Scientia Horticulturae 353 (2025) 114469

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Research Paper

Food waste-derived fertilizers as sustainable bio-based nutrient sources: Enhancing pak choi growth, nutrient concentrations, and physiological function

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ARTICLE INFO

Keywords:
Brassica rapa
Black soldier fly
Digestate
Frass
Microbiome
Photosynthesis

ABSTRACT

Managing post-consumer food waste requires strategies that enhance circularity within the food system. Anaerobic digestion of food waste produces digestate, a nutrient-rich byproduct, while black soldier fly (BSF) bioconversion generates frass, nutrient-rich larval excreta with potential agricultural applications. This study evaluated food-waste-derived solid digestate and frass as sustainable, bio-based nutrient sources for pak choi in a greenhouse pot trial using a soil–sand mixture. Results demonstrated that sole application of solid digestate improved plant growth and physiological performance, whereas frass alone reduced growth performance, indicating potential phytotoxicity. Specifically, the foliar CO_2 assimilation rate in frass fertilized plants was 13 μmol CO_2 m^{-2} s^{-1} whereas plants receiving digestate or mineral fertilizer exhibited higher rates, ranging from 18 to 20 μmol CO_2 m^{-2} s^{-1}. Germination bioassays showed greater seedling vigour with digestate than with frass, suggesting differences in nutrient availability and toxicity. The poor growth associated with frass application was attributed to high Na, K, and NH_4^+ levels compared to digestate, causing possible ionic stress and nutrient imbalances. Illumina metabarcoding (16S/ITS) further revealed that digestate harboured richer bacterial and fungal diversity across phylum, family, and genus levels, likely supporting improved plant performance via biostimulatory activity. Importantly, blending 25 % digestate and frass (N) with 75 % mineral N fertilizer maximized biomass yield, demonstrating that up to 25 % of mineral N inputs can be substituted by these bio-based resources. These findings underscore that selectively integrating organic amendments with mineral fertilizers can recycle food-waste streams into effective nutrient sources while maintaining productivity, advancing sustainability, and circular bioeconomy.

1. Introduction

Food waste constitutes a paradox in the contemporary world, where roughly one-third of all food produced globally for human consumption is lost or wasted (Chen et al., 2020). This food waste leads to substantial loss of critical resources, including water, land, and energy, and contributes significantly to greenhouse gas emissions when discarded or mismanaged (Gatto and Chepeliev, 2024). The pursuit of more sustainable food waste management methods, focusing on the recovery of energy and nutrients, has thus been identified as a pivotal component of sustainable development (Moraes et al., 2021). Transforming this waste into valuable agricultural inputs can mitigate negative environmental impacts, offering a promising solution to this multifaceted problem.

Anaerobic digestion (AD) and the utilization of black soldier fly (BSF, *Hermetia illucens*) larvae as tools for organic waste management are two innovative approaches within the emerging realms of circular economy and sustainable production. Anaerobic digestion is a biological process wherein microorganisms break down organic matter in the absence of oxygen, producing biogas and a nutrient-rich by-product known as digestate (Ren et al., 2018). Digestate is characterized by its high concentration of plant nutrients, such as nitrogen (N), phosphorus (P), and potassium (K), making it a potential alternative to mineral fertilizers (Risberg et al., 2017). The solid fraction of digestate (dewatered digestate fraction), rich in organic matter and nutrients like nitrogen,

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https://doi.org/10.1016/j.scienta.2025.114469
Received 1 March 2025; Received in revised form 19 October 2025; Accepted 24 October 2025
Available online 31 October 2025

phosphorus, and potassium, serves as a slow-release amendment that enhances soil structure and long-term fertility (Egene et al., 2021; Jin et al., 2022; Zilio et al., 2022). Similarly, BSF larvae-based waste management has emerged as a promising technology over the past decade due to its remarkable efficiency in bio-converting organic waste streams into valuable resources (Siddiqui et al., 2024). This approach yields a protein-rich larval biomass suitable as a sustainable animal feed ingredient and produces an organic fertilizer, known as frass. Frass is enriched with organic matter, essential plant nutrients, beneficial microorganisms, and bioactive compounds that collectively enhance plant growth and soil health (Lopes et al., 2022; Green, 2023).

Global agriculture still remains heavily reliant on mineral fertilizers, particularly nitrogen (N) synthesized via the Haber-Bosch process. This energy-intensive process relies on fossil fuels, causing significant environmental impacts, while prolonged mineral fertilizer use depletes soil organic matter, reducing soil fertility and health (Erisman et al., 2008; Mulvaney et al., 2009; Huang et al., 2019; Tyagi et al., 2022). Similarly, phosphorus (P) is extracted from phosphate rock, a finite resource concentrated in specific global regions (Elser and Bennett, 2011). Conversely, organic fertilizers contribute to long-term improvements in soil organic matter, fertility, nutrient availability, and microbial activity (Wong et al., 2015; Abbott et al., 2018; Allam et al., 2022). Furthermore, beyond providing essential plant nutrients, organic amendments and soil fertilizers frequently contain bioactive compounds (e.g. biostimulants) that promote plant growth, enhance soil microbial diversity, and contribute to improved soil structure and health (Wong et al., 2020; Sani et al., 2025). For instance, Du et al. (2023) reported biostimulants in dissolved organic matter recovered from AD sludge through alkali-hydrothermal treatment with bioactivity for rice growth, which was attributed to the abundance of amino compounds and humic substances. Another study found auxin-like activities in hydrophobic dissolved organic matter fractions extracted from digestate, comparable to recognized biostimulants (Scaglia et al., 2016). Similarly, Green (2023) reported the presence of beneficial bacteria and specific plant biostimulants (e.g. abscisic acid, jasmonic acid and gibberellins) in BSF frass, highlighting its potential in providing benefits to plants' metabolism.

Several benefits can arise from applying organic fertilizers and fertilizers with bioactive substances to the soil-plant system, including improved nutrient and water use efficiency, enhanced stomatal regulation and hormonal regulation, and increased photosynthetic performance (Yong et al., 2014; Sani and Yong, 2022; Wong et al., 2015). The potential benefits of organic inputs arise from the action of microorganisms, including both bacteria and fungi, present in these materials (Wong et al., 2016; de Vries et al., 2020). For instance, Poveda et al. (2019) demonstrated that the biostimulatory activity of mealworm frass was significantly diminished when the frass was sterilized. Similarly, Arabzadeh et al. (2024) found that filtered (0.22 μm) BSF frass extracts, lacking microorganisms, were less effective in inhibiting plant pathogens compared to unfiltered extracts. Furthermore, Radzikowska-Kujawska et al. (2023) evaluated the growth and performance of basil (*Ocimum basilicum* L.) under normal and water-deficit conditions, finding that plants fertilized with BSF frass exhibited improved growth and photosynthetic performance compared to unfertilized plants and those fertilized with manure. Similarly, digestate solutions significantly increased growth and photosynthetic efficiency in bush beans by improving the plant's metabolism as a result of increased antioxidant capacity, total phenolic content, and ascorbic acid (Lee et al., 2021).

Despite the potential benefits, the practical application of digestate and BSF frass in agriculture faces several challenges. One significant challenge lies in the variability of the nutrient composition of these fertilizers, which can be influenced by factors such as the type of organic waste used as feedstock and the specific process parameters during digestion (Möller and Müller, 2012; Sani et al., 2025) and BSF larvae production (Lopes et al., 2022). Furthermore, process variability can influence the composition of these fertilizers and thereby affecting the consistency and predictability of their impact on crop growth, physiological responses, and soil health. While the individual contributions of food waste-derived digestate (Sharma et al., 2023) and insect frass (Tan et al., 2021) to agricultural production are well documented, their combined use remains largely underexplored. Combining organic fertilizers and fertilizers from distinct origins and compositions offers the potential for synergistic benefits in promoting sustainable and efficient agricultural practices, due to the complementary properties of these materials (Abbott et al., 2018; Liang et al., 2022; Sani and Yong, 2022). Thus, understanding the physicochemical, microbial, and bioactive properties of organic fertilizers such as digestate and BSF frass would be crucial for developing best practices that support their safe and effective use in agriculture (Geissdoerfer et al., 2017; Huang et al., 2019).

This study explored the potential synergies arising from the combined application of food waste-derived digestate and BSF frass, focusing on their complementary nutrient and microbial profiles. It was hypothesized that digestate and BSF frass could serve as effective organic fertilizers for the cultivation of a vegetable, pak choi (*Brassica rapa* ssp. *chinensis*), either alone or in combination. Additionally, we hypothesized that combining these organic sources could partially or fully replace mineral fertilizers without compromising yield or crop quality. Therefore, this study investigated the physicochemical, microbial, and bioactive properties of digestate and BSF frass derived from food waste and assessed their impact on plant growth, photosynthetic efficiency, and nutrient concentration in shoots of pak choi, with particular focus on their potential to partially or entirely replace mineral fertilizers.

2. Material and methods

2.1. Solid digestate, frass and soil: sources and physiochemical characterisation

Solid digestate derived from the anaerobic digestion of food waste was collected from the municipality of Eskilstuna, Sweden. The digestate was collected in buckets directly from the output of the solid and liquid fractionation of the Eskilstuna Biogas AB plant, transported to the laboratory and immediately frozen. Black soldier fly larvae frass was obtained from an experimental waste management facility located at the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden, where BSF larvae were reared using pre-consumer food waste as a feedstock. The details of the waste composition and frass production were described by Lopes et al. (2024). Briefly, 12,000 BSF larvae (5-day-old) were fed 11.5 kg of food waste during 12 days in a climate-controlled room, according to the methodologies described in Lopes et al. (2023). Then, the larvae were separated from frass by sieving and the frass was frozen (−20 °C) until use. The materials including soil, sand, digestate, and frass, were analyzed at LMI AB, Helsingborg, Sweden, to determine their baseline physio-chemical properties following European standard methods. These included measurements of pH, electrical conductivity (EC) using a 1:5 (v) substrate-to-water extraction (SS-EN 13,037:2011, SS-EN 13,038:2011), and laboratory-compacted bulk density (SS-EN 13,040). Additionally, the Comprehensive Analysis of Trace Elements (CAT) methodology (SS-EN 13,651) was used to assess key nutrients and trace elements, such as NH_4-N, NO_3-N, Al, B, Cd, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, Si, and Zn. Furthermore, total nitrogen (N) and carbon (C) were quantified using the Elemental Particle Analyzer (EPA) method. Samples were combusted at 900–1000 °C in the Flash 2000 Elemental Analyzer (Thermo Scientific), and the resulting gases were analyzed by gas chromatography with thermal conductivity detection. The result of these analyses is presented in Table 1. In addition, the soil used in pot trial validation was collected from the agroecological research station at Lönnstorp in southern Sweden, dried and sieved (2-mm mesh). The soil contained approximately 15 % clay and 3 % organic matter. Sand was obtained from Hasselfords garden (1–3 mm size, volumetric weight 1.5 kg L^{-1}, pH 7.5, and EC 5 mS/m). Soil and

Table 1
Physio-chemical characterization of digestate, frass, soil, and soil-sand mix (70:30) on weight basis. Nutrients are represented by their potentially available amounts as determined by CAT analysis, except for total N and C.

Component	Digestate	BSF frass	Soil	Soil-sand mix
pH	7.50	7.60	5.3	5.5
EC (mS cm^{-2})	1.1	12	0.30	0.25
Bulk density (kg L^{-1})	0.56	0.48	1.22	1.42
Total N (%)	4.48	3.89	0.12	0.12
NH_4—N (mg kg^{-1})	1107.1	4166.66	< 0.5	0.35
NO_3—N (mg kg^{-1})	0.61	< 0.5	131.1	91.55
Total C (%)	22.87	40.96	1.68	1.62
C/N ratio	5.10	10.52	13.02	13.50
P (mg kg^{-1})	67.90	1645.8	9.0	9.15
K (mg kg^{-1})	162.50	9791.65	28.7	23.94
Mg (mg kg^{-1})	128.6	375.00	58.2	48.59
S (mg kg^{-1})	1017.85	1958.33	13.9	9.86
Na (mg kg^{-1})	114.30	20,833.33	22.9	19.01
Al (mg kg^{-1})	4.46	8.33	81.9	70.42
B (mg kg^{-1})	0.89	4.17	0.40	0.35
Cu (mg kg^{-1})	5.36	3.12	0.81	1.06
Fe (mg kg^{-1})	803.57	< 0.5	60.65	54.93
Mn (mg kg^{-1})	3.60	3.10	21.31	18.31
Mo (mg kg^{-1})	1.79	1.04	0.40	0.35
Si (mg kg^{-1})	21.43	25.00	8.19	6.34
Zn (mg kg^{-1})	25.00	17.71	0.81	0.70

inert sand were mixed in a 70/30 ratio (v/v), resulting in a soil-sand mix (Table 1).

2.2. Microbial profiling of solid digestate and frass

In addition to physio-chemical characterization, both fertilizers were also analysed for their microbial abundance and composition. The bacterial and fungal communities existing in both fertilizers were determined by metabarcoding with the Illumina technology (Illumina Inc., San Diego, CA, United States). Three steps were followed for this analysis: DNA extraction of the samples, carried out with the Qiagen Power Soil extraction kit using negative controls (extraction without sample) for controlling the presence of contaminants; amplification by PCR for the regions V4 of 16S (bacteria) and ITS of ITS (fungi), using the protocol defined by the Earth Microbiome Project (Gilbert et al., 2014); and DNA sequencing of the 16S and ITS barcodes in Illumina MiSeq, which produced paired-end reads of 250 pair bases. Sequencing was performed on all five independent biological replicates per fertilizer. After sequencing the samples' DNA, bioinformatics analysis of the data was performed. For that purpose, the quality control of sequences was carried out, followed by the deduction of Amplicon-Sequence Variants (ASV) and their taxonomic classification using the R library 'dada2'. Following which, the ASVs were identified, and contaminants were removed with the aid of the R library 'decontam'. Finally, data were analysed statistically for alpha and beta diversity and abundance of distinct groups of microorganisms in different taxonomic levels.

2.3. Germination bioassay

Frass and digestate were evaluated in a seed germination bioassay for verifying potential phytotoxicity traits, according to the procedures described in Luo et al. (2018). The bioassay was conducted with seeds of pak choi and watercress (*Nasturtium officinale*); which are two of the most sensitive species for germination trials (Luo et al., 2018). Five grams of each amendment were dissolved into 50 mL of deionized water, shaken horizontally (250 rotations per minute, rpm) for 1 h, then centrifuged at 4500 rpm for 15 min and filtered through 0.45 µm Whatman® filter paper. Then, 5 mL of each extract was poured on top of two layers of filter paper placed inside 90-mm Petri dishes (n = 3), together with 15 seeds per dish, with a 10-mm spacing between seeds. Three Petri dishes containing only deionized water were used as a control. Seeds were kept inside a dark incubator set at 25 °C for 5 days. Seed germination (SG) was determined by enumerating the germinated seeds (Eq. (1)) and radicle length was determined for each seedling. The relative seed germination (RSG) (Eq. (2)), relative radicle growth (RRG) (Eq. (3)) and the germination index (GI) (Eq. (4)) were used to evaluate the phytotoxicity of the organic fertilizers.

$$SG\ (\%) = 100 \times \frac{number\ of\ germinated\ seeds}{number\ of\ total\ seeds} \quad \text{(Eq. 1)}$$

$$RSG\ (\%) = 100 \times \frac{number\ of\ germinated\ seeds\ in\ sample}{number\ of\ germinated\ seeds\ in\ control} \quad \text{(Eq. 2)}$$

$$RRG\ (\%) = 100 \times \frac{total\ radicle\ length\ of\ germinated\ seeds\ (sample)}{total\ radicle\ length\ of\ germinated\ seeds\ (control)} \quad \text{(Eq. 3)}$$

$$GI\ (\%) = RSG \times RRG \times 100 \quad \text{(Eq. 4)}$$

2.4. Greenhouse bioassay: site, conditions and treatments

The well-known vegetable, pak choi (*Brassica rapa* ssp. *chinensis*) was grown in the 3-L plastic pot with soil-sand mix (Table 1). Each pot was filled with 3 kg of the mix. The experiment was conducted inside a climate-controlled greenhouse of the Department of Biosystems and Technology, at the Swedish University of Agricultural Sciences (SLU, Alnarp, Sweden), during September and October 2023. Greenhouse temperatures were set to 20/18 °C (day/night), with ventilation at 22/20 °C. No humidity control was implemented during this period. The addition of artificial light was set to start at 6 h and be turned off at 22 h. The spectral range of the 400 W High-Pressure Sodium (HPS) lamp used in this study spans from 350 nm to 850 nm.

The experiment was conducted in a completely randomized design (CRD) with five replicates. The quantity of each fertilizer or amendment used in the distinct treatments are presented in Table 2**.** Fertilization doses were calculated based on the nitrogen demand of the crop, established at 260 kg N ha^{-1}, based on the study of Chang et al. (2023), thus each pot received a total of 0.2785 g of N. Nitrogen was provided considering only the plant-available N in the products (NH_4^+ and NO_3^-), as pak choi is a short cycle crop and when applying organic fertilizers in such crops a high immobilization of nutrients can occur, hampering plants growth (Esteves et al., 2022). A commercial mineral fertilizer (NPK 11–5–18) was used as a positive control. The mineral fertilizer had a concentration of 110 g kg^{-1} of available N, while digestate had 1.11 g kg^{-1} (N—NH_4 1107.1 mg kg^{-1} and N—NO_3 1.1 mg kg^{-1}) and frass had 4.16 g kg^{-1} (solely as N—NH_4^+) of available N. The application of each fertilizer or amendment was performed in three distinct events: 50 % being provided at the same day seedlings were transplanted; 25 %

Table 2
Summary of the treatments established in the experiment and the total amounts in grams (g) of each fertilizer/amendment, including solid digestate, black soldier fly larvae frass and the mineral NPK fertilizer, added to each replicate (pot).

Treatment	Composition	NPK (g)	Digestate (g)	Frass (g)
T0	Control (unfertilized)	–	–	–
T1	Solid digestate (100 % N)	–	250.45	–
T2	BSF frass (100 % N)	–	–	66.82
T3	Digestate (50 % N) + Frass (50 % N)	–	125.2	33.41
T4	NPK (25 % N) + Digestate and Frass (75 % N)	0.633	93.9	25.06
T5	NPK (50 % N) + Digestate and Frass (50 % N)	1.266	62.61	16.70
T6	NPK (75 % N) + Digestate and Frass (25 % N)	1.899	31.3	8.35
T7	NPK (100 % N)	2.532	–	–

provided at 14 days after transplantation (DAT); and the remaining 25 % provided seven days later, at 21 DAT.

2.5. Plant measurement and analyses

The number of leaves on each plant was counted starting from 14 DAT and continued weekly until the time of harvest. During each counting session, only fully expanded leaves were included, in order to ensure consistency. Shoots were cut at the soil surface, and the roots were carefully sampled by removing the surrounding soil by washing. Both shoots and roots were weighed on a digital scale, all leaves were counted, and the area of all individual leaves was measured in a leaf area measuring meter (Li-3100, Licor Inc., NE, USA). Subsequently, roots and shoots were placed in individual paper bags and dried in an air-circulating oven set at 65 °C, until constant weight. Dry shoots were analysed for macronutrients (N, P, K, Ca, Mg and S), micronutrients (Mn, B, Zn, Cu, and Mo) and Na by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) by an accredited laboratory (LMI AB, Helsingborg, Sweden). All shoot nutrient concentrations were expressed on a dry-weight (DW) basis: macronutrients and Na in g kg^{-1} DW, and micronutrients in mg kg^{-1} DW.

2.6. Photosynthetic performance

2.6.1. Leaf gas exchange measurements

Gas exchange measurements, including net CO_2 assimilation (A) and stomatal conductance (gsw), were conducted using a portable, open-path and infrared gas analyzer system (LI-6800, Licor Biosciences Inc., Lincoln, NE, USA), aiming to evaluate the physiological responses of the plants cultivated under distinct fertilization regimes. These measurements were performed on the third fully developed leaf (leaf 3) at both 21 DAT (days after transplanting) and 30 DAT. The measurements took place between 09h30 and 15h30, a period chosen to capture peak photosynthetic activity under stable environmental conditions (Yong et al., 2014). For each measurement, the leaf was subjected to an optimal chamber setting of CO_2 concentration (400 ppm), temperature (25 °C), relative humidity (RH) (70 % or 1.2 kPa VPD), flow rate (500 μmols^{-1}) and fan speed (10,000 rpm) (Yong et al., 2010; Wang et al., 2020). The gas exchange system was calibrated according to the manufacturer's guidelines prior to measuring to ensure accuracy. Leaves were allowed to stabilize for several minutes before recording gas exchange parameters to reflect steady-state photosynthesis. Data was automatically logged by the gas exchange system; the gas exchange calculations of the instrument were in accordance with Farquhar et al. (1980). The collected data was analysed to compare gas exchange parameters across different treatments at both DAT, providing insights into the photosynthetic performance of the plants over time under different fertilization regimes.

2.6.2. Chlorophyll a fluorescence (Fv/Fm): PSII maximum efficiency

The dark-adapted maximum efficiency of photosystem II (PSII), denoted as Fv/Fm, was measured on the youngest, fully developed mature leaf (leaf 3) using a standardized chlorophyll a fluorescence protocol. Following the gas exchange measurements, the non-destructive assessment was performed with a fluorometer (Handy-PEA fluorometer, Hansatech Instruments, King's Lynn, UK) at midday between 11h00 and 15h00 on both 21 and 30 DAT (Zhou et al., 2015; Wang et al., 2020). For each measurement, leaves were dark-adapted for 30 min using dark clips to ensure reliable assessment of the maximum quantum efficiency of PSII (Maxwell and Johnson, 2000). The dark clips were placed on the middle part of the adaxial side of the leaf blade in order to avoid major veins while ensuring consistency and accuracy. Following dark adaptation, chlorophyll a fluorescence was induced using a saturating light pulse with an intensity of 3500 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) for a duration of five seconds. This intensity is sufficient to close all PSII reaction centres, enabling the accurate determination of the Fv/Fm ratio (Strasser et al., 2004; Ma et al., 2021). The Handy-PEA fluorometer was calibrated prior to use, and regular maintenance and calibration checks were performed to maintain measurement accuracy (Zhou et al., 2015). Environmental conditions were kept consistent across both measurement days to minimize variability due to external factors such as light intensity, temperature, and humidity. The Fv/Fm ratio was calculated using the equation below (Eq. (5)).

$$Fv \Big/ Fm = \frac{Fm - F_o}{Fm} \tag{Eq. 5}$$

where Fm represents the maximum fluorescence and F_o the minimum fluorescence from dark-adapted leaves.

2.6.3. Foliar chlorophyll content

The foliar chlorophyll content was assessed optically and non-destructively using a portable meter (chlorophyll meter MC-100, Apogee Instruments, North Logan, UT, USA) on the same dates and same leaves used earlier for gas exchange, and the later Fv/Fm measurements. The purpose of carrying out the gas exchange assessments in leaves prior to foliar chlorophyll fluorescence and content measurements was to minimize perturbations to the stomata (Yong et al., 2010; Zhou et al., 2015). The chlorophyll meter, which operates by measuring the absorbance of two wavelengths of light (red and near-infrared) transmitted through the leaf, provided a non-destructive estimation of the chlorophyll content index (CCI). Each reading was taken in triplicate per leaf to ensure accuracy and reproducibility of the data (Richardson et al., 2002). The data collected were then averaged for further statistical analysis.

2.7. Statistical analysis

The experimental data from the experiment were analyzed and visualized using different packages in RStudio (version 4.2.3). Data were subjected to both Shapiro-Wilk's and Levene's tests to assess normality of errors and homoscedasticity of variances, respectively. When the assumptions were satisfied, variables were analysed by one-way analysis of variance (ANOVA) followed by the multiple comparison of means by Tukey's honestly significant difference (HSD) test at a 5 % significance level. Seed germination indexes were evaluated by means of a *t*-test at a 5 % probability level. The "corrplot" package in R was used to estimate the Pearson correlation among the variables. To identify the most contributing response variables and the association among them, principal component analysis (PCA) was performed on the Pearson correlation matrix with variables standardized (centered and scaled with no post-hoc rotation applied), and the results were visualised using the "factoextra" package.

The microbial composition and abundance data for frass and digestate were carried out by using generalized linear models (GLM) for assessing differences among fertilizers in terms of microbial composition. GLM quasipoisson was used for assessing the observed numbers of Amplicon Sequence Variants (ASV), while a Gaussian GLM (Zhang et al., 2020) was adopted for evaluating the Shannon Index, which indicates the biodiversity of the fertilizers' microbiome. In addition, the beta-diversity of samples was analysed by means of a Principal Coordinates Analysis (PCoA) by means of three distinct methods, namely the Jaccard index that quantifies the similarity between samples based on the presence and absence of ASVs, the Bray-Curtis index, which quantifies the similarity between samples considering the abundance of ASVs, and also the Aitchinson distance that quantifies similarity considering the abundance of ASVs and the data composition nature. These indexes were further analysed by a PERMANOVA in order to test differences in the beta-diversity within groups.

3. Results

3.1. Abundance of microorganisms in frass and digestate

The solid digestate derived from food waste exhibited a significantly higher bacterial and fungal diversity compared to BSF frass at the phylum, family, and genus levels (Figs. 1 and 2; **Figures S1 & S2**). The number of bacterial and fungal ASVs observed in digestate were approximately 1000 and 650, respectively, while for frass it was approximately 100 and 65, respectively. Similarly, the Shannon index, which indicates the diversity of species in each amendment was significantly lower ($p < 0.0001$) for frass in relation to digestate (Figs. 1 and 2). Beta diversity analysis on rarefied data showed significant compositional differences between frass and digestate samples in both bacterial and fungal communities (**Figure S1**).

Frass was dominated by Actinobacteria, Firmicutes and a small concentration of Proteobacteria, while digestate displayed a much higher abundance of phyla, including Bacteroidota, Chloroflexi and others (**Figures S1 & S2**). The same trend was verified at the family level, in which frass displayed a predominance of Bacillaceae and Corynebacteriaceae, while digestate had a more balanced and abundant diversity. Finally, at the genus level, frass was dominated by *Corynebacterium* and *Pseudogracilibacillus*, while digestate delivered a higher diversity (Figs. 1 and 2). It is noteworthy that at the genus level, digestate was associated with over 50 % of unknown groups in comparison to around 8 % in frass (Figs. 1 and 2). In relation to fungal groups, the same trends were observed. Frass displayed a much lower diversity of groups in all taxonomic levels, with an almost complete dominance of Ascomycota at the phylum level, while digestate had a dominance of Ascomycota but also the presence of Basidiomycota. Similarly, at the family and genus levels, solid digestate displayed a higher and more balanced diversity in relation to frass (Figs. 1 and 2; **Figures S1&S2**).

3.2. Seed germination bioassay

Seed germination (SG), relative seed germination (RSG), relative root growth (RRG), and germination index (GI) showed clear differences between digestate and frass treatments across both test crops (Table 3). Digestate consistently supported high germination and growth, with SG values of 100.0 ± 0.0 % in pak choi and 96.7 ± 4.7 % in watercress, while frass resulted in markedly lower values of 68.9 ± 12.9 % and 12.2 ± 11.3 %, respectively. RSG followed the same trend, remaining close to 100 % under digestate for both species, but declining to 68.9 ± 6.8 % in pak choi and 13.1 ± 6.1 % in watercress with frass. Similarly, RRG was highest under digestate, reaching 118.1 ± 3.7 % for pak choi and 90.5 ± 3.8 % for watercress, compared with 78.8 ± 5.9 % and 30.0 ± 10.2 % under frass. Correspondingly, GI values exceeded 100 % in pak choi and

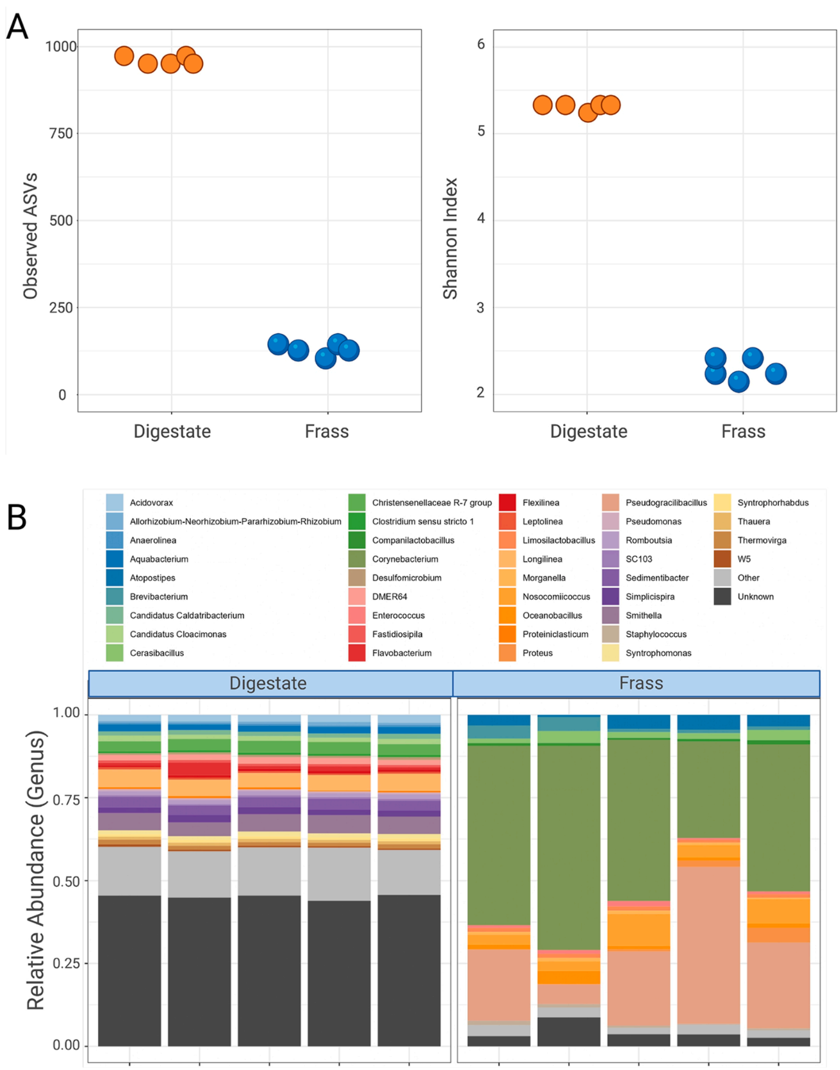

Fig. 1. Microbial characterization of frass and digestate ($n = 5$). (**A**) alpha diversity of bacterial groups (16S); (**B**) relative abundance of bacteria genera.

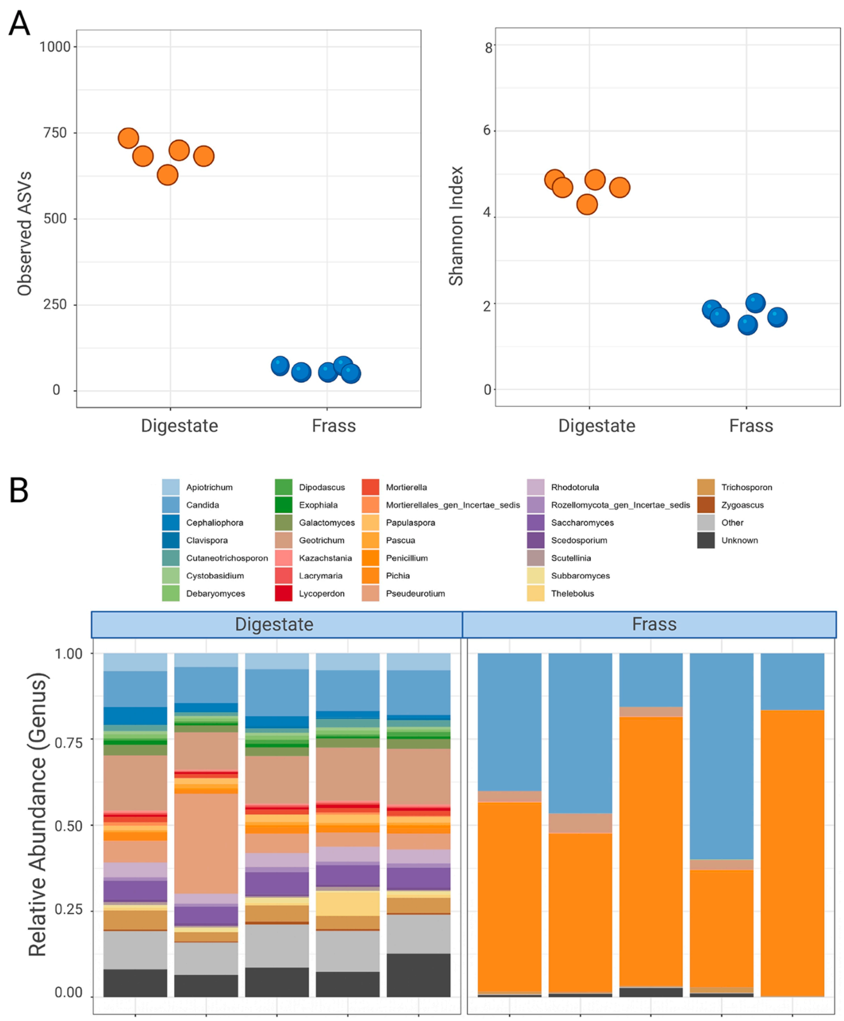

Fig. 2. Microbial characterization of frass and digestate ($n = 5$). (**A**) alpha diversity of fungal groups (ITS); (**B**) relative abundance of fungi genera.

Table 3
Indexes related to the seed germination bioassay conducted with pak choi and water cress seeds.

		SG (%)	RSG (%)	RRG (%)	GI (%)
Digestate	Pak choi	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	118.1 ± 3.7^{a}	118.1 ± 3.7^{a}
	Water cress	96.7 ± 4.7^{a}	100.0 ± 2.8^{a}	90.5 ± 3.8^{a}	90.5 ± 4.5^{a}
Frass	Pak choi	68.9 ± 12.9^{b}	68.9 ± 6.8^{b}	78.8 ± 5.9^{b}	54.6 ± 9.3^{b}
	Water cress	12.2 ± 11.3^{b}	13.1 ± 6.1^{b}	30.0 ± 10.2^{b}	4.4 ± 3.0^{b}

SG: seed germination; **RSG**: relative seed germination; **RRG**: relative radicle growth; **GI**: germination index. Distinct superscript letters indicate statistically significant differences between fertilizers (digestate and frass) within the same index and plant species, at 5 % probability level. Values are shown as mean ± standard deviation; means not sharing a letter differ significantly.

approached 91 % in watercress with digestate, whereas frass reduced GI to 54.6 ± 9.3 % and 4.4 ± 3.0 %, respectively.

3.3. Vegetative growth attributes

The number of leaves per plant increased progressively across all treatments, with significant differences emerging from 21 DAT onward (Fig. 3). By 21 DAT, T7 produced the highest leaf count, followed by T6 and T5, whereas T0 and T2 consistently recorded the lowest values. At 35 DAT, the highest number of leaves (12.8 ± 1.1 leaves per plant) was registered for T7 (positive control), being statistically similar to T5 (11.4 ± 1.1 leaves per plant) and T6 (12.2 ± 0.8 leaves per plant) but higher than T1-T4. Across all observation dates, T0 consistently produced the lowest leaf numbers, whereas T7 and T6 remained at the upper range.

Shoot fresh weight differed significantly among treatments (Fig. 4A). The lowest shoot fresh weight was recorded in T0 followed by T2 whereas the highest was observed in T6 and T7, which were statistically similar to T4 and T5. A similar trend was verified for the shoots' dry weight, with the lowest values registered for the negative control (T0) and the highest values being observed in T6 and T7 (Fig. 4B). The maximum root fresh and dry weight was recorded in T3 which was statistically similar to T2 and T4-T7, while the exclusive application of solid digestate (T1) resulted in the lowest root fresh and dry weight (Fig. 4C-D). Furthermore, for root-to-shoot ratio, the lowest values were registered for exclusive application of digestate (T1) or mineral fertilizer (T7) (Fig. 4E). The total leaf area (cm^2 per plant) was lowest in T0, followed by T2 and peaked in T3 which was statistically similar to T4-T7 (Fig. 4F). Although sole digestate treatment T1 exhibited a slightly lower total leaf area, it was statistically similar to the mineral treatment T7.

3.4. Photosynthetic efficiency

Net CO_2 assimilation (A) and stomatal conductance (gsw) by plants fertilized with BSF frass, solid digestate, or combinations differed

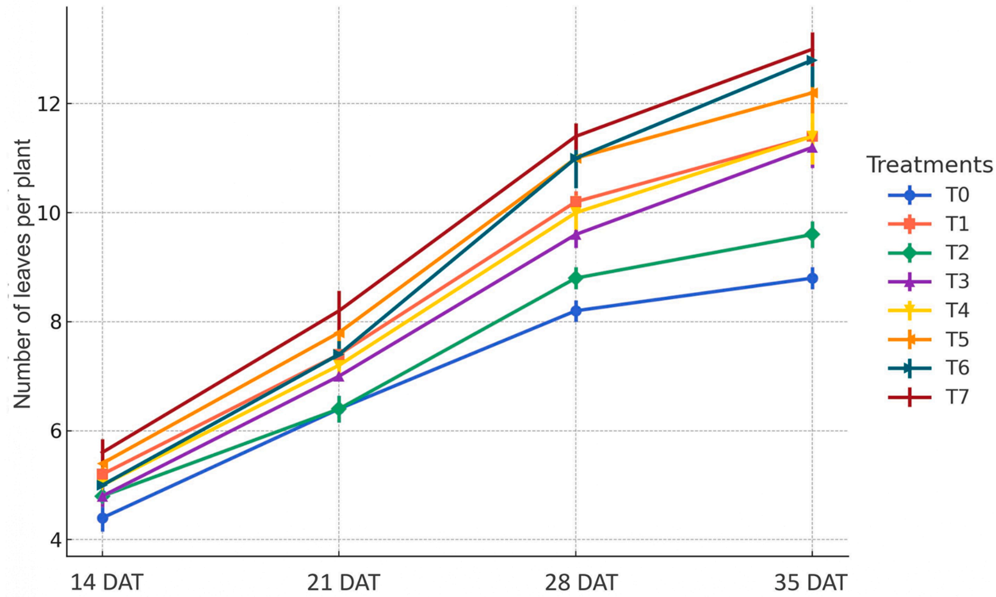

Fig. 3. The number of leaves of pak choi plants cultivated in a soil-sand mixture with distinct fertilization regimes, at different days after transplantation (DAT). Treatments: T0 = *C* (Control, unfertilized); T1 = Digestate 100 % N; T2 = Frass 100 % N; T3 = Digestate 50 % *N* + Frass 50 % N; T4 = NPK 25 % *N* + Digestate and Frass 75 % N; T5 = NPK 50 % *N* + Digestate and Frass 50 % N; T6 = NPK 75 % *N* + Digestate and Frass 25 % N; T7 = NPK 100 % N.

significantly (Fig. 5A–D). Both parameters were significantly reduced in older leaves compared to younger ones, as indicated by the comparison of A and gsw values at 21 DAT and 30 DAT (Fig. 5A–D). However, plants fertilized exclusively with digestate (T1) exhibited higher A and gsw values at 21 DAT, which persisted even in the older leaves at 30 DAT. Overall, all treatments presented an assimilation, ranging from 17 to 20 μmol CO_2 m^{-2} s^{-1}, with a notably lower assimilation (around 13 μmol CO_2 m^{-2} s^{-1}) by plants fertilized exclusively with BSF frass (T2) (Fig. 5A-B). The same trend was verified for the stomatal conductance (Fig. 5C-D).

The treatment T1 (Digestate 100 % N) consistently yielded the highest CCI values (Fig. 6A-D). Conversely, treatment T2 (Frass 100 % N) resulted in the lowest CCI values. Other treatments, including those combining solid digestate and BSF frass (T3) and those incorporating varying proportions of mineral fertilizer (T4, T5, T6, T7), demonstrated intermediate effects with a gradual improvement in CCI, with T7 (NPK 100 % N) reaching the highest values in CCI (30 DAT). The Fv/Fm ratio, an indicator of the maximum efficiency of Photosystem II, showed no significant differences among treatments at 21 DAT (Fig. 6C). However, at 30 DAT, treatment T2 exhibited a significant decline in Fv/Fm compared to T1, T6, and T7 treatments (Fig. 6D).

3.5. Nutrient concentrations in shoots

For macronutrients, sole digestate treatment T1 exhibited the highest levels of N, P, Ca, S, and Mg (Figure 7; Table 4). Conversely, treatment T0 (unfertilized control) consistently showed lower concentrations of macronutrients, particularly in N and P, making it the least nutrient-rich treatment. However, the sole application of BSF frass (T2) resulted in the highest concentration of K in the shoots whereas sole digestate application T1 showed the lowest concentration of K. In the micronutrient profile, sole digestate treatment T1 again stands out with the highest concentrations of Cu, Fe, Mo, and Zn, while T0 and T2 showed comparatively lower concentrations. Sodium (Na) concentrations peaked in T3 (combined frass digestate application), followed by T4 and T2. Across measured elements, T1 yielded the highest tissue concentrations across macro- and micronutrients, whereas T0 consistently exhibited the lowest levels (Fig. 7). Furthermore, T7, which represents the complete mineral fertilizer treatment, demonstrated a balanced nutrient profile with relatively high concentrations of both macronutrients and micronutrients.

3.6. Interactions between plant nutrients and photosynthetic parameters

Significant positive correlations were observed between N concentration in the shoots and net assimilation of CO_2, stomatal conductance, total leaf area, and number of leaves, while negative correlations were found between Na and K concentrations in the shoots and the chlorophyll content in the pak choi plants (Fig. 8). Morphological traits, including total leaf area, and number of leaves, exhibited strong correlations. Similarly, the N, P, and S concentrations in shoots showed positive correlation. The foliar chlorophyll fluorescence was also positively correlated with growth parameters such as shoot fresh and dry weight. We found a strong negative correlation between high sodium (Na) and potassium (K) levels in shoots with physiological parameters such as net assimilation, stomatal conductance, and chlorophyll content index.

In addition to identifying possible correlations among variables, an exploratory PCA analysis was performed, which displayed the relationships among the response variables (Fig. 9). On the biplot, Dim1 and Dim2, which represent the first and second principal components, accounted for 33.6 % and 19.3 % of the data variance, totalling 52.9 % of the total variance of all data considered. It was verified that the shoots' concentration of Na and K were oppositely related to parameters such as net CO_2 assimilation, stomatal conductance and CCI, as well as for N and S uptake. Conversely, root development and consequently the root-to-shoot ratio was correlated to the concentrations of K, Na and Fe in the shoots. The factor loadings of the PCA demonstrated the strong influence of the root-to-shoot ratio, K concentration and root growth on the second dimension of the PCA, while CCI, A, total N, P and S strongly affected the first dimension of the PCA, contributing significantly to the data variance.

4. Discussion

The utilization of diverse organic by-products and residual materials as soil or biological amendments in agricultural and horticultural systems has emerged as a scientifically validated strategy to ameliorate

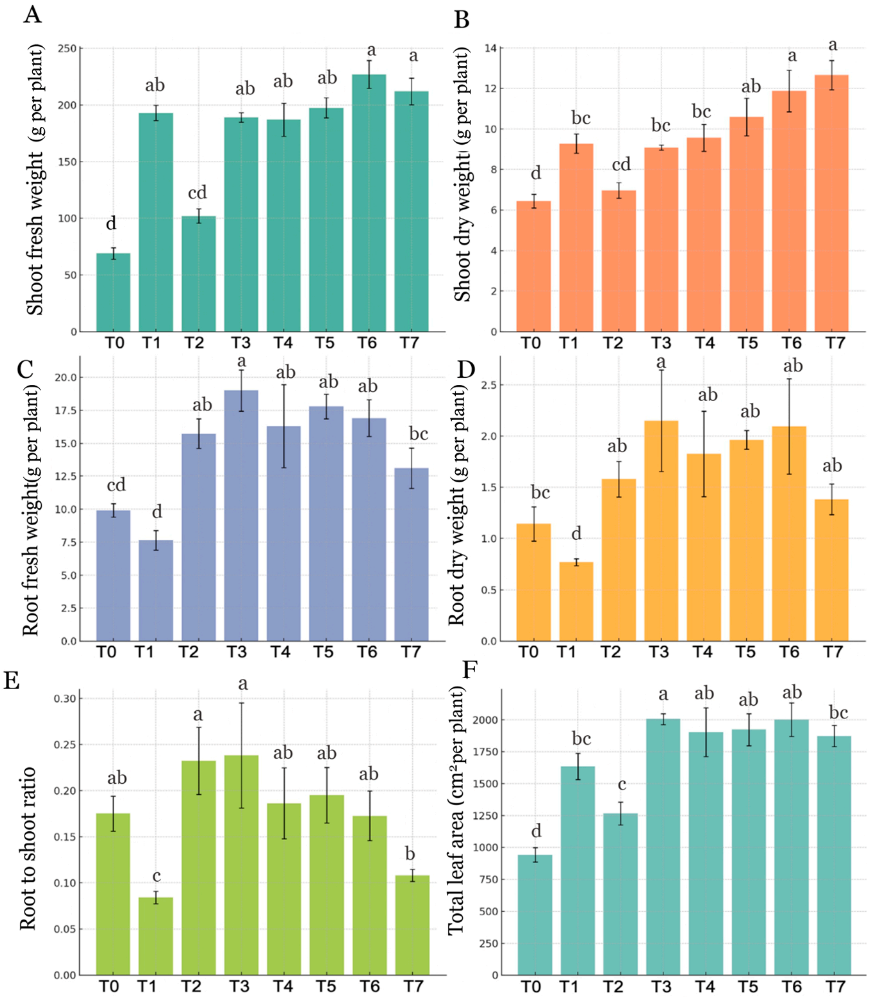

Fig. 4. Vegetative growth traits of pak choi at 35 days after transplanting (DAT) in a soil–sand mixture under eight fertilizer treatments. (A) Shoot fresh weight (g per plant); (B) Shoot dry weight (g per plant); (C) Root fresh weight (g per plant); (D) Root dry weight (g per plant); (E) Root-to-shoot ratio (dry-weight basis); (F) Total leaf area (cm^2 per plant). Values are mean ± standard error (SE); means not sharing a letter differ significantly at the 5 % significance level ($P < 0.05$). Treatments: T0 = *C* (Control, unfertilized); T1 = Digestate 100 % N; T2 = Frass 100 % N; T3 = Digestate 50 % *N* + Frass 50 % N; T4 = NPK 25 % *N* + Digestate and Frass 75 % N; T5 = NPK 50 % *N* + Digestate and Frass 50 % N; T6 = NPK 75 % *N* + Digestate and Frass 25 % N; T7 = NPK 100 % N.

nutrient limitations, enhance nutrient use efficiency, and optimize productivity (Wong et al., 2016; Abbott et al., 2018, 2020; Sani et al., 2025). These practices also align with circular bioeconomy principles by valorizing nutrient-rich waste streams and reintegrating them into production systems, thereby contributing to nutrient cycling and reducing reliance on mineral fertilizers (Abbott et al., 2018; Sani et al., 2025). Among such resources, solid digestate is widely available and commonly applied, whereas black soldier fly (BSF) frass remains comparatively novel and less studied (Lopes et al., 2022; Basri et al., 2022). The present study demonstrated that combining these two organic fertilizers derived from post-consumer food waste enhances plant growth and serves as a more sustainable alternative nutrient source to mineral fertilizers.

4.1. Food waste-derived solid digestate delivered improved performance and had higher microbial abundance than BSF frass

Phytotoxicity in organic fertilizers and fertilizers might occur due to several parameters, either alone or in combination (de Bang et al., 2021; Wang et al., 2022; Wu et al., 2023). According to Wichuk and McCartney (2010), indicators of lack of maturity (readiness for use) and stability (degradability of organic matter) in organic materials include high NH_4^+ concentrations coupled with elevated pH leading to NH_3 formation, low NO_3^- concentrations, high electrical conductivity due to sodium (Na) presence, and elevated microbial activity. In this study, BSF frass exhibited several of these unfavourable traits, notably high NH_4^+, elevated Na, and low NO_3^-, whereas solid digestate displayed a more balanced nutrient profile with higher NO_3^- and lower NH_4^+ and Na. This difference highlights the mechanism by which frass imposed phytotoxic stress: excessive NH_4^+ can acidify the rhizosphere and inhibit root respiration, while Na accumulation induces osmotic stress and disrupts K^+ and Mg^{2+} uptake, collectively impairing germination and seedling establishment. Lopes et al. (2022) previously underscored the instability of BSF frass, proposing that the conventional 10- to 12-day bioconversion period with BSF larvae is inadequate for achieving organic matter stabilization and initiating humification processes. Even extended stabilization would not remove non-volatile ions such as Na, meaning that phytotoxicity risks remain when food waste is the feed substrate. Consequently, frass phytotoxicity may reach critical thresholds depending on the substrate provided to the larvae; for instance, food waste as a feed source could result in elevated phytotoxicity levels.

Our findings therefore support the interpretation that frass phytotoxicity limits nutrient uptake and growth. This is consistent with Song et al. (2021), who showed that composting fresh frass increased the germination index of pak choi from <20 % to nearly 100 %, and Lopes et al. (2024), who demonstrated that recirculating frass through BSF

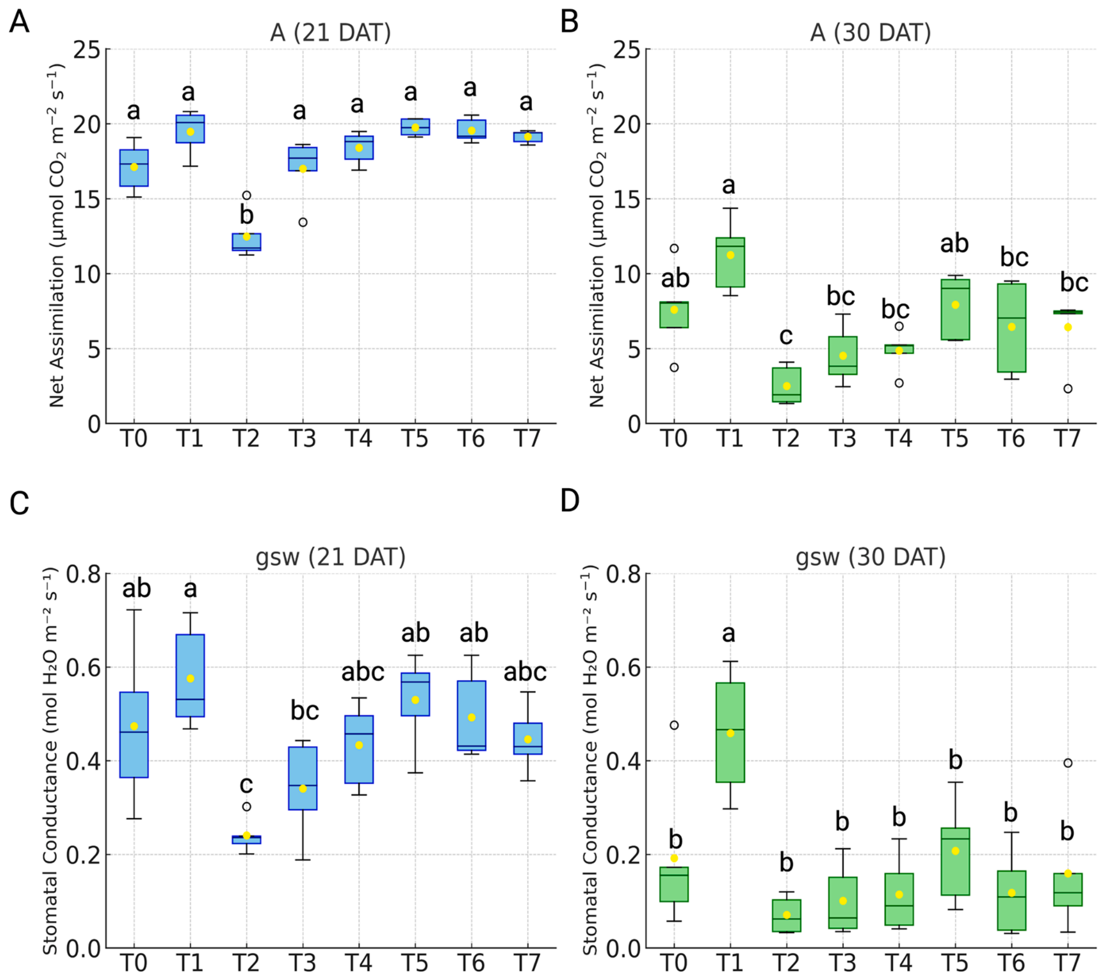

Fig. 5. The physiological dynamics of pak choi plants cultivated with distinct fertilization regimes. (A) Net assimilation (A) at 21 days after transplanting (DAT); (B) Net assimilation at 30 DAT; (C) stomatal conductance (gsw) at 21 DAT; (D) stomatal conductance at 30 DAT. $n = 5$. Treatments: T0 = *C* (Control, unfertilized); T1 = Digestate 100 % N; T2 = Frass 100 % N; T3 = Digestate 50 % *N* + Frass 50 % N; T4 = NPK 25 % *N* + Digestate and Frass 75 % N; T5 = NPK 50 % *N* + Digestate and Frass 50 % N; T6 = NPK 75 % *N* + Digestate and Frass 25 % N; T7 = NPK 100 % N.

bioconversion improved stability. Together, these studies suggest that post-treatment (e.g. composting, anaerobic digestion or frass recirculation) of frass is essential before agronomic application, as also discussed thoroughly by Lopes et al. (2025). The microbial composition also differentiated the two materials. Whereas frass microbes largely reflected larval gut microbiota and raw compost-associated groups (e.g., Acidobacteria, Proteobacteria, Actinobacteria), digestate contained a broader and more abundant microbial community shaped by the extended retention time and mixed feedstocks of anaerobic digestion (Gold et al., 2020; Yan et al., 2023). This higher microbial abundance may enhance soil processes such as nutrient mineralization, organic matter turnover, and production of phytohormones, thereby supporting plant growth (Van Midden et al., 2023).

The presence of microorganisms in organic fertilizers and their interaction within the rhizosphere-root continuum is strongly related to a plant's development, and a significant part of microbial interaction with plants relates to the production of biostimulants, especially phytohormones, by the microbes (Yong et al., 2014; Wong et al., 2016; de Vries et al., 2020; Hua et al., 2024). Furthermore, Lu et al. (2021) demonstrated positive correlations between microbial groups (e.g. real counts of Acidobacteria, Actinobacteria and Firmicutes, highly abundant in both fertilizers used in this study and the concentration of specific hormones such as auxins, gibberellins and cytokinins in the bulk soil, rhizosphere and roots. These phytohormones (e.g., cytokinins, auxins, and gibberellins) are directly involved in processes such as cell division and elongation, vascular differentiation, and root development (Wong et al., 2015; de Vries et al., 2020; Gupta et al., 2020; Wong et al., 2020). It is thus plausible that the microbial diversity of digestate contributed to pak choi growth by stimulating phytohormone-mediated processes, whereas these potential benefits in frass were counteracted by its high salinity and NH_4^+ load, which can suppress microbial activity and hormone synthesis. Nevertheless, as de Vries et al. (2020) noted, many beneficial traits are shared across microbial taxa, complicating attribution of specific effects. This is consistent with Poveda et al. (2019), who showed that the positive effects of mealworm frass on bean growth diminished when sterilized, indicating that microbial contributions are essential but can be masked by unfavourable substrate chemistry. Future research should therefore isolate key microbial strains from both digestate and frass to clarify their role in phytohormone production, nutrient cycling, and plant regulation, while also considering how physicochemical stressors (e.g. Na, NH_4^+) modulate these effects.

4.2. Pak choi photosynthetic performance was improved by solid digestate but reduced by BSF frass

Solid digestate (T1) enhanced photosynthesis in pak choi by increasing net assimilation, stomatal conductance, and chlorophyll content index. Mechanistically, this is attributable to digestate's provision of readily available nitrogen to support Rubisco activity and micronutrients such as Mg and Fe that are essential for chlorophyll

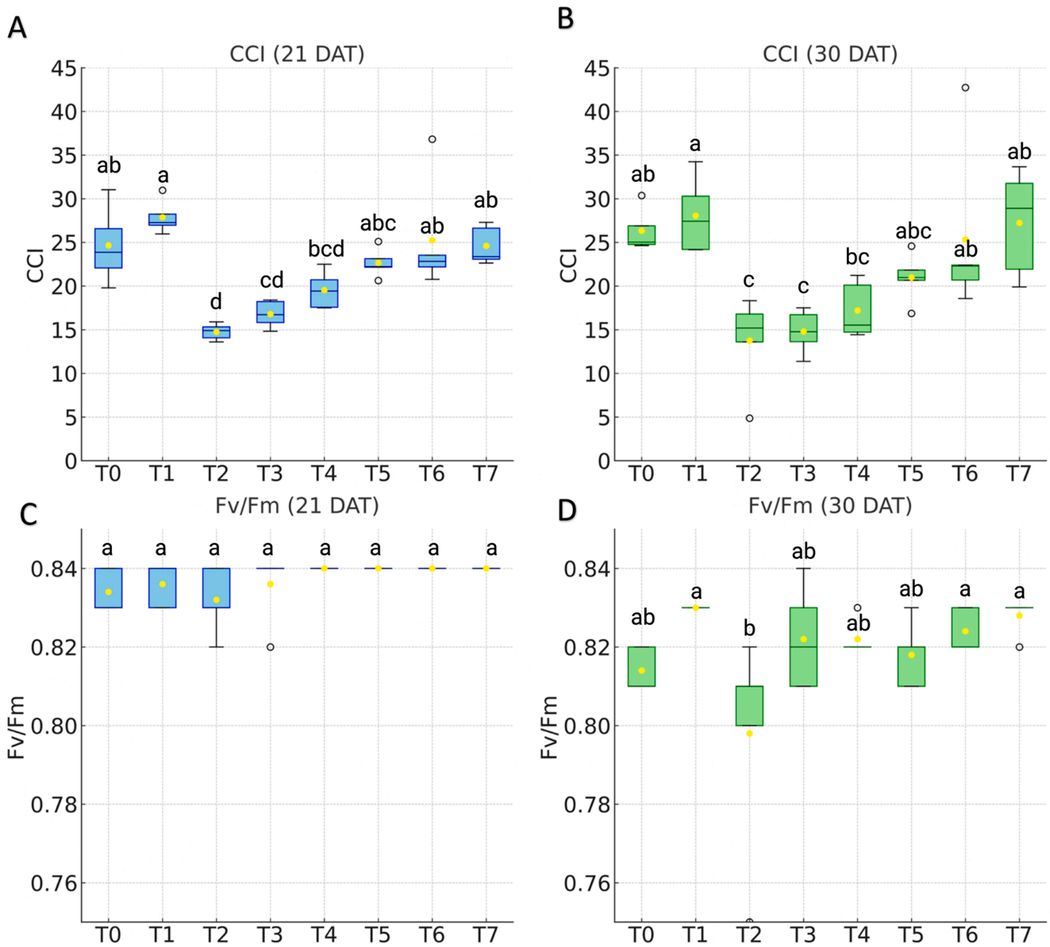

Fig. 6. The foliar chlorophyll content index (CCI) and fluorescence in pak choi plants cultivated with distinct fertilization regimes ($N = 5$). Fv/Fm: maximum efficiency of Photosystem (II). DAT: Days after transplanting. Treatments: T0 = *C* (Control, unfertilized); T1 = Digestate 100 % N; T2 = Frass 100 % N; T3 = Digestate 50 % *N* + Frass 50 % N; T4 = NPK 25 % *N* + Digestate and Frass 75 % N; T5 = NPK 50 % *N* + Digestate and Frass 50 % N; T6 = NPK 75 % *N* + Digestate and Frass 25 % N; T7 = NPK 100 % N.

Table 4
Concentration of macronutrients and micronutrients in the shoots of pak choi plants cultivated under distinct fertilization regimes.

	T0	T1	T2	T3	T4	T5	T6	T7
N ($g\ kg^{-1}$)	22.0 ± 3.1^{c}	57.2 ± 2.0^{a}	30.9 ± 3.8^{bc}	39.9 ± 2.6^{b}	37.0 ± 2.8^{b}	33.3 ± 9.7^{b}	39.1 ± 8.6^{b}	39.3 ± 4.7^{b}
P ($g\ kg^{-1}$)	3.9 ± 0.3^{b}	8.9 ± 0.2^{a}	4.2 ± 0.3^{a}	5.6 ± 0.1^{a}	5.6 ± 0.5^{a}	5.80 ± 1.1^{a}	6.0 ± 1.8^{a}	6.1 ± 0.7^{a}
K ($g\ kg^{-1}$)	44.3 ± 5.8^{cd}	26.9 ± 2.5^{d}	95.5 ± 11.6^{a}	70.8 ± 4.5^{b}	72.5 ± 5.9^{b}	66.0 ± 16.0^{b}	74.2 ± 15.9^{b}	63.4 ± 7.7^{bc}
Ca ($g\ kg^{-1}$)	28.7 ± 2.8^{d}	41.7 ± 2.4^{a}	30.8 ± 2.4^{cd}	37.3 ± 1.0^{ab}	35.6 ± 1.9^{bc}	34.3 ± 2.8^{bc}	32.6 ± 3.4^{bcd}	31.3 ± 2.5^{cd}
Mg ($g\ kg^{-1}$)	2.7 ± 0.3^{cd}	3.9 ± 0.3^{a}	2.9 ± 0.5^{bcd}	3.5 ± 0.3^{ab}	3.3 ± 0.3^{abc}	3.0 ± 0.3^{bcd}	2.8 ± 0.2^{cd}	2.5 ± 0.2^{d}
S ($g\ kg^{-1}$)	3.5 ± 0.6^{e}	16.9 ± 1.1^{a}	5.7 ± 0.6^{d}	8.6 ± 0.3^{c}	9.5 ± 0.5^{c}	9.2 ± 1.4^{c}	11.7 ± 0.6^{b}	11.5 ± 0.4^{b}
B ($mg\ kg^{-1}$)	80.4 ± 4.9^{b}	99.2 ± 11.6^{a}	69.4 ± 8.6^{b}	80.0 ± 3.8^{b}	83.4 ± 4.6^{ab}	82.0 ± 12.8^{b}	82.2 ± 3.4^{b}	77.6 ± 8.0^{b}
Cu ($mg\ kg^{-1}$)	5.4 ± 2.4^{b}	10.0 ± 1.0^{a}	5.3 ± 0.6^{b}	6.8 ± 0.3^{b}	6.5 ± 0.4^{b}	5.9 ± 0.8^{b}	6.1 ± 1.4^{b}	5.7 ± 1.1^{b}
Mn ($mg\ kg^{-1}$)	54.0 ± 8.3^{b}	114.0 ± 15.1^{a}	109.4 ± 31.7^{a}	120.0 ± 15.8^{a}	100.2 ± 13.1^{a}	96.8 ± 22.02^{a}	128.0 ± 23.8^{a}	136.0 ± 15.1^{a}
Mo ($mg\ kg^{-1}$)	0.92 ± 0.10^{d}	2.00 ± 0.16^{a}	0.80 ± 0.09^{d}	1.28 ± 0.11^{b}	1.18 ± 0.1^{bc}	0.95 ± 0.15^{cd}	0.79 ± 0.08^{d}	0.50 ± 0.11^{e}
Na ($g\ kg^{-1}$)	5.2 ± 1.9^{de}	10.9 ± 0.7^{bc}	16.1 ± 3.6^{ab}	26.9 ± 1.1^{a}	20.6 ± 2.3^{a}	14.4 ± 5.4^{bc}	9.9 ± 22^{cd}	4.1 ± 0.4^{e}
Zn ($mg\ kg^{-1}$)	26.0 ± 2.8^{d}	76.4 ± 3.9^{a}	39.4 ± 3.6^{c}	54.2 ± 3.2^{b}	47.4 ± 3.6^{bc}	41.0 ± 5.4^{c}	40.6 ± 8.1^{c}	41.2 ± 5.8^{c}

N: nitrogen; **P**: phosphorus; **K**: potassium; **Ca**: calcium; **Mg**: magnesium; **S**: sulphur; **B**: boron; **Cu**: copper; **Mn**: manganese; **Mo**: molybdenum; **Na**: sodium; **Zn**: zinc. Treatments: T0 = *C* (Control, unfertilized); T1 = Digestate 100 % N; T2 = Frass 100 % N; T3 = Digestate 50 % *N* + Frass 50 % N; T4 = NPK 25 % *N* + Digestate and Frass 75 % N; T5 = NPK 50 % *N* + Digestate and Frass 50 % N; T6 = NPK 75 % *N* + Digestate and Frass 25 % N; T7 = NPK 100 % N. Distinct letters indicate significant differences among treatments for the same nutrient, according to the Tukey's test, at a 5 % probability level.

biosynthesis and electron transport. Previous studies similarly reported that digestates enhance photosynthesis by supplying balanced nutrients and improving soil structure (Song et al., 2021). Wang et al. (2023) showed that digestate compost increased pak choi biomass and chlorophyll concentration, highlighting how nutrient-rich inputs stimulate chlorophyll accumulation and thereby improve light harvesting efficiency. Other studies with potatoes (Khan et al., 2024), bush beans (Lee et al., 2021), and pak choi (Cheong et al., 2020) consistently demonstrated that digestates can substitute or complement mineral fertilizers in maintaining photosynthetic competence. Collectively, these studies suggest that digestate enhances photosynthesis by sustaining chlorophyll content, boosting CO_2 fixation, and improving water use

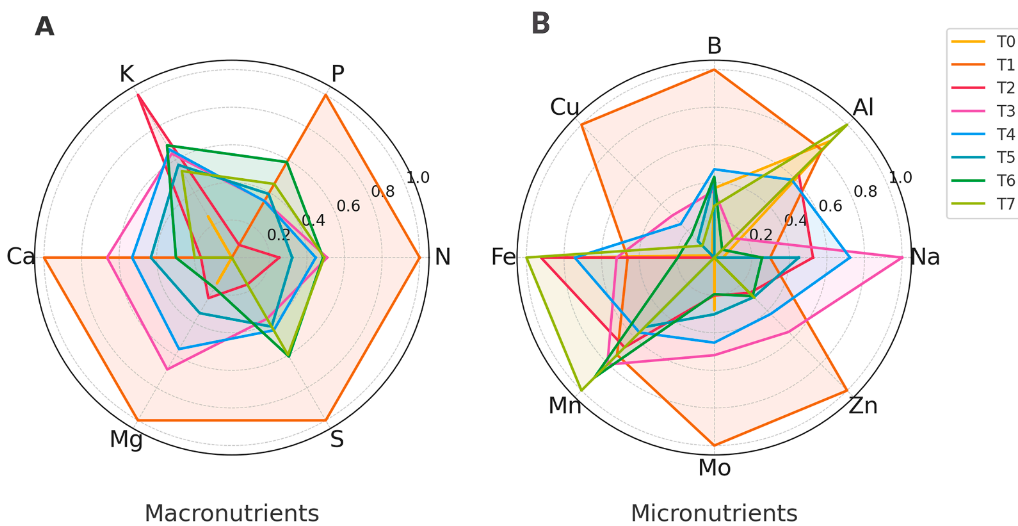

Fig. 7. The radar plots showing the relative macronutrient (A), micronutrient and Na (B) concentration per unit dry mass in pak choi shoots across different fertilization treatments. Each axis represents a specific nutrient, with values normalized from 0 to 1 to highlight differences between treatments.

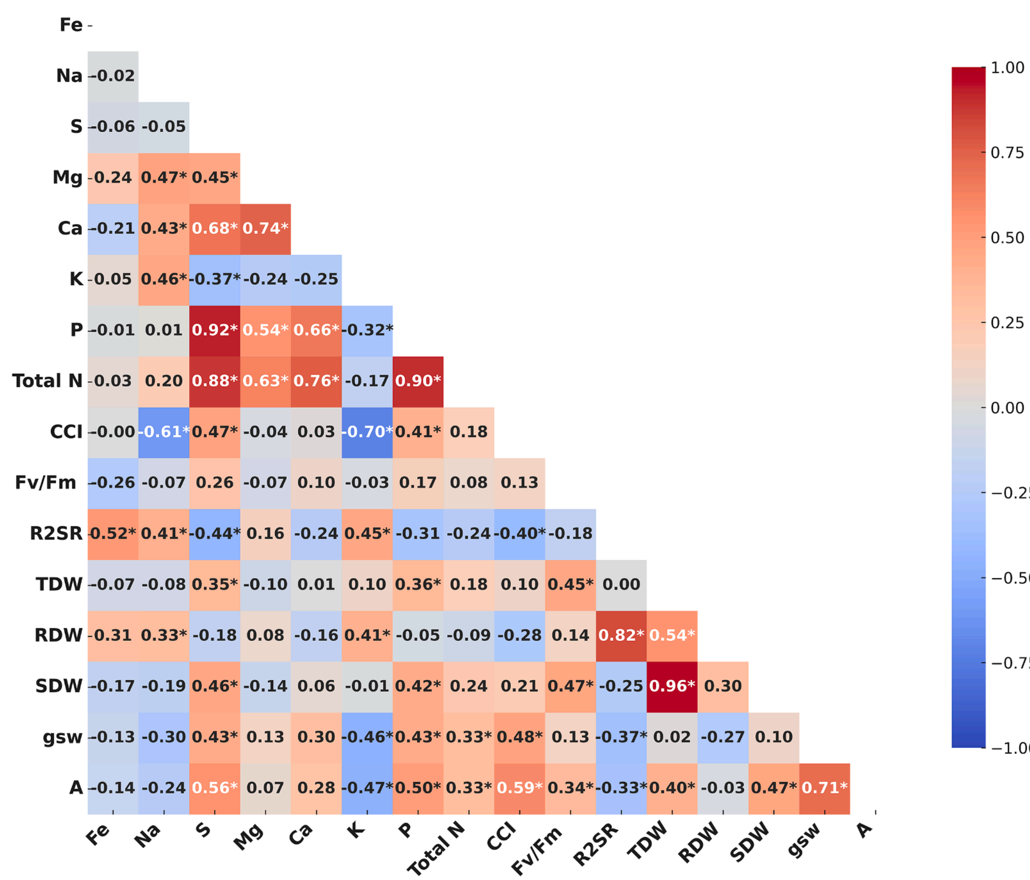

Fig. 8. The correlation plot including macronutrients and micronutrients of the pak choi shoots and photosynthetic parameters ($N = 5$) cultivated with distinct fertilization regimes. **RDW**: root dry weight, **SDW**: shoot dry weight, **TDW**: total dry weight, **R2SR**: root to shoot ratio, **CCI**: chlorophyll content index, **A**: Net assimilation; **gsw**: stomatal conductance; **Fv/Fm**: chlorophyll fluorescence. Asterisks represent significant interactions according to the Pearson's correlation test at a 5 % probability level.

efficiency, underpinning its potential as a sustainable alternative to mineral fertilizers.

By contrast, BSF frass reduced photosynthesis due to its high Na and NH_4^+ content. Elevated Na induces osmotic stress and ionic toxicity, leading to stomatal closure and reduced CO_2 uptake . At the same time, NH_4^+ in excess acidifies the rhizosphere, disrupts carbon–nitrogen balance, and interferes with root respiration, further limiting nutrient absorption. These mechanisms explain the phytotoxic effects observed in our study, consistent with previous findings that frass with high EC or high NH_4-N content inhibits growth (Setti et al., 2019; Bohm et al.,

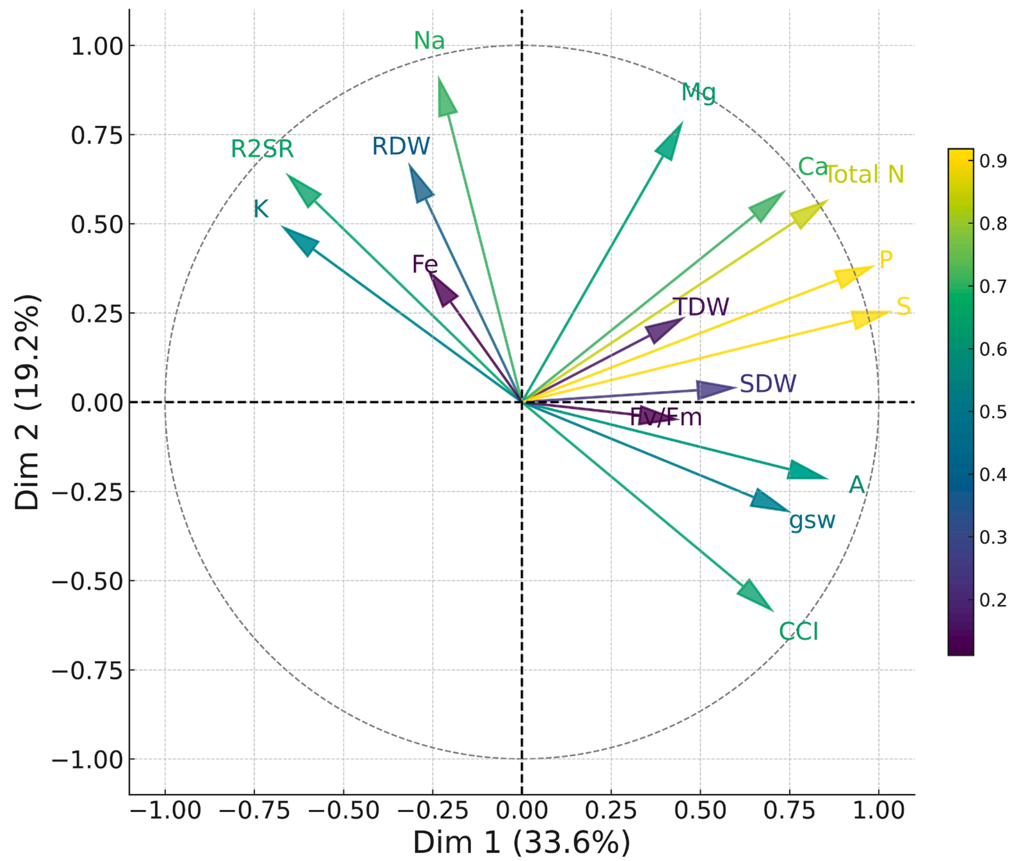

Fig. 9. PCA highlighted the contribution and relationship among the individual variables of pak choi plants cultivated with distinct fertilization regimes. **RDW**: root dry weight, **SDW**: shoot dry weight, **TDW**: total dry weight, **R2SR**: root to shoot ratio, **CCI**: chlorophyll content index, **A**: Net assimilation; **gsw**: stomatal conductance; **Fv/Fm**: chlorophyll fluorescence.

2023). The unusually high Na concentration in our frass (~3.7 % DM) likely reflects the post-consumer food waste origin, which contained large amounts of added salt. This suggests a parallel concentration of Cl^-, which was not measured but may have intensified ionic stress. Similar findings have been reported for food waste composts with elevated salinity (Yang et al., 2021). High concentrations of Na^+, Cl^-, and NH_4^+ compete with essential nutrients on soil colloids, disturb ionic balance, and impair photosynthetic metabolism (Munns and Tester, 2008; Leogrande and Vitti, 2019; Wang et al., 2022; Wu et al., 2023). These results highlight the need for stabilization or dilution strategies before frass can be applied effectively as a fertilizer, particularly when derived from salt-rich waste substrates.

4.3. *Excessive sodium (Na) and potassium (K) in BSF frass impaired pak choi photosynthesis and physiology*

Excessive Na and K supplied by frass disrupted ionic balance in pak choi, impairing photosynthetic parameters such as net assimilation and chlorophyll content index (CCI). While K concentration remained within tolerable ranges, Na concentrations approached toxicity thresholds, leading to osmotic stress, ionic toxicity, and reduced photosynthetic efficiency. Na stress inhibits key enzymes of the Calvin cycle, promotes stomatal closure, and reduces CO_2 availability for fixation (Munns and Tester, 2008; Shabala and Munns, 2017). Furthermore, excessive Na concentration in leaves not only disrupts ionic homeostasis but also induces oxidative stress that damages chloroplast ultrastructure, accelerates chlorophyll degradation, and limits light capture (Zuo et al., 2024). The resulting overproduction of reactive oxygen species (ROS) impairs key enzymes in the Calvin cycle and electron transport chain, thereby reducing photosynthetic efficiency. In addition, Na stress interferes with Rubisco activation by disturbing ionic balance and decreasing Mg^{2+} availability, while destabilizing ATP synthase and other thylakoid enzymes through ionic toxicity (Pecherina et al., 2022). Such multifaceted damage underscores how salinity stress compromises photosynthetic machinery at both structural and biochemical levels, ultimately constraining plant productivity. These findings are consistent with recent reports highlighting the central role of ROS and ionic imbalance in the molecular dissection of salinity tolerance (Atta et al., 2023). Although PSII efficiency (Fv/Fm) was largely maintained, CCI declined sharply with rising Na, reflecting Na-induced chlorophyll degradation and oxidative stress (Wu et al., 2023; Wang et al., 2024). This indicates that Na toxicity limits light captures and CO_2 assimilation even before PSII photochemistry is severely impaired. Our findings align with Huang et al. (2024), who observed that frass improved soil fertility but also warned that high salt contents cause growth inhibition. These results emphasize that the ionic composition of frass must be carefully monitored and managed to prevent salinity stress in crops.

Excessive K concentration also contributed to reduced photosynthesis. While K is essential for osmoregulation and enzyme activation, oversupply disrupts Mg uptake, destabilizes chlorophyll molecules, and promotes oxidative stress (Vandegeer et al., 2021; Zhao et al., 2020; Pantha et al., 2023). This imbalance triggered stomatal regulation responses that reduced conductance and CO_2 assimilation (Hasanuzzaman et al., 2018; Zhao et al., 2020). Similar to Na stress, high K induced ROS generation, which damaged chlorophyll and further limited photosynthetic capacity (Mostofa et al., 2022; Johnson et al., 2022). The problem was exacerbated under conditions of low Mg, as observed in frass-applied plants, since Mg is critical for chlorophyll stability and Rubisco activation. The imbalance between excess K and insufficient Mg intensified oxidative stress, impairing the photosynthetic machinery

(Tränkner et al., 2018; de Bang et al., 2021). Other ions, including Cl^- and NH_4^+/NH_3, may also have contributed to the observed physiological depression. High Cl^- disrupts ionic balance, while excess NH_4^+ acidifies cytosolic pH and interferes with carbon–nitrogen metabolism, collectively reducing growth and photosynthesis (White and Broadley, 2001; Britto and Kronzucker, 2002; Esteban et al., 2016). Taken together, these findings reveal that the combined effects of Na, K, Cl^-, and NH_4^+ drive physiological inhibition in frass-treated plants through osmotic stress, ionic toxicity, and oxidative damage. Careful management and stabilization of frass are therefore essential to mitigate these risks.

4.4. Combined digestate, frass, and mineral nitrogen (N) enhanced pak choi performance

Digestate alone improved growth and physiological traits due to its nutrient-rich profile and diverse microbial community, whereas frass alone suppressed growth because of its high NH_4^+, Na, and salinity levels. The nutrient properties of digestate, containing both readily available and slow-release components, provided a continuous and balanced supply of essential nutrients to the plant's activity (Brychkova et al., 2024; Hua et al., 2024). This dual nutrient release mechanism enhances nutrient use efficiency and supports sustained plant growth over time (Möller and Müller, 2012; Horta and Carneiro, 2021). Furthermore, Cheong et al. (2020) demonstrated that incorporating food waste-derived anaerobic digestate into soil can enhance the growth of pak choi (*Brassica rapa*) by supplying essential nutrients, particularly nitrogen and phosphorus, as an effective substitute for synthetic fertilizers. Moreover, several studies demonstrated that the presence of plant growth-promoting bacterial communities in anaerobic digestates promote growth and physiological dynamics (Mukhuba et al., 2018; Lu et al., 2021; Roopnarain et al., 2023; Hua et al., 2024). Although plants treated with sole digestate exhibited higher growth than the unfertilized control and sole frass treated plants, their fresh biomass was significantly lower compared to the synthetic fertilizer control. While digestate alone provides a rich source of plant nutrients and improves soil structure, it may not supply all the essential mineral nutrients in the optimal balance required for maximal plant growth. For instance, the low mineralisation rates of solid digestates, coupled with soil immobilisation of mineral nitrogen, limited the growth of lettuce (*Lactuca sativa* L.) and kale (*B. oleracea* var. Winterborn) (Horta and Carneiro, 2021). This underscores that digestate cannot fully substitute mineral N without supplementation.

Frass alone, despite containing beneficial components such as N and chitin, did not support growth because its nutrient release pattern was poorly matched to plant demand and its high NH_4^+ load induced phytotoxicity. Previous studies also showed that fresh frass releases N slowly (Gebremikael et al., 2022) and that stabilisation (e.g., composting) can reduce phytotoxic effects and improve plant responses (Song et al., 2021). Further studies also demonstrated that either a nutrient deficiency or the phytotoxic effects of the frass could negatively impact plant growth (Esteves et al., 2022; Chavez et al., 2023). Interestingly, a 50/50 combination of digestate and frass did not consistently enhance the foliar net assimilation rate, indicating that the interaction between these two organic fertilizers was not additive. The phytotoxicity of BSF frass likely plays a role in this complex interaction, potentially counteracting some of the beneficial effects of the solid digestate. This non-additive interaction suggested that the presence of toxic compounds in the frass might have interfered with nutrient uptake and physiological processes, indicating the need for careful management of the ratios and application methods when using these fertilizers in combination.

When combined with mineral fertilizer, however, digestate and frass interacted synergistically. Digestate supplied a mixture of fast- and slow-release nutrients, frass contributed organic matter and chitin that support microbial activity, and mineral N provided immediate availability. Together, these inputs balanced NO_3^- and NH_4^+ levels, improved nutrient uptake, and sustained plant growth. Growth and photosynthetic performance improved progressively with increasing mineral N, with the strongest effects observed at 75 % supplementation. The synergistic effects of combining organic and synthetic fertilizers enhance nutrient use efficiency, reduce nutrient losses, and promote overall plant health (Sani and Yong, 2022), even though these parameters were not investigated extensively for the present study. The application of digestate and frass as organic fertilizers offers a slow-release source of essential nutrients, which, when combined with synthetic fertilizers, can create a synergistic effect that optimises nutrient availability and uptake by plants (Beesigamukama et al., 2021; Barragán-Fonseca et al., 2022). When appropriately diluted and stabilized, digestate and BSF frass can partially substitute mineral fertilizers without yield penalties. These findings support integrated nutrient management strategies that combine organic and synthetic inputs to lessen reliance on mineral fertilizers while advancing more sustainable production systems.

5. Conclusion

This study provides new mechanistic insight into how food-waste-derived amendments shape crop performance, demonstrating that solid digestate and BSF frass occupy very different positions within sustainable nutrient management. Digestate consistently enhanced germination, photosynthetic efficiency, and biomass production, supported by its balanced nutrient profile and diverse microbial community, confirming its suitability as a reliable organic fertilizer. In contrast, BSF frass derived from post-consumer food waste imposed phytotoxic stress, driven by excessive Na, K, and NH_4^+, which overrode potential microbial and nutritional benefits. We identify Na-driven phytotoxicity from salt-rich post-consumer food waste as one of the key under-recognized constraints to BSF frass utility in horticulture.

Importantly, the study also demonstrated that strategic integration of organic amendments with mineral fertilizers can maintain crop yields while reducing reliance on synthetic inputs. In controlled pot trials, partial substitution of mineral fertilizer (~25 %) with digestate and frass sustained high productivity, underscoring the potential of combined nutrient strategies to advance circular and climate-smart agriculture. Limitations include the pot-scale experimental design, the use of a single crop species, and frass sourced from one feedstock stream. Multi-site field trials with diverse crops and waste substrates will be essential to validate these findings at scale.

Future research should therefore prioritize (i) frass processing technologies to mitigate ionic toxicity, (ii) microbial isolation to harness biostimulant functions, and (iii) system-level evaluations of organic–mineral integration under field conditions. These insights can also inform policy frameworks and certification thresholds for salinity and NH_4^+ in frass, accelerating safe adoption of waste-derived fertilizers. By addressing these gaps, digestate and processed frass can be positioned as key components of sustainable horticultural nutrient management and circular bioeconomy frameworks.

Funding

This work was supported by the Biogas Solution Research Center (BSRC), project number 52,669–1, co-financed by the Swedish Energy Agency, Linköping University and the Swedish University of Agricultural Sciences. This work was also supported financially in part by Formas (grant number 2021–02001) and by the Carl-Tryggers Foundation (project number CTS21:1483).

CRediT authorship contribution statement

Md. Nasir Hossain Sani: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ivã Guidini Lopes:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Siri Caspersen:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Karl-Johan Bergstrand:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Thomas Prade:** Writing – review & editing, Validation, Supervision, Funding acquisition. **Jean Wan Hong Yong:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2025.114469.

Data availability

Data will be made available on request.

References

Abbott, L.K., Macdonald, L.M., Wong, M.T.F., Webb, M.J., Jenkins, S.N., Farrell, M., 2018. Potential roles of biological fertilizers for profitable grain production - A review. Agric. Ecosyst. Environ. 256, 34–50. https://doi.org/10.1016/j.agee.2017.12.021.

Allam, M., Radicetti, E., Quintarelli, V., Petroselli, V., Marinari, S., Mancinelli, R., 2022. Influence of organic and mineral fertilizers on soil organic carbon and crop productivity under different tillage systems: a meta-analysis. Agriculture 12, 464. https://doi.org/10.3390/agriculture12040464.

Arabzadeh, G., Delisle-Houde, M., Dorais, M., Deschamps, M.-H., Derome, G.W., Tweddell, R.J., 2024. Evaluation of the antagonistic activity of black soldier fly frass extracts against plant pathogens using single- and double-layer agar bioassays. J. Insects Food Feed 10, 1731–1740. https://doi.org/10.1163/23524588-20230022.

... & Atta, K., Mondal, S., Gorai, S., Singh, A.P., Kumari, A., Ghosh, T., Jespersen, D., 2023. Impacts of salinity stress on crop plants: improving salt tolerance through genetic and molecular dissection. Front. Plant Sci. 14, 1241736. https://doi.org/10.3389/fpls.2023.1241736.

Barragán-Fonseca, K.Y., Nurfikari, A., van de Zande, E.M., van Loon, J.J.A., de Boer, W., Dicke, M., 2022. Insect frass and exuviae to promote plant growth and health. Trends. Plant Sci. 27, 646–654. https://doi.org/10.1016/j.tplants.2022.01.007.

Basri, N.E.A., Azman, N.A., Ahmad, I.K., Suja, F., Jalil, N.A.A., Amrul, N.F., 2022. Potential applications of frass derived from black soldier fly larvae treatment of food waste: a review. Foods 11, 2664. https://doi.org/10.3390/foods11172664.

Beesigamukama, D., Mochoge, B., Korir, N., Ghemoh, C.J., Subramanian, S., Tanga, C.M., 2021. In situ nitrogen mineralization and nutrient release by soil amended with black soldier fly frass fertilizer. Sci. Rep. 11, 14799. https://doi.org/10.1038/s41598-021-94269-3.

Bohm, K., Hatley, G.A., Robinson, B.H., Gutiérrez-Ginés, M.J., 2023. Analysis of chemical and phytotoxic properties of frass derived from black soldier fly-based bioconversion of biosolids. Sustainability 15, 11526. https://doi.org/10.3390/su151511526.

Britto, D.T., Kronzucker, H.J., 2002. NH_4^+ toxicity in higher plants: a critical review. J. Plant Physiol. 159, 567–584. https://doi.org/10.1078/0176-1617-0774.

Brychkova, G., McGrath, A., Larkin, T., Goff, J., McKeown, P.C., Spillane, C., 2024. Use of anaerobic digestate to substitute inorganic fertilizers for more sustainable nitrogen cycling. J. Clean. Prod. 446, 141016. https://doi.org/10.1016/j.jclepro.2024.141016.

Chang, L., Xiong, X., Hameed, M.K., Huang, D., Niu, Q., 2023. Study on nitrogen demand model in pak choi (*Brassica campestris* ssp. *Chinensis* L.) based on nitrogen contents and phenotypic characteristics. Front. Plant Sci. 14, 1111216. https://doi.org/10.3389/fpls.2023.1111216.

Chavez, M.Y., Uchanski, M., Tomberlin, J.K., 2023. Impacts of black soldier fly, (Diptera: stratiomyidae) larval frass on tomato production. J. Econ. Entomol. 116, 1490–1495. https://doi.org/10.1093/jee/toad150.

Chen, C., Chaudhary, A., Mathys, A., 2020. Nutritional and environmental losses embedded in global food waste. Resour. Conserv. Recycl. 160, 104912. https://doi.org/10.1016/j.resconrec.2020.104912.

Cheong, J.C., Lee, J.T., Lim, J.W., Song, S., Tan, J.K., Chiam, Z.Y., Tong, Y.W., 2020. Closing the food waste loop: food waste anaerobic digestate as fertilizer for the cultivation of the leafy vegetable, xiao bai cai (*Brassica rapa*). Sci. Total Environ. 715, 136789. https://doi.org/10.1016/j.scitotenv.2020.136789.

de Vries, F.T., Griffiths, R.I., Knight, C.G., Nicolitch, O., Williams, A., 2020. Harnessing rhizosphere microbiomes for drought-resilient crop production. Science 368, 270–274. https://doi.org/10.1126/science.aaz5192.

de Bang, T.C., Husted, S., Laursen, K.H., Persson, D.P., Schjoerring, J.K., 2021. The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. New. Phytol. 229, 2446–2469. https://doi.org/10.1111/nph.17074.

Du, Z., Zhao, P., Fu, Q., Wang, Q., Hu, A., Zhang, W., Wang, D., 2023. Biostimulants in dissolved organic matters recovered from anaerobic digestion sludge with alkali-hydrothermal treatment: nontarget identification by ultrahigh-resolution mass spectrometry. Environ. Int. 173, 107813. https://doi.org/10.1016/j.envint.2023.107813.

Egene, C.E., Sigurnjak, I., Regelink, I.C., Schoumans, O.F., Adani, F., Michels, E., Meers, E., 2021. Solid fraction of separated digestate as soil improver: implications for soil fertility and carbon sequestration. J. Soils Sediments 21, 678–688. https://doi.org/10.1007/s11368-020-02792-z.

Elser, J., Bennett, E., 2011. A broken biogeochemical cycle. Nature 478 (7367), 29–31. https://doi.org/10.1038/478029a.

Erisman, J.W., Sutton, M.A., Galloway, J., Klimont, Z., Winiwarter, W., 2008. How a century of ammonia synthesis changed the world. Nat. Geosci. 1 (10), 636–639. https://doi.org/10.1038/ngeo325.

Esteban, R., Ariz, I., Cruz, C., Moran, J.F., 2016. Mechanisms of ammonium toxicity and the quest for tolerance. Plant Sci. 248, 92–101. https://doi.org/10.1016/j.plantsci.2016.04.008.

Esteves, C., Fareleira, P., Castelo-Branco, M.A., Lopes, I.G., Mota, M., Murta, D., Menino, R., 2022. Black soldier fly larvae frass increases the soil's residual nutrient content and enzymatic activity–a lettuce production trial. J. Insects Food Feed 8, 1431–1440. https://doi.org/10.3920/JIFF2022.0005.

Farquhar, G.D., von Caemmerer, S., Berry, J.A., 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149, 78–90. https://doi.org/10.1007/BF00386231.

Gatto, A., Chepeliev, M., 2024. Global food loss and waste estimates show increasing nutritional and environmental pressures. Nat. Food 5, 136–147. https://doi.org/10.1038/s43016-023-00915-6.

Gebremikael, M.T., van Wickeren, N., Hosseini, P.S., De Neve, S., 2022. The impacts of black soldier fly frass on nitrogen availability, microbial activities, C sequestration, and plant growth. Front. Sustain. Food Syst. 6, 795950. https://doi.org/10.3389/fsufs.2022.795950.

Geissdoerfer, M., Savaget, P., Bocken, N.M., Hultink, E.J., 2017. The circular economy – a new sustainability paradigm? J. Clean. Prod. 143, 757–768. https://doi.org/10.17863/CAM.7193.

Gilbert, J.A., Jansson, J.K., Knight, R., 2014. The earth microbiome project: successes and aspirations. BMC. Biol. 12 (1). https://doi.org/10.1186/s12915-014-0069-1.

Gold, M., von Allmen, F., Zurbrügg, C., Zhang, J., Mathys, A., 2020. Identification of bacteria in two food waste black soldier fly larvae rearing residues. Front. Microbiol. 11, 582867. https://doi.org/10.3389/fmicb.2020.582867.

Green, T., 2023. A biochemical analysis of Black Soldier fly (*Hermetia illucens*) larval frass plant growth promoting activity. PLoS. One 18, e0288913. https://doi.org/10.1371/journal.pone.0288913.

Gupta, A., Rico-Medina, A., Cano-Delgado, A.I., 2020. The physiology of plant responses to drought. Science 368, 266–269. https://doi.org/10.1126/science.aaz7614.

Hasanuzzaman, M., Bhuyan, M.B., Nahar, K., Hossain, M.S., Mahmud, J.A., Hossen, M.S., Masud, A.A.C., Moumita, Fujita, M., 2018. Potassium: a vital regulator of plant responses and tolerance to abiotic stresses. Agronomy 8, 31. https://doi.org/10.3390/agronomy8030031.

Horta, C., Carneiro, J.P., 2021. Use of digestate as organic amendment and source of nitrogen to vegetable crops. Appl. Sci. 12, 248. https://doi.org/10.3390/app12010248.

Hua, Y., Chen, S., Tong, T., Li, X., Ji, R., Xu, Q., Zhang, Y., Dai, X., 2024. Elucidating the molecular mechanisms and comprehensive effects of sludge-derived plant biostimulants on crop growth: insights from metabolomic analysis. Adv. Sci., 2404210 https://doi.org/10.1002/advs.202404210.

Huang, R., McGrath, S.P., Hirsch, P.R., Clark, I.M., Storkey, J., Wu, L., Zhou, J., Liang, Y., 2019. Plant-microbe networks in soil are weakened by century-long use of inorganic fertilizers. Microb. Biotechnol. 12, 1464–1475. https://doi.org/10.1111/1751-7915.13487.

Huang, Z., Xu, S., Zheng, N., Yin, X., Yang, Y., Yao, H., 2024. Salty bio-converted organic fertilizer modulates soil greenhouse gas emissions. J. Clean. Prod. 445, 141192. https://doi.org/10.1016/j.jclepro.2024.141192.

Jin, K., Ran, Y., Alengebawy, A., Yang, G., Jia, S., Ai, P., 2022. Agro-environmental sustainability of using digestate fertilizer for solanaceous and leafy vegetables cultivation: insights on fertilizer efficiency and risk assessment. J. Environ. Manage 320, 115895. https://doi.org/10.1016/j.jenvman.2022.115895.

... & Johnson, R., Vishwakarma, K., Hossen, M.S., Kumar, V., Shackira, A.M., Puthur, J.T., Hasanuzzaman, M., 2022. Potassium in plants: growth regulation, signaling, and environmental stress tolerance. Plant Physiol. Biochem. 172, 56–69. https://doi.org/10.1016/j.plaphy.2022.01.001.

... & Khan, R.U., Ullah, I., Khurshid, G., Suboktagin, S., Khan, A.R., Zeb, I., Ahmad, R., 2024. Stimulatory effects of smoke solution and biogas digestate slurry application on photosynthesis, growth, and methylation profiling of solanum tuberosum. Plant Signal. Behav. 19, 2336724. https://doi.org/10.1080/15592324.2024.2336724.

Lee, M.E., Steiman, M.W., Angelo, S.K.S., 2021. Biogas digestate as a renewable fertilizer: effects of digestate application on crop growth and nutrient composition. Renew. Agric. Food Syst. 36, 173–181. https://doi.org/10.1017/S1742170520000186.

Leogrande, R., Vitti, C., 2019. Use of organic fertilizers to reclaim saline and sodic soils: a review. Arid Land Res. Manag. 33, 1–21. https://doi.org/10.1080/15324982.2018.1498038.

Liang, Z., Jin, X., Zhai, P., Zhao, Y., Cai, J., Li, S., Li, C., 2022. Combination of organic fertilizer and slow-release fertilizer increases pineapple yields, agronomic efficiency

and reduces greenhouse gas emissions under reduced fertilization conditions in tropical areas. J. Clean. Prod. 343, 131054. https://doi.org/10.1016/j.jclepro.2022.131054.
Lopes, I.G., Gómez-Brandón, M., Praeg, N., Claeys, J., Yakti, W., Bitterlich, M., Geilfus, C.M., Klammsteiner, T., 2025. BugBook: critical considerations for evaluating and applying insect frass. J. Insects Food Feed 1–28. https://doi.org/10.1163/23524588-bja10254.
Lopes, I.G., Wiklicky, V., Ermolaev, E., Lalander, C., 2023. Dynamics of black soldier fly larvae composting - impact of substrate properties and rearing conditions on process efficiency. Waste Manag. 172, 25–32. https://doi.org/10.1016/j.wasman.2023.08.045.
Lopes, I.G., Wiklicky, V., Vinnerås, B., Yong, J.W.H., Lalander, C., 2024. Recirculating frass from food waste bioconversion using black soldier fly larvae: impacts on process efficiency and product quality. J. Environ. Manage 366, 121869. https://doi.org/10.1016/j.jenvman.2024.121869.
Lopes, I.G., Yong, J.W.H, Lalander, C., 2022. Frass derived from black soldier fly larvae treatment of biodegradable wastes. A critical review and future perspectives. Waste Manag. 142, 65–76. https://doi.org/10.1016/j.wasman.2022.02.007.
Lu, Y., Wang, E., Tang, Z., Rui, J., Li, Y., Tang, Z., Dong, W., Liu, X., George, T.S., Song, A., Fan, F., 2021. Roots and microbiome jointly drive the distributions of 17 phytohormones in the plant soil continuum in a phytohormone-specific manner. Plant Soil 470, 153–165. https://doi.org/10.1007/s11104-021-04898-w.
Luo, Y., Liang, J., Zeng, G., Chen, M., Mo, D., Li, G., Zhang, D., 2018. Seed germination test for toxicity evaluation of compost: its roles, problems and prospects. Waste Manag. 71, 109–114. https://doi.org/10.1016/j.wasman.2017.09.023.
Ma, M., Liu, Y., Bai, C., Yang, Y., Sun, Z., Liu, X., Zhang, S., Han, X., Yong, J.W.H., 2021. The physiological functionality of PGR5/PGRL1-dependent cyclic electron transport in sustaining photosynthesis. Front. Plant Sci. 12, 702196. https://doi.org/10.3389/fpls.2021.702196.
Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence - a practical guide. J. Exp. Bot. 51 (345), 659–668. https://doi.org/10.1093/jexbot/51.345.659.
Möller, K., Müller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. Eng. Life Sci. 12, 242–257. https://doi.org/10.1002/elsc.201100085.
Moraes, N.V., Lermen, F.H., Echeveste, M.E.S., 2021. A systematic literature review on food waste/loss prevention and minimization methods. J. Environ. Manage 286, 112268. https://doi.org/10.1016/j.jenvman.2021.112268.
Mostofa, M.G., Rahman, M.M., Ghosh, T.K., Kabir, A.H., Abdelrahman, M., Khan, M.A.R., Tran, L.S.P., 2022. Potassium in plant physiological adaptation to abiotic stresses. Plant Physiol. Biochem. 186, 279–289. https://doi.org/10.1016/j.plaphy.2022.07.011.
Mukhuba, M., Roopnarain, A., Adeleke, R., Moeletsi, M., Makofane, R., 2018. Comparative assessment of bio-fertilizer quality of cow dung and anaerobic digestion effluent. Cogent. Food Agric. 4, 1435019. https://doi.org/10.1080/23311932.2018.1435019.
Mulvaney, R.L., Khan, S.A., Ellsworth, T.R., 2009. Synthetic nitrogen fertilizers deplete soil nitrogen: a global dilemma for sustainable cereal production. J. Environ. Qual. 38, 2295–2314. https://doi.org/10.2134/jeq2008.0527.
Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annu Rev. Plant Biol. 59, 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911.
Pantha, P., Oh, D.H., Longstreth, D., Dassanayake, M., 2023. Living with high potassium: balance between nutrient acquisition and K-induced salt stress signaling. Plant Physiol. 191, 1102–1121. https://doi.org/10.1093/plphys/kiac564.
Pecherina, A., Grinberg, M., Ageyeva, M., Zanegina, D., Akinchits, E., Brilkina, A., Vodeneev, V., 2022. Salt-induced changes in cytosolic pH and photosynthesis in tobacco and potato leaves. Int. J. Mol. Sci. 24 (1), 491. https://doi.org/10.3390/ijms24010491.
Poveda, J., Jiménez-Gómez, A., Saati-Santamaría, Z., Usategui-Martín, R., Rivas, R., García-Fraile, P., 2019. Mealworm frass as a potential biofertilizer and abiotic stress tolerance-inductor in plants. Appl. Soil Ecol. 142, 110–122. https://doi.org/10.1016/j.apsoil.2019.04.016.
Radzikowska-Kujawska, D., Sawinska, Z., Grzanka, M., Kowalczewski, P.Ł., Sobiech, Ł., Świtek, S., Nowicki, M., 2023. *Hermetia illucens* frass improves the physiological state of basil (*Ocimum basilicum* L.) and its nutritional value under drought. PLoS. One 18 (1), e0280037. https://doi.org/10.1371/journal.pone.0280037.
Ren, Y., Yu, M., Wu, C., Wang, Q., Gao, M., Huang, Q., Liu, Y., 2018. A comprehensive review on food waste anaerobic digestion: research updates and tendencies. Bioresour. Technol 247, 1069–1076. https://doi.org/10.1016/j.biortech.2017.09.109.
Richardson, A.D., Duigan, S.P., Berlyn, G.P., 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytol. 153, 185–194. https://doi.org/10.1046/j.0028-646X.2001.00289.x.
Risberg, K., Cederlund, H., Pell, M., Arthurson, V., Schnürer, A., 2017. Comparative characterization of digestate versus pig slurry and cow manure–Chemical composition and effects on soil microbial activity. Waste Manag. 61, 529–538. https://doi.org/10.1016/j.wasman.2016.12.016.
Roopnarain, A., Akindolire, M.A., Rama, H., Ndaba, B., 2023. Casting light on the micro-organisms in digestate: diversity and untapped potential. Fermentation 9, 160. https://doi.org/10.3390/fermentation9020160.
Sani, M.N.H., Amin, M., Bergstrand, K.J., Caspersen, S., Prade, T., Yong, J.W.H., 2025. Harnessing biostimulants from biogas digestates for high-value resource recovery: a review. Environ. Chem Lett. https://doi.org/10.1007/s10311-024-01801-8. *in press*.
Sani, M.N.H., Yong, J.W.H., 2022. Harnessing synergistic biostimulatory processes: a plausible approach for enhanced crop growth and resilience in organic farming. Biol. (Basel) 11, 41. https://doi.org/10.3390/biology11010041.
Scaglia, B., Nunes, R.R., Rezende, M.O.P., Tambone, F., Adani, F., 2016. Investigating organic molecules responsible of auxin-like activity of humic acid fraction extracted from vermicompost. Sci. Total Environ. 562, 289–295. https://doi.org/10.1016/j.scitotenv.2016.03.212.
Setti, L., Francia, E., Pulvirenti, A., Gigliano, S., Zaccardelli, M., Pane, C., Ronga, D., 2019. Use of black soldier fly (*Hermetia illucens* (L.), Diptera: stratiomyidae) larvae processing residue in peat-based growing media. Waste Manag. 95, 278–288. https://doi.org/10.1016/j.wasman.2019.06.017.
Shabala, S., Munns, R., 2017. Salinity stress: physiological constraints and adaptive mechanisms. Plant Stress Physiology. Cabi, Wallingford UK, pp. 24–63. https://doi.org/10.1079/9781845939953.0059.
Sharma, P., Bano, A., Verma, K., Yadav, M., Varjani, S., Singh, S.P., Tong, Y.W., 2023. Food waste digestate as biofertilizer and their direct applications in agriculture. Bioresour. Technol. Rep. 23, 101515. https://doi.org/10.1016/j.biteb.2023.101515.
Siddiqui, S.A., Harahap, I.A., Osei-Owusu, J., Saikia, T., Wu, Y.S., Fernando, I., Câmara, J.S., 2024. Bioconversion of organic waste by insects–A comprehensive review. Process Saf. Environ. Prot. https://doi.org/10.1016/j.psep.2024.04.122.
Song, S., Ee, A.W.L., Tan, J.K.N., Cheong, J.C., Chiam, Z., Arora, S., Lam, W.N., Tan, H.T.W., 2021. Upcycling food waste using black soldier fly larvae: effects of further composting on frass quality, fertilising effect and its global warming potential. J. Clean. Prod. 288, 125664. https://doi.org/10.1016/j.jclepro.2020.125664.
Strasser, R.J., Tsimilli-Michael, M., Srivastava, A., 2004. Analysis of the chlorophyll a fluorescence transient. Chlorophyll a fluorescence: a Signature of Photosynthesis. Springer Netherlands, Dordrecht, pp. 321–362. https://doi.org/10.1007/978-1-4020-3218-9_12.
Tan, J.K.N., Lee, J.T.E., Chiam, Z., Song, S., Arora, S., Tong, Y.W., Tan, H.T.W., 2021. Applications of food waste-derived black soldier fly larval frass as incorporated compost, side-dress fertilizer and frass-tea drench for soilless cultivation of leafy vegetables in biochar-based growing media. Waste Manag. 130, 155–166. https://doi.org/10.1016/j.wasman.2021.05.025.
Tränkner, M., Tavakol, E., Jákli, B., 2018. Functioning of potassium and magnesium in photosynthesis, photosynthate translocation and photoprotection. Physiol. Plant 163, 414–431. https://doi.org/10.1111/ppl.12747.
Tyagi, J., Ahmad, S., Malik, M., 2022. Nitrogenous fertilizers: impact on environment sustainability, mitigation strategies, and challenges. Int. J. Environ. Sci. Technol. 19, 11649–11672. https://doi.org/10.1007/s13762-022-04027-9.
Van Midden, C., Harris, J., Shaw, L., Sizmur, T., Pawlett, M., 2023. The impact of anaerobic digestate on soil life: a review. Appl. Soil Ecol. 191, 105066. https://doi.org/10.1016/j.apsoil.2023.105066.
Vandegeer, R.K., Zhao, C., Cibils-Stewart, X., Wuhrer, R., Hall, C.R., Hartley, S.E., Johnson, S.N., 2021. Silicon deposition on guard cells increases stomatal sensitivity as mediated by K^+ efflux and consequently reduces stomatal conductance. Physiol. Plant 171, 358–370. https://doi.org/10.1111/ppl.13202.
Wang, F., Gao, J., Yong, J.W.H., Wang, Q., Ma, J., He, X., 2020. Higher atmospheric CO_2 levels favor C_3 plants over C_4 plants in utilizing ammonium as a nitrogen source. Front. Plant Sci. 11, e537443. https://doi.org/10.3389/fpls.2020.537443.
Wang, F., Wang, Q., Yu, Q., Ye, J., Gao, J., Liu, H., Yong, J.W.H., Yu, Y., Liu, X., Kong, H., He, X., Ma, J., 2022. Is the NH_4^+-induced growth inhibition caused by the NH4+ form of the nitrogen source or by soil acidification? Front. Plant Sci. 13, e968707. https://doi.org/10.3389/fpls.2022.968707.
Wang, N., Bai, X., Huang, D., Chen, Q., Shao, M., Xu, Q., 2023. Impacts of digestate-based compost on soil property and nutrient availability. Environ. Res. 234, 116551. https://doi.org/10.1016/j.envres.2023.116551.
Wang, X., Chen, Z., Sui, N., 2024. Sensitivity and responses of chloroplasts to salt stress in plants. Front. Plant Sci. 15, 1374086. https://doi.org/10.3389/fpls.2024.1374086.
White, P.J., Broadley, M.R., 2001. Chloride in soils and its uptake and movement within the plant: a review. Ann. Bot. 88, 967–988. https://doi.org/10.1006/anbo.2001.1540.
Wichuk, K.M., McCartney, D., 2010. Compost stability and maturity evaluation - a literature review. Can. J. Civ. Eng. 37, 1505–1523. https://doi.org/10.1139/L10-101.
Wong, W.S., Tan, S.N., Ge, L., Chen, X., Yong, J.W.H., 2015. The importance of phytohormones and microbes in biofertilizers: a critical review. In: Maheshwari, D.K. (Ed.), Bacterial Metabolites in Sustainable Agroecosystem. Springer International, Switzerland, pp. 105–158. https://doi.org/10.1007/978-3-319-24654-3_6.
Wong, W.S., Tan, S.N., Ge, L., Chen, X., Letham, D.S., Yong, J.W.H., 2016. The importance of phytohormones and microbes in biostimulants: mass spectrometric evidence and their positive effects on growth. Acta Hortic. 1148, e6. https://doi.org/10.17660/ActaHortic.2016.1148.6.
Wong, W.S., Zhong, H.T., Cross, A.T., Yong, J.W.H., 2020. Plant biostimulants in vermicomposts: characteristics and plausible mechanisms. In: Geelen, D., Xu, L. (Eds.), The Chemical Biology of Plant Biostimulants, 1st Ed. Wiley, pp. 155–180. https://doi.org/10.1002/9781119357254.ch6.
Wu, D., Chen, C., Liu, Y., Yang, L., Yong, J.W.H., 2023. Iso-osmotic calcium nitrate and sodium chloride stresses have differential effects on growth and photosynthetic capacity in tomato. Sci. Hortic. 312, 111883. https://doi.org/10.1016/j.scienta.2023.111883.
Yan, J., Chen, X., Wang, Z., Zhang, C., Meng, X., Zhao, X., Ma, X., Zhu, W., Cui, Z., Yuan, X., 2023. Effect of temperature and storage methods on liquid digestate: focusing on the stability, phytotoxicity, and microbial community. Waste Manag. 159, 1–11. https://doi.org/10.1016/j.wasman.2023.01.023.
Yang, J.W., Luyima, D., Park, S.J., Kim, S.H., Oh, T.K., 2021. Mixing sodium-chloride-rich food waste compost with livestock manure composts enhanced the agronomic performance of leaf lettuce. Sustainability 13 (23), 13223. https://doi.org/10.3390/su132313223.

Yong, J.W.H., Ng, Y.F., Tan, S.N., Chew, A.Y.L., 2010. Growth observations and the effects of fertilizer applications on physiology of *Jatropha curcas* L. Photosynthetica 48, 208–218. https://doi.org/10.1007/s11099-010-0026-3.

Yong, J.W.H., Letham, D.S., Wong, S.C., Farquhar, G.D., 2014. *Rhizobium*-induced elevation in xylem cytokinin delivery in pigeonpea induces changes in shoot development and leaf physiology. Funct. Plant Biol. 41, 1323–1335. https://doi.org/10.1071/FP14066.

Zhang, X., Guo, B., Yi, N., 2020. Zero-inflated gaussian mixed models for analyzing longitudinal microbiome data. PLoS. One 15 (11), e0242073. https://doi.org/10.1371/journal.pone.0242073.

Zhao, C., Zhang, H., Song, C., Zhu, J.K., Shabala, S., 2020. Mechanisms of plant responses and adaptation to soil salinity. Innovation 1 (1). https://doi.org/10.1016/j.xinn.2020.100017.

Zhou, R., Yu, X., Kjaer, K.H., Rosenqvist, E., Ottosen, C.-O., Wu, Z., 2015. Screening and validation of tomato genotypes under heat stress using F_v/F_m to reveal the physiological mechanism of heat tolerance. Environ. Exp. Bot. 118, 1–11. https://doi.org/10.1016/j.envexpbot.2015.05.006.

Zilio, M., Pigoli, A., Rizzi, B., Herrera, A., Tambone, F., Geromel, G., Adani, F., 2022. Using highly stabilized digestate and digestate-derived ammonium sulphate to replace synthetic fertilizers: the effects on soil, environment, and crop production. Sci. Total Environ. 815, 152919. https://doi.org/10.1016/j.scitotenv.2022.152919.

Zuo, G., Huo, J., Yang, X., Mei, W., Zhang, R., Khan, A., Feng, N., Zheng, D., 2024. Photosynthetic mechanisms underlying NaCl-induced salinity tolerance in rice (*Oryza sativa*). BMC. Plant Biol. 24 (1), 41. https://doi.org/10.1186/s12870-024-04723-3.