka, Japan) and centrifuged at $15,000 \times g$ for 5 min. The resulting supernatant was collected for subsequent analyses.

TSS was determined using a digital refractometer (PR-101 α , ATAGO, Tokyo, Japan) and expressed as %Brix. The citric acid concentration was quantified using a capillary electrophoresis (CE) system (Model 7100, Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector, as described by Horie (2009). The CE analysis used a 100-cm uncoated fused-silica capillary (91.5 cm effective length, 50 μm i.d.; GL Sciences, Tokyo, Japan). A mixture of 20 μL of the supernatant, 80 μL of ultrapure water, and 100 μL of 2 mg mL^{-1} fucose (Tokyo Chemical Industry, Tokyo, Japan) was prepared, resulting in a final fucose concentration of 1 mg mL^{-1} as an internal standard. The running buffer consisted of 20 mM 2,6-pyridinedicarboxylic acid and 0.5 mM hexadecyltrimethylammonium bromide (pH 12.1). Samples were injected at 50 mbar for 5 s, with separation performed at -30 kV. Detection was performed at 350 nm with a reference wavelength of 270 nm, and the capillary temperature was maintained at 25°C, following the analytical conditions reported by Wang et al. (2018). GABA was analyzed using a high-performance liquid chromatography (HPLC) system (Model 1260 Infinity, Agilent Technologies) with precolumn derivatization. The procedure followed the method described by Horie (2012), with the exception that 3-aminobutyric acid (Tokyo Chemical Industry) was used as the internal standard. For sample preparation, 50 μL of the supernatant was mixed with 440 μL of ultrapure water and 10 μL of 1.0 mg mL^{-1} 3-aminobutyric acid, producing a final concentration of 20 $\mu\text{g mL}^{-1}$. Amino acid derivatization using *o*-phthalaldehyde (OPA) was performed via the HPLC injector program. A mixture of 5 μL of 0.4 N borate buffer (pH 10.2), 1 μL of the sample, 0.5 μL of OPA reagent (Agilent Technologies), and 8 μL of 5 mM citrate buffer (pH 6.3) containing 2% phosphoric acid was prepared at room temperature and injected immediately.

2.7 Statistical analyses

Statistical analyses were performed using Microsoft Excel (Microsoft 365 MSO, version 2408). Differences between treatments were assessed using independent t-tests at a significance level of $p < 0.01$.

3 Results

Daily average environmental conditions during the cultivation period are shown in Figure 1. Daily average air temperature decreased slightly at the beginning of cultivation and remained relatively constant thereafter, with minimum, maximum, and mean temperatures of 13.7, 37.9, and 20.5°C, respectively. The average outside solar radiation during cultivation was 10 MJ m⁻² d⁻¹, and the mean daytime CO₂ concentration was 423.3 ppm.

3.1 Nitrification and nutrient solution management

Microbial processing of methane fermentation digestate using the MPM method maintained nitrate–nitrogen concentrations of approximately 226 mg L⁻¹ in the MPM-digestate nutrient solution, while the CF nutrient solution maintained concentrations of approximately 222 mg L⁻¹ throughout the cultivation period (Figure 2). Nitrite (NO₂⁻) and NH₄⁺ were below the detection

limits of the analytical methods used and were therefore not detected during cultivation (data not shown). The EC of the irrigation and drainage solutions is shown in Figure 3. Irrigation EC values for the MPM-digestate (MPM-In) and CF (CF-In) treatments were maintained at approximately 2.0 and 1.8 mS cm⁻¹, respectively, indicating comparable nitrate supply between treatments. In contrast, the drainage EC of the MPM-digestate treatment (MPM-Out) increased during Stage II, reaching a peak value of approximately 5.0 mS cm⁻¹. In contrast, the drainage EC of the CF treatment (CF-Out) remained at approximately 2.9 mS cm⁻¹ (Table 2). Following the observed increase in drainage EC under the MPM-digestate treatment, irrigation volumes were adjusted to increase the drainage rate to greater than 30%. As a result, drainage EC decreased after reaching its maximum value. These adjustments were applied daily until drainage EC values stabilized. Analysis of the initial nutrient solutions showed that the MPM-digestate solution contained higher concentrations of sodium (Na) and chloride (Cl), and lower concentrations of calcium (Ca) and magnesium (Mg), compared with the CF nutrient solution (Table 1).

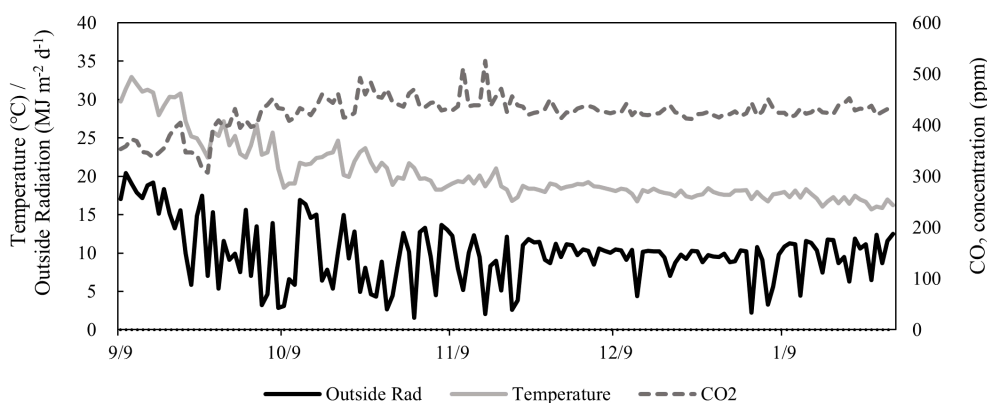


FIGURE 1

The daily average environmental data (temperature inside the greenhouse, outside radiation, and CO₂ concentration) during cultivation.

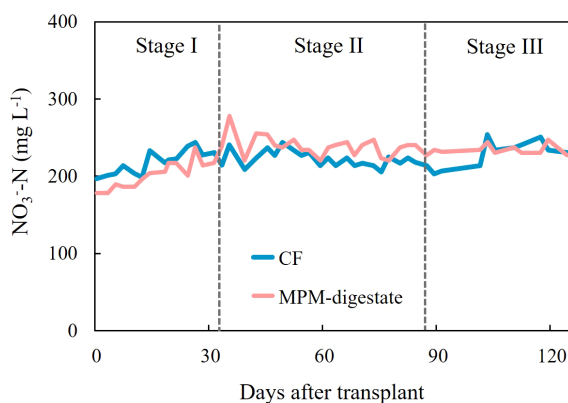


FIGURE 2

Daily nitrate-nitrogen (NO₃-N) ion concentrations in the MPM digestate and CF treatments supplied during tomato cultivation.

TABLE 2 Electric conductance and ion compositions of drainage solutions 84 days after transplant.

Composition	Unit	Chemical fertilizer	MPM-digestate
EC	mS cm ⁻¹	2.9	5.08
NO ₃ ⁻ -N	mg L ⁻¹	270	360
NH ₄ ⁺ -N		1.6	2.3
K ⁺		390	800
Na ⁺		57	460
Cl ⁻		29	510
Ca ²⁺		190	70
Mg ²⁺		66	22
Fe		1.9	2.6
Mn		0.28	<0.05
Mo		0.06	<0.05
Zn		<0.05	0.97
Cu		0.06	0.20
B		1.0	0.83

3.2 Aboveground plant biomass and fruit quality

Aboveground plant biomass and yield components were analyzed based on the hierarchical framework of tomato growth and yield formation (Heuvelink and Dorais, 2005; Higashide and Heuvelink, 2009; Higashide, 2022). Representative plant development during Stage II (46 DAT) is shown in Figure 4. The CY, DM distribution to fruits, LAI, cumulative IL, and LUE for each growth stage (Stages I–III) and for the entire cultivation period are summarized in Table 3. Total dry matter (TDM) accumulation by

stage and for the entire period is shown in Figures 5A, B. Fruit harvest began during Stage II (Table 3), while no marketable yield was obtained during Stage I. The dry weights of fruits present during Stage I were derived from immature fruits collected at destructive sampling. Total CY did not differ significantly between treatments over the entire cultivation period; however, the MPM-digestate treatment produced 9.5 kg m⁻² compared with 10.7 kg m⁻² under the CF treatment. Analysis of CY by growth stage revealed a significant difference only during Stage III, during which the MPM-digestate treatment resulted in lower yield (4.6 kg m⁻²) than the CF treatment (5.8 kg m⁻²).

The TDM of the total stages under MPM-digestate treatment was significantly lower than that under CF treatment, demonstrating an 18.2% reduction (Figure 5B). For each growth stage, a significant difference was observed only during Stage III (Figure 5A; $p < 0.01$), with values of 0.17 kg m⁻² under the MPM-digestate treatment and 0.35 kg m⁻² under the CF treatment. Figure 5A shows that, during Stage III, the stem and leaf DM of the MPM-digestate treatment were significantly lower than those of the CF treatment, while no significant difference was observed in fruit dry weight. Accordingly, the distribution of DM to the fruit in the MPM-digestate treatment during Stage III was higher than that observed in the CF treatment, at 45.6% and 27.5%, respectively. The trend was also observed for DM distribution to fruits for the total of all stages. The LAI of the total stages in the MPM-digestate treatments was significantly lower than that in the CF treatment, with values of 3 and 4.1 m² m⁻², respectively. For individual stages, LAI under the MPM-digestate treatment was significantly lower than that under the CF treatment during Stages I and III (Table 3). The cumulative IL was consistently lower under the MPM-digestate treatment than under the CF treatment across all stages, resulting in a significantly lower total cumulative IL under the MPM-digestate treatment. In addition, the LUE was significantly reduced under the MPM-digestate treatment in all stages. This difference was particularly notable during Stage III, where LUE values were 1.8 kg MJ⁻¹ under the MPM-digestate treatment and 3.4 kg MJ⁻¹ under

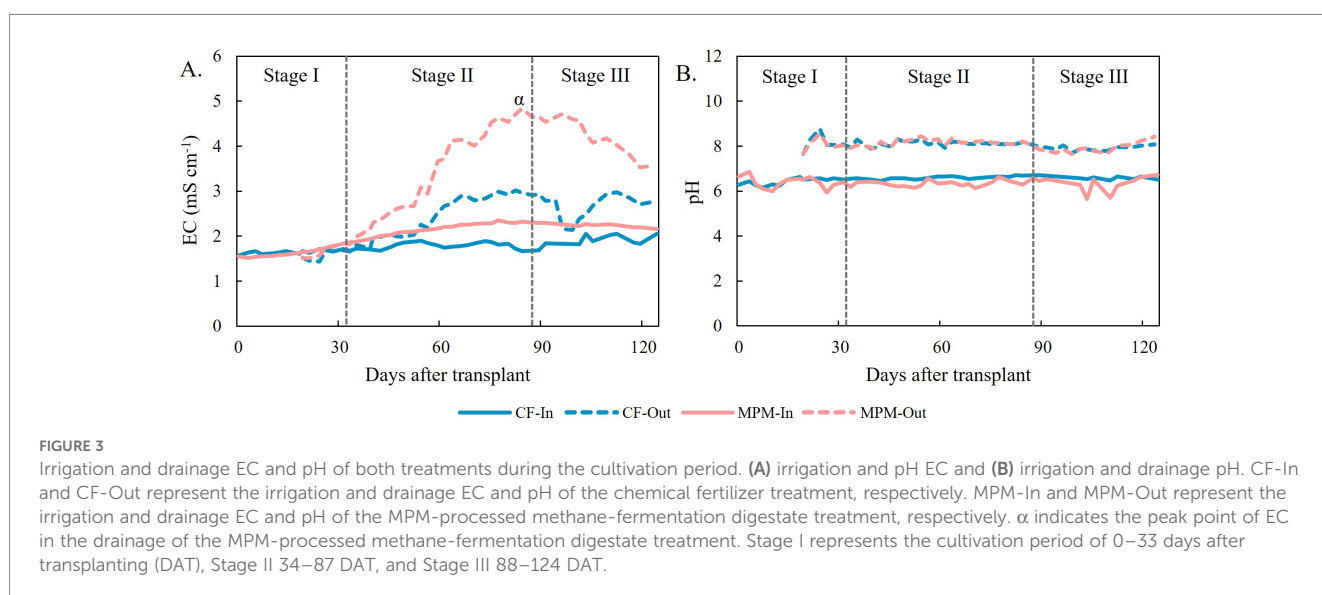


TABLE 3 Plant aboveground biomass under chemical (CF) and MPM-processed methane fermentation digestate (MPM-digestate) treatments at each stage.

Stage	Treatment	Cumulative yield (kg m ⁻²)	DM to fruit (%)	Leaf area index (m ² m ⁻²)	Cumulative intercepted light (MJ m ⁻²)	Light use efficiency (kg MJ ⁻¹)
Stage I	CF	0	38.7 ± 0.1	2.4 ± 0.1 a	56.1 ± 1.6 a	4.1 ± 0.5
	MPM-Digestate	0	44.6 ± 0.04	1.7 ± 0.02 b	48.7 ± 0.4 b	4.7 ± 0.4
Stage II	CF	4.9 ± 0.7	66.9 ± 1.4	0.8 ± 0.8	134.0 ± 4.4 a	4.6 ± 1.1
	MPM-Digestate	4.9 ± 0.8	67.3 ± 2.9	1.0 ± 0.5	126.3 ± 4.3 b	4.6 ± 0.3
Stage III	CF	5.8 ± 0.9 a	27.5 ± 12.2 b	0.9 ± 0.7 a	100.0 ± 1.4 a	3.4 ± 1.2 a
	MPM-Digestate	4.6 ± 1.0 b	45.6 ± 15.7 a	0.3 ± 0.5 b	96.2 ± 1.6 b	1.8 ± 0.8 b
Total	CF	10.7 ± 1.0	50.8 ± 0.02 b	4.1 ± 0.7 a	290.1 ± 1.4 a	4.2 ± 0.4 a
	MPM-Digestate	9.5 ± 0.6	57.5 ± 0.03 a	3.0 ± 0.5 b	271.2 ± 1.6 b	3.7 ± 0.3 b

the CF treatment. Therefore, the reductions observed in TDM, cumulative IL, and LUE under the MPM-digestate treatment corresponded with the significantly lower LAI values recorded during Stage III, which substantially influenced the total stage results.

Figure 6 shows the effect of MPM-digestate treatment on the growth and yield of tomato based on the hierarchy of yield components, growth, and fruit characteristics of tomato

(Higashide, 2022). The figure highlights the contribution of each component to the overall performance of the tomato plants for the combined stages under the MPM-digestate treatment. The fruit quality of both treatments was analyzed. Figure 7 shows fruit quality such as TSS, lycopene, GABA, and citric acid. Fruit trusses 1 and 3 were harvested during Stage II, and 5 and 7 were harvested during Stage III. Although most variables showed no statistically significant differences between treatments within each truss, the citric acid in truss 7 (Figure 7) was significantly higher under the MPM-digestate treatment with an average concentration of 551.3 mg 100 g FW⁻¹, compared to 435.1 mg 100 g FW⁻¹ under the CF treatment. An increasing trend was observed in TSS from Stages II to III in both treatments. On average, the TSS under the MPM-digestate treatment increased from 5.21 to 5.88% Brix, and under the CF treatment, it increased from 5.11 to 5.88% Brix. Conversely, a decreasing trend was observed in lycopene from Stage II to Stage III in both treatments. On average, the lycopene under the MPM-digestate treatment decreased from 7.27 to 5.93 mg 100 g FW⁻¹, whereas under the CF treatment it decreased from 7.41 to 5.99 mg 100 g FW⁻¹.

4 Discussion

Nitrogen availability plays a central role in regulating vegetative growth, chlorophyll formation, and yield in tomatoes (Ye et al., 2022). In hydroponic systems, nitrogen is predominantly supplied in nitrate form, whereas organic fertilizers often contain nitrogen primarily as ammonium, which can inhibit plant growth at elevated concentrations (Rachma et al., 2025). In the present study, methane fermentation digestate was processed using the MPM method, which converted ammonium-rich digestate into a nitrate-dominated nutrient solution suitable for hydroponic application. As shown in Table 1, Figure 2, raw digestate contained high NH₄⁺ concentrations with negligible NO₃⁻, whereas MPM processing substantially reduced NH₄⁺ levels and increased NO₃⁻-N

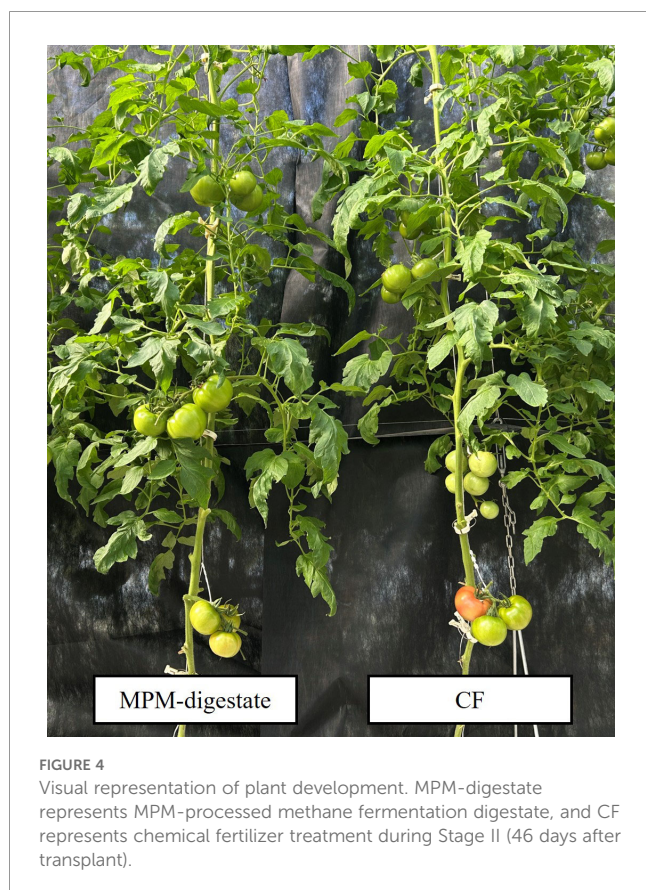


FIGURE 4 Visual representation of plant development. MPM-digestate represents MPM-processed methane fermentation digestate, and CF represents chemical fertilizer treatment during Stage II (46 days after transplant).

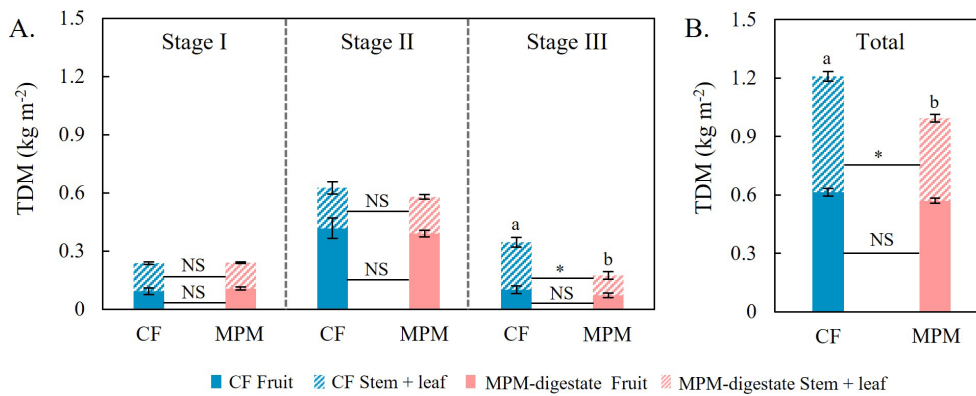


FIGURE 5 Distribution of dry matter to the stem + leaf and fruit. **(A)** Distribution of dry matter in each stage, and **(B)** distribution of dry matter in the total stage. Blue represents CF and pink represents MPM-digestate treatment. The striped area represents the dry matter of the stem + leaf. Letters (a) and (b) indicate significant differences between treatments at each stage; p -value < 0.01. Asterisks (*) indicate significant differences in dry matter of plant parts between the treatments with p -value < 0.001, and (NS) indicates no significant difference. Stage I represents the cultivation period 0–33 DAT, Stage II 34–87 DAT, and Stage III 88–124 DAT.

concentrations to values exceeding 100 mg L^{-1} , within the recommended range for tomato cultivation (Kageyama, 1991). These results indicate that the MPM method can convert methane fermentation digestate into a nitrate-containing nutrient solution appropriate for hydroponic use, although quantitative nitrification efficiency was not evaluated in this study.

Across the entire cultivation period, total CY and most fruit quality parameters did not differ significantly between the MPM-digestate and CF treatments. The yield of hydroponic tomatoes is a complex trait influenced by a hierarchy of physiological and morphological components, including light interception, biomass production, and dry matter partitioning (Higashide and Heuvelink, 2009). The effect of the MPM-digestate treatment on the growth and yield of tomato in the total stage was analyzed based on the hierarchy of yield components, growth, and fruit characteristics (Figure 5; Heuvelink and Dorais, 2005; Higashide and Heuvelink, 2009; Higashide, 2022). When assessed at the whole-season scale, comparable responses of cumulative yield, total fruit dry weight, and fruit dry matter content were observed between treatments (Figure 6). These associations do not imply causality, but they are consistent with the absence of significant differences in total CY.

A significant reduction in total stage TDM was observed due to the substantial decrease during Stage III (Figure 5). Both LUE and cumulative IL influenced TDM. As presented in Table 3, LUE was significantly reduced under the MPM-digestate treatment throughout the entire growth period, with a notable decline during Stage III. Cumulative IL was consistently lower under the MPM-digestate treatment throughout all the stages, particularly during Stage III, when light interception is critical in fruit expansion and ripening. Although LUE is determined by factors such as photosynthetic rate and the light extinction coefficient, these parameters were not directly measured in this study. As shown in Figure 3, the increased drainage EC was observed during Stage II under the MPM-digestate treatment.

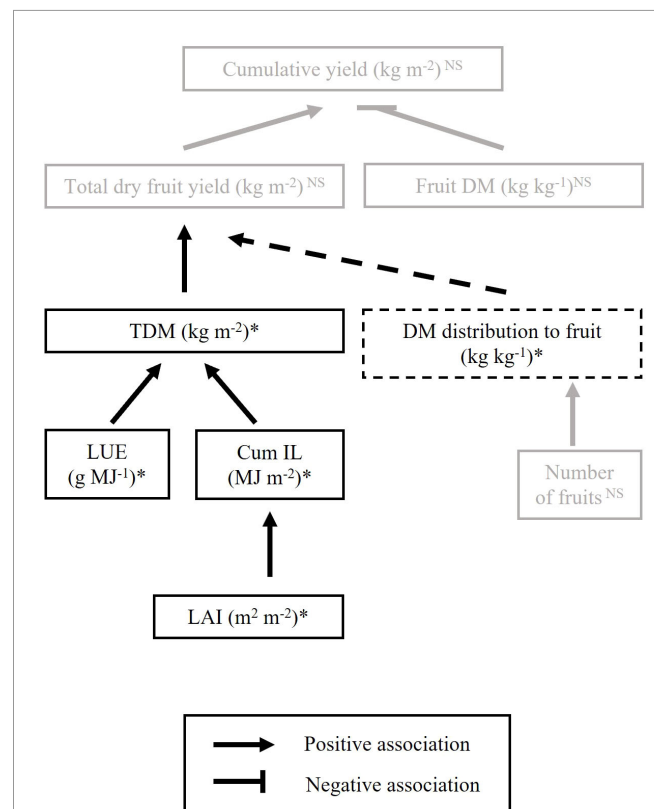


FIGURE 6 The hierarchy of yield components and related traits in tomato from the total stage of cultivation under chemical fertilizer and MPM-digestate treatments, based on Higashide (2022). The asterisks (*) represent significant differences between the treatments at p -value < 0.01, (NS), and gray lines represent no significant difference. Solid black arrows indicate significantly higher values in the CF treatment, while dashed black arrows indicate significantly higher values in the MPM-digestate treatment.

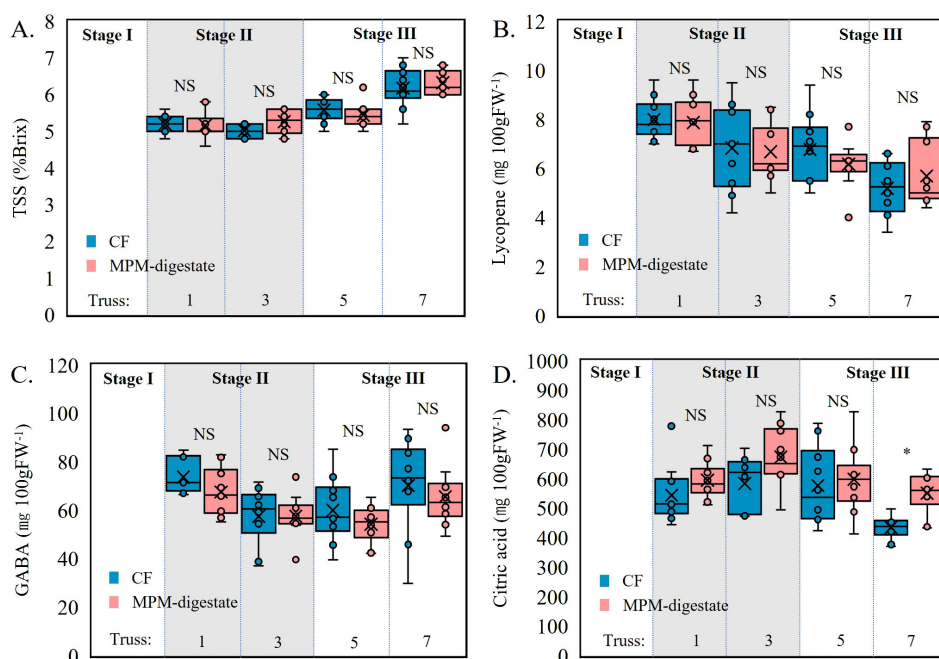


FIGURE 7

The quality of tomato fruits. (A) total soluble solids (TSS), (B) lycopene, (C) GABA, and (D) citric acid concentrations of trusses 1, 3, 5, and 7 of both treatments. Pink represents MPM-digestate treatment, and blue represents CF treatment. The asterisks (*) represent significant differences between the treatments at p -value < 0.01 , and (NS) indicates values are not significantly different between treatments in each truss. Stage I represents the cultivation period 0–33 days after transplanting (DAT), Stage II 34–87 DAT, and Stage III 88–124 DAT.

This peaked at approximately 5 mS cm^{-1} in the MPM-digestate treatment, exceeding that detected for CF treatment (2.9 mS cm^{-1}) and the commonly reported threshold for tomato growth inhibition ($\sim 4 \text{ mS cm}^{-1}$; Azarmi et al., 2010). Ion composition analysis indicated that this increase was primarily attributable to the accumulation of Na^+ and Cl^- , which were present at higher concentrations in the MPM-digestate treatment irrigation solution (Table 1), progressively increasing root-zone salinity. Tomatoes are known to suffer growth inhibition when EC exceeds 4 mS cm^{-1} (Azarmi et al., 2010). Beyond this threshold, osmotic and ionic stress impairs cell expansion. Stage III growth suppression appears to be a delayed consequence of salinity stress experienced during Stage II. Prolonged exposure to sodium chloride can impair root hydraulic conductivity and limit water uptake (Rodriguez et al., 1997), disturb ion homeostasis (Loudari et al., 2020), and reduce photosynthetic efficiency (Zhang et al., 2022). This stress may disrupt photosynthesis and consequently reduce LUE (Wu and Kubota, 2008). Schwarz and Kläring (2002) demonstrated that increased EC levels resulted in reductions in whole-plant photosynthesis and dry weight, primarily owing to a decrease in leaf area. These findings explained that the elevated drainage EC in Stage II leads to reduced growth and yield of tomato plants under MPM-digestate treatment in Stage III, illustrating both lag effects of salt accumulation and long-term physiological damage. Additionally, lower Ca^{2+} and Mg^{2+} concentrations in the MPM-digestate treatment (Table 1) may have exacerbated stress by impairing cell structure and photosynthetic function (Zhu et al., 2025; Soyama et al., 2025). However, because

root-zone EC, leaf ion concentrations, and photosynthetic rates were not directly measured, this interpretation remains inferential, warranting further studies for validation.

The light extinction coefficient and LAI determine the cumulative IL. In this study, LAI was significantly reduced under the MPM-digestate treatment during Stages I and III (Table 3), resulting in lower cumulative IL. Consistent with previous studies, reductions in LAI were closely associated with decreased biomass accumulation and yield formation (Higashide and Heuvelink, 2009). Although vegetative biomass was reduced during Stage III under the MPM-digestate treatment, fruit dry matter did not differ significantly from the CF treatment. Consequently, the proportion of dry matter allocated to fruits was approximately 18% higher. This increase reflects a relative shift caused by reduced stem and leaf biomass rather than enhanced fruit sink strength (Higashide, 2022), as fruit number was standardized through pruning and fruit dry weight remained unchanged. These findings indicate that stress imposed by elevated salinity disproportionately affected vegetative growth while allowing fruit biomass to be maintained.

Under salinity or nutrient stress, plants may alter assimilate partitioning through hormonal regulation, including abscisic acid-mediated responses, which can favor reproductive development under adverse conditions (Munns and Tester, 2008; Li et al., 2020). This prioritization of reproductive growth under stress has been documented in tomatoes and other crops, where salinity or water deficit triggers increased allocation of photo assimilates to fruits at the expense of vegetative tissues (Zhang et al., 2022). Similar

partitioning responses have also been reported under moderate stress or suboptimal nutrient availability (Heuvelink and Dorais, 2005; Itoh et al., 2020), in which plants maintain fruit development at the expense of stem and leaf growth. Consequently, the increased fruit DM distribution observed in the MPM-digestate treatment suggests that plants under mild salinity stress from Na^+ and Cl^- accumulation prioritized resource allocation to reproductive structures. Yield reductions during Stage III were likely driven by salinity-induced limitations in light interception and biomass production, as reflected in reduced LAI and vegetative growth.

The assessment of fruit quality parameters, including TSS, lycopene, GABA, and citric acid, revealed no significant differences between treatments in each truss except for citric acid in the seventh truss (Figure 7). This pattern aligns with previous findings (Ehret et al., 2005; Kechasov et al., 2021), which showed that fruit quality in tomatoes tends to remain stable under moderate environmental or nutritional variation, particularly when nitrate availability is sufficient. Furthermore, stage-specific harvesting at trusses 1, 3, 5, and 7 provided no evidence of treatment-induced quality deterioration during fruit development. Elevated EC, owing to increased salinity, when maintained within threshold levels, can enhance the TSS content of tomato fruits with minimal yield reduction (Heuvelink and Dorais, 2005; Itoh et al., 2020). In the present study, although drainage EC increased under the MPM-digestate treatment, the extent of this elevation was relatively mild and primarily reflected salt accumulation rather than severe salinity stress. In contrast, TSS did not increase significantly despite elevated EC, likely because drainage EC values did not reach the higher threshold range (8–12 mS cm^{-1}) typically required to induce sugar accumulation through osmotic adjustment (Itoh et al., 2020).

An increased citric acid concentration was observed in the seventh truss fruits under the MPM-digestate treatment. Organic acids, particularly citric and malic acids, are known to increase in tomato fruits in response to salinity-related stress (Zushi and Matsuzoe, 2005; Saito et al., 2008). This pattern suggests that the elevated drainage EC observed during Stage II under the MPM-digestate treatment may have contributed to enhanced organic acid accumulation at later fruit developmental stages. Such responses are consistent with salt-induced modulation of metabolic pathways associated with the tricarboxylic acid (TCA) cycle, as salt stress has been shown to alter the expression of multiple TCA-cycle-related enzymes during tomato fruit development (Yin et al., 2010). Nonetheless, while this mechanism provides a plausible explanation for the increased citric acid content, the specific biochemical or genetic basis cannot be confirmed in the present study, as molecular analyses were not conducted.

This study has several limitations that should be acknowledged. First, the MPM processing period required approximately three months to produce a usable nutrient solution, which may limit scalability for commercial production. Second, the study focused exclusively on the tomato cultivar ‘Momotaro Hope.’ Because tomato cultivars differ markedly in their tolerance to salinity and in their physiological and fruit-quality responses, the generalizability of the present findings should be interpreted with caution. Future research

that includes cultivars with diverse salt-stress sensitivities will be important to confirm the broader applicability of the conclusions. Third, the study did not include direct physiological measurements, such as continuous root-zone EC monitoring, leaf tissue ion analysis, or assessments of photosynthetic activity. The absence of these measurements limits the ability to elucidate the mechanistic pathways underlying the plant responses observed. Incorporating these physiological indicators in future experiments would provide deeper insights into how moderate salinity and nutrient solution type affect plant growth and fruit quality. In conclusion, MPM-processed methane fermentation digestate can be used as a nitrate-containing nutrient source for hydroponic tomato cultivation when salinity is carefully managed. Although late-stage biomass accumulation and yield were reduced under elevated EC conditions, overall fruit quality was maintained. For practical application, drainage EC should be maintained below approximately 4 mS cm^{-1} through management practices such as increasing irrigation volume to enhance leaching, periodic substrate flushing, and frequent EC monitoring. These measures are essential to minimize salinity stress and support stable production when using digestate-based nutrient solutions in hydroponic 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.

Author contributions

DR: Methodology, Writing – review & editing, Investigation, Writing – original draft, Supervision, Software, Resources, Validation, Data curation, Visualization, Conceptualization, Formal analysis. KM: Writing – review & editing, Supervision, Resources, Software, Conceptualization, Investigation, Formal analysis, Methodology, Validation, Visualization. YY: Formal analysis, Writing – review & editing. HU: Formal analysis, Writing – review & editing. KY: Formal analysis, Writing – review & editing. MS: Methodology, Writing – review & editing, Resources. MI: Resources, Supervision, Writing – review & editing. D-HA: Supervision, Writing – review & editing, Conceptualization, Resources.

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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.

Correction note

A correction has been made to this article. Details can be found at: [10.3389/fhort.2026.1813573](https://doi.org/10.3389/fhort.2026.1813573).

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

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