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Research article

Primary study on frass fertilizers from mass-reared insects: Species variation, heat treatment effects, and implications for soil application at laboratory scale

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ABSTRACT

Insect farming has gained popularity as a resource-efficient and eco-friendly method for managing organic wastes by converting them into high-quality protein, fat, and frass. Insect frass is a powerful organic fertilizer that enriches the soil with essential plant nutrients and enhances plant defense mechanisms through chitin stimulation. Given the importance of frass commercialization for many insect farmers and the use of increasingly diverse organic wastes as insect feedstocks, there is a need for legal guidelines to enable clean production practices. The recent introduction of a legal definition for frass and heat treatment requirements by the EU commission marks a significant step towards standardizing its quality; however, little is known about the processes shaping its nutritional profiles and contributing to its maturation. Our study addresses key knowledge gaps in frass composition and production practices. Here, we analyzed the physicochemical, plant-nutritional, and microbiological properties of black soldier fly, yellow mealworm, and Jamaican field cricket frass from mass-rearing operations and assessed the impact of hygienizing heat treatment on fertilizer properties and frass microbiota. The results showed that frass properties varied significantly across insect species, revealing concentrations of plant-available nutrients as high as 7000 μg NH_4^+-N, 150 μg NO_2–NO_3^--N, and 20 mg available P per g of total solids. Heat treatment reduced microbial activity, biomass, and viable counts of pathogenic *Escherichia coli* and *Salmonella* spp. In terms of frass microbiome composition, alpha diversity showed no significant differences between fresh and heat-treated frass samples; however, significant differences in microbial community composition were observed across the three insect species. Despite heat treatment, soil application of frass reactivated and boosted soil microbial activity, inducing up to a 25-fold increase in microbial respiration, suggesting no long-term detrimental effects on microorganisms. These findings not only enhance our understanding of insect frass as a nutrient-rich organic fertilizer but also have implications for regulatory frameworks, underscoring its promising potential for soil health and nutrient cycling. However, it is important to recognize the primary nature of this research, conducted at laboratory scale and over a short term. Future studies should aim to validate these findings in agricultural settings and explore additional factors influencing frass properties and its (long-term) interaction with soil ecosystems.

1. Introduction

Large-scale insect farming has emerged as a promising means to address prevalent socio-ecological problems. Compared to traditional livestock, it requires less land and water, yet achieves higher reproduction and conversion rates, while generating lower greenhouse gas emissions (van Huis and Oonincx, 2017). Furthermore, it could play a pivotal role in promoting a circular economy by transforming organic waste (e.g., food waste and manure) into valuable proteins and fats, thereby minimizing resource consumption and establishing an efficient loop within the food and feed production system (Cadinu et al., 2020; Walter et al., 2020). Among the vast range of edible insects (Jongema, 2017), species such as the black soldier fly (BSF; *Hermetia illucens*, Linnaeus 1758), the yellow mealworm (YMW; *Tenebrio molitor*; Linnaeus, 1758), and Jamaican field cricket (JFC; *Gryllus assimilis*; Fabricius, 1775) have become popular among insect farmers in Western countries

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(Wilkie, 2018). Their popularity is primarily owed to their ability to efficiently convert organic matter and their beneficial nutritional content (van Huis, 2013). Although insect products for human consumption are still viewed as niche products that often evoke aversion among potential consumers, insects are more readily accepted as feed for aquaculture, poultry, and pigs (Verbeke et al., 2015).

Insect farming primarily focuses on protein and fat production; however, it inevitably generates rearing residues that may significantly contribute to farm profitability (Niyonsaba et al., 2021). These residues include excrements, exuviae, undigested substrates, and dead insects and represent the main side stream of the process (Klammsteiner et al., 2020a). This so-called insect frass has been shown to have wide-ranging beneficial effects on plants and is mainly sold as an organic fertilizer (Ferruzca-Campos et al., 2023; Houben et al., 2020; Menino et al., 2021). The general composition of frass can be highly diverse, depending on the farmed species and substrate used to grow the insects. Despite its diversity, the microbiota associated with frass, in addition to insect-specific nutritional contents, are relatively understudied. However, the fertilizing effect of frass is associated with a high content of organic carbon, nitrogen, and phosphorus, which is comparable to that of other organic fertilizers (Beesigamukama et al., 2022).

Microorganisms introduced into the frass, primarily via insect feces, play a crucial role in enhancing the decomposition process (Houben et al., 2020; Klammsteiner et al., 2020b). These microbes may also contribute to making the frass more similar to the insect gut microbiome (Gold et al., 2020). However, the rapid development of farmed insect species, geared toward shortening rearing cycles (BSF: 20 days, YMW: 67 days, JFC: 60 days) (Heussler et al., 2022; Rumbos et al., 2021; Kulma et al., 2022), limits the time for microbes and insects to effectively modify the accumulated frass, resulting in a comparatively immature compost (Beesigamukama et al., 2022; Liu et al., 2022). While BSF larvae naturally aggregate and generate temperatures of up to 50 °C (Shishkov et al., 2019; Ushakova et al., 2018), these temperatures are insufficient for microbiological stabilization. This is in contrast to traditional composting methods that reach 70 °C or higher, ensuring the elimination of potentially harmful pathogens and weed seeds, promoting the microbiological maturation of the compost, and producing a stable end product (Insam et al., 2023; Zhou et al., 2022).

To increase product safety and reduce potential health hazards from pathogens in insect products, the EU Commission established a detailed definition of insect frass, categorizing it in the same group as processed animal manure. This regulation introduced hygienic standards for insect frass, requiring farmers to heat-treat frass at 70 °C for at least 60 min (Regulation, 2021). Although this mandatory pretreatment should ensure pathogen removal, it may also inhibit microbial activity beneficial to frass nutrient content, potentially altering its value as a soil fertilizer. To date, only one study has investigated the effect of heat treatment on the frass of black soldier fly larvae and found that it was successful in reducing Enterobacteriaceae, *Salmonella*, and *Clostridium perfringens* below the detection limit (Van Looveren et al., 2022). However, the total viable counts decreased by only 1-log and bacterial endospores were unaffected, and soil fertilizer quality was not assessed. With the rapid expansion of the insect farming sector and the value of commercializing rearing residues, a thorough assessment of risks and opportunities has become imperative.

In this study, we comprehensively characterized and compared the physicochemical and microbiological features of frass from three widely farmed insect species (BSF, YMW, and JFC). To explore the effects of hygienization, we subjected the frass to heat treatment following legislative guidelines. Using 16S rRNA gene amplicon sequencing, we evaluated differences in bacterial communities between insect species before and after heat treatment. Furthermore, we assessed how this hygienization process influences the suitability of frass as an organic soil amendment by conducting a soil incubation trial and screening frass-amended soils. We hypothesized that I) the physicochemical and microbiological properties of frass will strongly vary among the investigated insect species, II) heat treatment will have an extensive effect on the microbiological activity of frass, and III) while heat treatment can effectively reduce specific pathogens, there might be a compromise in overall microbial diversity and activity, affecting the value of frass as a soil fertilizer.

2. Materials and methods

2.1. Origin and pretreatment of the frass

Fresh untreated frass from BSF (Fig. 1A), YMW (Fig. 1B), and JFC (Fig. 1C) was obtained from commercial insect farmers in Austria (BSF and YMW) and Croatia (JFC). Upon arrival, the frass was stored at −20 °C and, prior to its use, gently thawed over 24 h at 4 °C. For the heat treatment, the frass was evenly spread on large glass petri dishes at a layer height of approx. 1 cm and incubated at 70 °C for 1 h in a preheated drying cabinet (Memmert, Schwabach, Germany). Following this procedure, the heat-treated frass was transferred into paper bags and left to cool to room temperature before further use.

2.2. Physicochemical parameters

2.2.1. Water, total solids, volatile solids, and ash content

The water and total solids (TS) contents were determined gravimetrically by calculating the loss in mass before and after drying the samples (n = 3) at 105 °C for 24 h in a drying cabinet (UN110, Memmert, Schwabach, Germany) (Schinner, 1996). To determine the volatile solids (VS) and ash contents, the TS fraction was finely ground using a mortar and pestle and incinerated in a muffle furnace (CWF 1000, Carbolite, Neuhausen, Germany) at 550 °C for 5 h (n = 3) (Schinner, 1996). The loss in mass was interpreted as the VS fraction, whereas the residue was considered as the ash content.

2.2.2. pH, electrical conductivity, and salinity

Samples (n = 4) were weighed into 50 mL plastic falcon tubes, mixed with a. deion. in a ratio of 1:12.5 (w/v), and incubated at room temperature overnight before measurement. A 774 pH meter (Metrohm, Herisau, Switzerland) was used to measure the pH of diluted samples. Electrical conductivity, salinity, and total dissolved solids were measured for the same samples using an LF330/SET conductivity electrode (WTW, Weilheim in Oberbayern, Germany).

2.2.3. Elemental analysis (CHNS)

Part of the dried biomass resulting from the TS determination was ground and sent to the Department of Waste and Resource Management (TU Wien, Vienna, Austria) for elemental analysis. The CHNS content was determined using a Vario MACRO elemental analyzer (Elementar, Langenselbold, Germany). First, 15 mg of the sample material wrapped in a tin capsule was combusted at 1150 °C, and the resulting combustion gas was separated through an adsorption column, reducing NOx to Cu and subsequently to N_2. Sequential desorption was induced by heating the absorption column and gases were measured using a thermal conductivity detector. He 5.0 was used as carrier gas.

2.2.4. Plant-available ammonium, nitrate and phosphorus content

Plant-available ammonium (NH_4^+-N; μg g^{-1} TS) and nitrate (NO_2–NO_3-N; μg g^{-1} TS) were determined using a modified Berthelot and cadmium reduction method, respectively, after shaking 2 g frass or 7.5 g soil:frass mixture in 30 mL KCl [1 M] for 1 h at 120 rpm (Schinner, 1996). Plant-available phosphorus (ortho-phosphate, μg g^{-1} TS) was determined by applying the Olsen method and shaking 0.4 g frass or 2 g soil:frass mixture in 40 ML LiCl [0.4 M] for 16 h at 150 rpm (Schinner, 1996). All extracts from frass and soil:frass mixtures were filtered (Macherey & Nagel 615¼, 150 mm filter paper) and analyzed for their respective nutrient contents using a Continuous Flow Analyzer (CFA, Skalar, Netherlands).

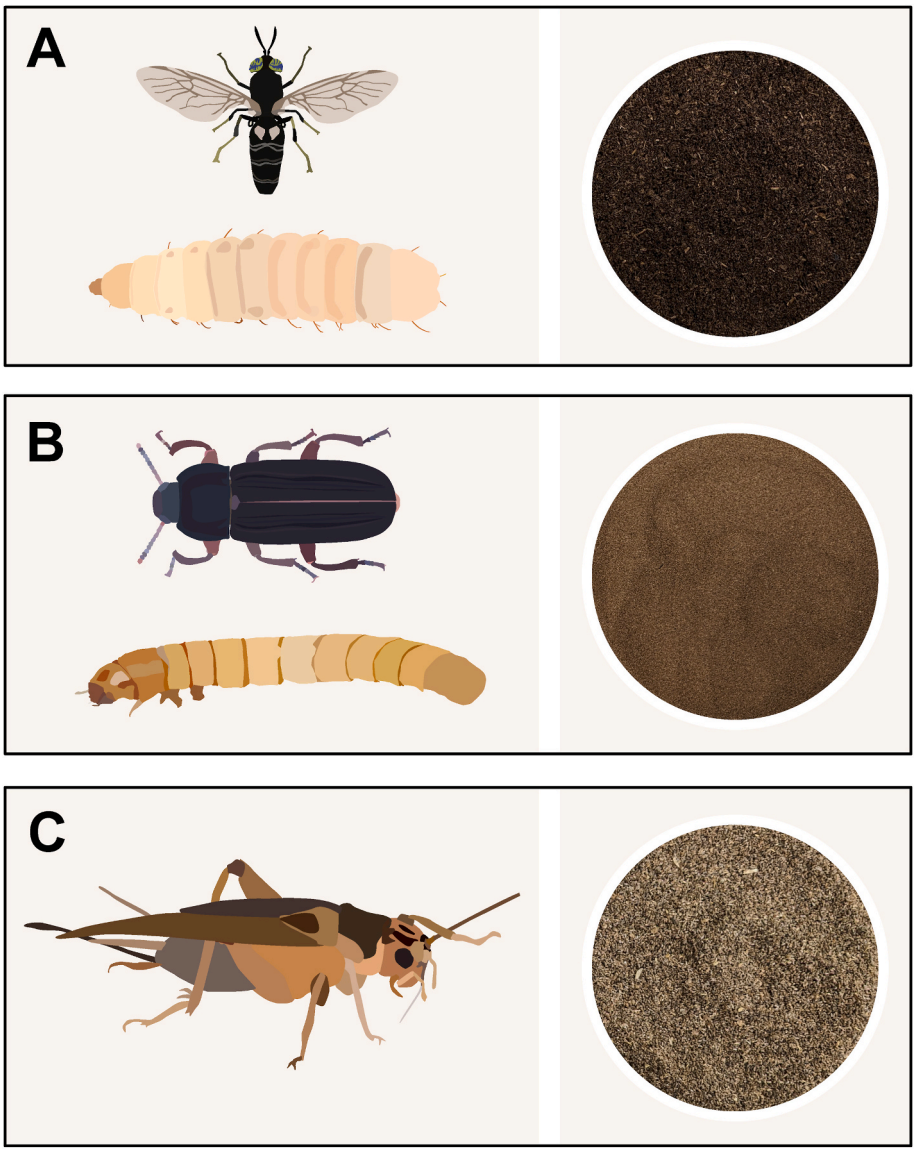

Fig. 1. Three insect species that are approved in the EU for food and feed applications, and also serve as frass producers. **A.** Black soldier fly (*Hermetia illucens*; Linnaeus, 1758) adult, larva, and its frass **B.** Yellow mealworm (*Tenebrio molitor*; Linnaeus, 1758) adult, larva, and its frass **C.** Jamaican field cricket (*Gryllus assimilis*; Fabricius, 1775) adult and its frass. The photos of frass represent the actual material used in this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.3. Microbiological parameters

2.3.1. Microbial respiration and biomass

Microbial respiration and substrate-induced respiration (for the calculation of microbial biomass) were determined using an EGA61-Soil respiration Device (ADC BioScientific, UK). Control soil, frass, and soil: frass mixtures were filled into acrylic glass tubes, closed with polystyrene foam pads, and aerated with a continuous stream of ambient air (humidified and tempered to 22 °C). The CO_2 released from the samples was recorded for 6 h using an infrared gas analyzer to calculate the microbial respiration (MR [μg CO2 g^{-1} TS h^{-1}]). Subsequently, glucose (1%, w/w dry weight) was added to the samples, and the CO_2 release was recorded for 12 h (substrate-induced respiration method). The maximum CO_2 release was used to calculate the microbial biomass (C_{mic} [μg C g^{-1} TS]), according to Anderson and Domsch (1978). The metabolic quotient (MQ) was calculated as the quotient of the MR and microbial biomass (C_{mic}).

2.3.2. Microbial counts

Standard I nutrient agar was prepared from 15 g peptone, 3 g yeast extract, 6 g NaCl, 1 g glucose, and 12 g agar, and adjusted to a pH of 7.5 ± 0.2 using HCl [0.5 M] to determine the total plate count of viable aerobic bacteria (Corry et al., 2011). Chromocult® Tryptone Bile X-glucuronide (TBX) agar (Merck, Darmstadt, Germany) and XLT4 agar (Merck) prepared according to the manufacturer's instructions, were used to detect and quantify *Escherichia coli* and *Salmonella* spp. For the dilution series, 1 g of sample biomass was sequentially diluted in sterile Ringer's solution (Merck, Darmstadt, Germany) to a dilution level of 10^{-8}. After selecting three appropriate dilution levels for each type of medium, 50 μL of the resulting dilutions were plated onto the respective agar plates. All plates were incubated at 37 °C and inspected after 24 and 48 h for quantification of colony-forming units (CFUs) and detection of pathogens.

2.3.3. DNA extraction and marker gene sequencing for bacterial and fungal communities

DNA was extracted from 150 (BSF), 200 (YMW), and 300 mg (JFC) of

both fresh and treated frass (n = 3) using the NucleoSpin Soil Kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's protocol. Lysis buffer SL2 was used for cell lysis, and the washed extracts were eluted in MN elution buffer. DNA yield and purity were assessed by UV–vis spectrophotometry using a NanoDrop 2000c device (Thermo Fisher Scientific, Waltham, MA, USA). All samples that passed the quality control were sent for 16S rRNA gene amplicon sequencing. Sequencing was carried out on the NovaSeq6000 platform (Illumina, San Diego, CA, USA) following a 2 × 250 bp approach and using the primer pair 515f (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806r (5′-GGACTACHVGGGTWTCTAAT-3′) to target the V4 region of the 16S rRNA gene. Raw reads were processed using '*dada2*' v.1.26.0 (Callahan et al., 2016) and classified into amplicon sequence variants following the latest standard operating procedure (https://benjjneb.github.io/dada2/tutorial.html). Briefly, adapter- and primer-free reads were truncated to a length of 200 bp based on the inspected quality profiles, and any reads containing Ns were discarded. After learning the error rates and sample inference using default settings, paired-end reads were merged to construct the sequence table. The chimeras were removed using the removeBimeraDenovo() command by applying the "consensus" method. Taxonomy was assigned to the amplicon sequence variants based on SILVA trainset version 138.1 (Quast et al., 2013). Amplicon sequence variants consisting of less than three reads that were not detected in at least 10% of the samples were removed from the data.

2.4. Soil incubation trial

Soil was collected from nearby agricultural land in Austria (Tyrol, Innsbruck; 47°15′47.6″N 11°20′24.0″E) and stored at 4 °C overnight. For the mesocosm experiment, any plant residues and stones were removed by passing the soil through a 4 mm sieve. Detailed information about the land use history, soil type, and soil physicochemical properties can be found in Table S1.

Soil:frass mixtures were prepared according to the recommended dosage for each type of fresh frass. For the heat-treated frass, the amounts were adjusted to match the total solids content of the untreated frass, as indicated in Table S2. For each replicate (n = 4), 200 g of sieved soil was thoroughly mixed with frass at the recommended ratio in a plastic bucket. The mixtures were transferred into nursery pots for plants ($Ø_{top}$ = 90 mm, $Ø_{bottom}$ = 60 mm, h = 80 mm), evenly moistened with a. deion. using a spray bottle, and loosely covered with cling foil. The pots were incubated in a shaded greenhouse for 14 days at 20 °C and 70% relative humidity. Water loss due to evaporation was monitored continuously by weighing the pots on a portable scale (KF6000A, G&G, Kaarst, Germany). Soil moisture within the pots decreased by max. 25%. To maintain consistency, the soil moisture was continuously adjusted to its initial value (Fig. S1).

2.5. Statistics and data analysis

All statistical calculations and visualizations were performed using R v.4.1.2 (R Core Team, 2021). The normal distribution of the data was tested using the Shapiro-Wilk test (Shapiro and Wilk, 1965). Non-normally distributed data were transformed using either the Box-Cox transformation (physicochemical parameters) or square root transformation (microbiological parameters) (Box and Cox, 1964). To assess significant differences in the physicochemical composition of the frass types and soil:frass mixtures, analysis of variance was calculated using the aov() function from the R '*stats*' package. For overall pairwise comparisons, Tukey's Honestly Significant Difference (Tukey HSD) posthoc test was calculated using the glht() function in the '*multcomp*' package (Hothorn et al., 2008), and summaries for statistical similarities and differences were generated using the multcompletters4() function in the '*multcompView*' package (Graves et al., 2019). To assess the variability of physicochemical parameters, principal component analysis was performed on the normalized data (TS, H_2O, VS, ash, pH, electrical conductivity, salinity, C, H, N, S, NH_4^+-N, NO_2–NO_3^--N, P, MR, MQ, and C_{mic}) using the prcomp() function in the R '*stats*' package. The results from the principal component analysis were visualized using the fviz_pca_biplot() function in the R '*factoextra*' package (Kassambara and Mundt, 2020). Differences in diversity (alpha diversity) of microbial communities of the tested frass types were calculated via analysis of variance and pairwise Wilcoxon rank-sum test using the pairwise.wilcox.test() function with Bonferroni correction from the '*stats*' package. To compare the microbial community structure among the samples, beta diversity was calculated and visualized via principal coordinates analysis using the amp_ordinate() function in '*ampvis2*' (Andersen et al., 2018). Linear discriminant analysis of effect size for the identification of biomarkers in microbial community data was calculated using the run_lefse() function from the '*microbiomeMarker*' package (Yang, 2021). To test the differences between groups of samples, permutational multivariate analysis of variance based on Bray-Curtis dissimilarity matrices was calculated using the adonis2() function in the '*vegan*' package (Oksanen et al., 2020). Redundancy analysis integrating frass microbiome data, physicochemical parameters, and measurements of microbial activity was calculated using the '*microeco*' package (Liu et al., 2021).

3. Results

The aim of this study was to characterize the physicochemical and microbiological properties of frass samples obtained from three industrially exploited insect species (Fig. 1), and to emphasize the key points of differentiation among them. Given the increasing concerns regarding the safety of utilizing untreated rearing residues from insect farming as agricultural fertilizer, we further examined the effects of heat treatment at 70 °C for 1 h on the physicochemical and microbiological characteristics of the frass.

3.1. Physicochemical characterization of fresh and heat-treated frass

The frass samples varied significantly in their general physicochemical composition, with the primary differentiating factor being the insect species of origin, as highlighted by the first two principal components of the principal component analysis, which explained 85% of the variance in the data (Fig. 2A). Heat treatment had a comparably minor impact on the distribution of the samples, as they showed minimal divergence from their corresponding fresh counterparts upon pairwise comparison of the respective groups (Fig. 2B). A detailed analysis of the physicochemical drivers is presented in Table 1. The fresh frass samples represented the conditions in which the untreated material was sold by the producers (Fig. 1A–C); however, they significantly varied in their water content. While the BSF frass was comparatively humid, with a water content of 42.65%, the other two types of frass ranged between 12.12 and 14.57%. The heat treatment significantly reduced the water content in the BSF frass by 12.17%, whereas YMW and JFC lost 3.72% and 6.43%, respectively. Accordingly, BSF frass exhibited the lowest total solids (TS) content of 57.35%. Comparable patterns applied to the relationship between volatile solids (VS) and ash content.

Elemental analysis revealed no significant differences in the relative contents of C, H, and S among the three types of frass, both before and after heat treatment. However, significant differences in the N content were observed, with JFC frass containing up to twice as much N, significantly shifting its C:N ratio to 6.38–6.82 as opposed to an average of 15.20 in BSF and 10.93 in YMW frass.

Fresh BSF frass demonstrated the highest NH_4^+-N content, reaching 6988.55 μg g^{-1} TS. Notably, it was also the only type of frass where the NH_4^+-N content was significantly reduced following heat treatment. YMW samples contained NO_2–NO_3^--N levels that were 3–7 times higher than those in JFC samples and 50–55 times higher than those in BSF, thereby contributing to the distinctiveness of this particular type of frass. Generally, NO_2–NO_3^--N concentrations were not affected by heat

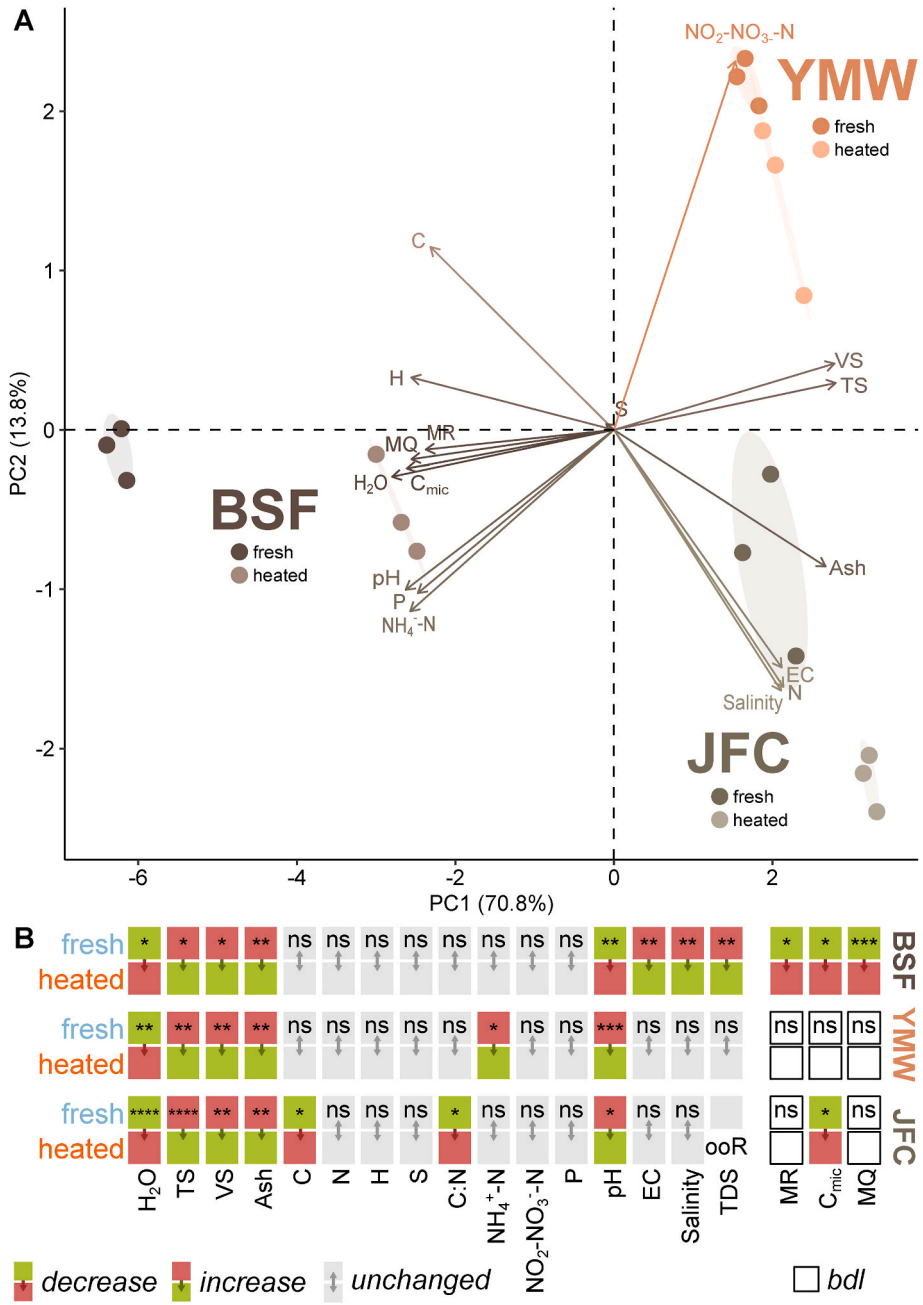

Fig. 2. A. Principal component analysis of physicochemical and microbiological parameters measured in fresh and heat-treated frass samples of black soldier fly (BSF), yellow mealworm (YMW), and Jamaican field cricket (JFC) (n = 3). **B**. Heatmap showing results of pairwise t-tests for fresh and heat-treated frass samples. TS = total solids, VS = volatile solids, C:N = carbon to nitrogen ratio, NH_4^+-N = ammonium nitrogen, NO_2–NO_3^--N = nitrite and nitrate nitrogen, EC = electrical conductivity, TDS = total dissolved solids, MR = microbial respiration, C_{mic} = microbial biomass carbon, MQ = metabolic quotient. ns: $p > 0.05$, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, ****: $p \leq 0.0001$, ooR = out of range, bdl = below detection limit. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

treatment, except for the JFC samples, which showed a 2.7-fold decrease from an average of 51.74 to 18.83 μg g^{-1} TS (Table 1). With more than 20 mg g^{-1} TS, plant-available P in the BSF frass samples exceeded the concentrations of the other two types of frass. The plant-available P content remained unaffected by heat treatment across all frass types.

The pH of fresh frass ranged between 6.24 and 7.66, and after heat treatment, it slightly decreased in BSF, slightly increased in YMW, and remained unchanged in JFC frass. The electrical conductivity slightly increased in all samples after heat treatment but remained between 4.55 and 5.09. As measurements of salinity and total dissolved solids are functions of the electrical conductivity, they followed the same patterns.

3.2. Microbiological characterization of fresh and heat-treated frass

Microbial activity was mainly observed in fresh BSF frass, and heat treatment significantly reduced this activity (Table 2). In these samples, the MR decreased by a factor of 23 after heating and reduced the C_{mic} to a third. In turn, MQ as a function of MR and C_{mic} decreased from 30.19 to 4.56 μg CO_2–C h^{-1} mg^{-1} C_{mic} after the treatment. Although significantly less microbial biomass (C_{mic}) was measured in fresh JFC frass, no physiologically active microbial activity (MR) was detected in these samples. Microbial activity in fresh and heat-treated frass of YMW was below the detection limit.

Table 1
Physicochemical parameters (mean ± standard deviation) before and after treating the frass at 70 °C for 1 h. Statistical differences were calculated via analysis of variance followed by Tukey HSD posthoc tests for pairwise comparison of sample groups (n = 4). TS = total solids, ooR = out of range, ns = not significant.

	BSF frass		YMW frass		JFC frass		*F-value*
	Fresh	Heated	Fresh	Heated	Fresh	Heated	
Water content [%]	42.65 ± 3.30^{a}	30.48 ± 1.00^{b}	12.12 ± 0.08^{d}	8.40 ± 0.40^{e}	14.57 ± 0.22^{c}	8.14 ± 0.20^{e}	*F = 855.90****
TS [%]	57.35 ± 3.30^{d}	69.52 ± 1.00^{c}	87.88 ± 0.08^{b}	91.60 ± 0.40^{a}	85.43 ± 0.22^{b}	91.86 ± 0.20^{a}	*F = 473.20****
Volatile solids [%]	50.36 ± 3.06^{e}	61.15 ± 0.92^{d}	78.67 ± 0.06^{b}	82.02 ± 0.36^{a}	75.58 ± 0.14^{c}	80.80 ± 0.38^{ab}	*F = 456.02****
Ash [%]	6.99 ± 0.25^{e}	8.37 ± 0.10^{d}	9.21 ± 0.03^{c}	9.58 ± 0.04^{bc}	9.85 ± 0.20^{b}	11.06 ± 0.20^{a}	*F = 223.27****
C [%]	42.79 ± 0.29^{a}	42.80 ± 0.27^{a}	42.06 ± 0.05^{b}	42.04 ± 0.07^{b}	41.33 ± 0.15^{c}	40.83 ± 0.25^{d}	*F = 73.37****
H [%]	5.90 ± 0.05^{a}	5.83 ± 0.02^{a}	5.55 ± 0.07^{bc}	5.60 ± 0.03^{b}	5.50 ± 0.07^{cd}	5.45 ± 0.03^{d}	*F = 62.35****
N [%]	2.84 ± 0.05^{c}	2.80 ± 0.14^{c}	3.84 ± 0.03^{b}	3.85 ± 0.03^{b}	6.07 ± 0.15^{a}	6.41 ± 0.20^{a}	*F = 648.35****
S [%]	0.92 ± 0.17^{ab}	0.77 ± 0.01^{b}	0.92 ± 0.17^{ab}	0.78 ± 0.01^{b}	0.98 ± 0.18^{a}	0.88 ± 0.01^{ab}	F = 3.94**
C:N ratio	15.09 ± 0.40^{a}	15.30 ± 0.84^{a}	10.95 ± 0.08^{b}	10.91 ± 0.07^{b}	6.82 ± 0.19^{c}	6.38 ± 0.23^{c}	*F = 462.91****
NH_4^+-N [µg g^{-1} TS]	6988.55 ± 371.51^{a}	5877.16 ± 90.10^{b}	848.24 ± 7.58^{d}	916.37 ± 15.91^{d}	2599.08 ± 44.07^{c}	2549.70 ± 40.75^{c}	*F = 3224.22****
NO_2–NO_3-N [µg g^{-1} TS]	2.95 ± 0.95^{d}	2.61 ± 0.40^{d}	153.28 ± 13.23^{a}	145.74 ± 7.55^{a}	51.74 ± 24.12^{b}	18.83 ± 1.97^{c}	*F = 135.31****
$P_{plant\ available}$ [mg g^{-1} TS]	20.34 ± 1.56^{a}	20.86 ± 0.55^{a}	10.16 ± 0.14^{c}	10.95 ± 0.30^{c}	12.73 ± 1.20^{b}	12.91 ± 0.98^{b}	*F = 74.03****
pH	7.66 ± 0.07^{a}	7.34 ± 0.01^{b}	6.24 ± 0.02^{e}	6.39 ± 0.03^{d}	6.57 ± 0.02^{c}	6.62 ± 0.01^{c}	*F = 896.38****
Electrical conductivity [mS cm^{-1}]	4.13 ± 0.11^{d}	4.55 ± 0.06^{bc}	4.43 ± 0.04^{c}	4.66 ± 0.16^{bc}	4.82 ± 0.26^{a}	5.09 ± 0.01^{ab}	*F = 12.03****
Salinity	2.13 ± 0.08^{d}	2.38 ± 0.05^{bc}	2.30 ± 0.00^{c}	2.43 ± 0.13^{bc}	2.50 ± 0.14^{b}	2.70 ± 0.00^{a}	*F = 12.15****
Total dissolved solids [mg L^{-1}]	1647.25 ± 42.17^{c}	1816.00 ± 24.43^{ab}	1766.50 ± 16.42^{b}	1862.75 ± 65.51^{ab}	1881.33 ± 57.07^{a}	ooR	*F = 16.99****

Table 2
Microbiological parameters (mean ± standard deviation) before and after treating the frass at 70 °C for 1 h. Statistical differences were calculated via analysis of variance followed by Tukey HSD posthoc tests for pairwise comparison of sample groups (n = 4). TS = total solids, MR = microbial respiration, C_{mic} = microbial biomass carbon, MQ = metabolic quotient, bdl = below detection limit.

	BSF frass		YMW frass		JFC frass		F-value
	Fresh	Heated	Fresh	Heated	Fresh	Heated	
MR [µg CO_2 g^{-1} TS frass h^{-1}]	318.30 ± 64.14^{a}	13.83 ± 1.83^{b}	bdl^{c}	bdl^{c}	bdl^{c}	bdl^{c}	*F = 283.46****
C_{mic} [µg C g^{-1} TS frass]	10,626.51 ± 2662.60^{a}	3064.24 ± 682.43^{b}	bdl^{c}	bdl^{c}	202.65 ± 49.27^{c}	bdl^{c}	*F = 157.88****
MQ [µg CO_2–C h^{-1} mg^{-1} C_{mic}]	30.19 ± 1.46^{a}	4.56 ± 0.38^{b}	bdl^{c}	bdl^{c}	bdl^{c}	bdl^{c}	*F = 3493.71****

Only in BSF frass, CFU counts of aerobically cultivable bacteria were significantly reduced from 1.3 10^9 to 3.8 10^8 after heat treatment (Fig. 3, Table S3, Table S4). No significant reduction was observed in YMW and JFC frass. CFUs of *E. coli* were found in neither fresh nor heat-treated samples of any of the three insect species. However, *Salmonella* spp. was detected in fresh JFC frass, with comparably low CFU counts of 1.7 10^3, which were reduced to below the detection limit after heat treatment.

3.3. Analysis of microbial communities in fresh and heat-treated frass

The initial 137,110 ± 4633 raw reads were reduced to 125,474 ± 6322 merged reads per sample after pre-processing. Alpha diversity, as measured by observed species (Fig. 4A) and the Shannon-Wiener index (Fig. 4B), showed no significant differences between fresh and heated frass samples within each insect species, as confirmed by the Wilcoxon rank-sum test. However, the frass samples, whether fresh or heated, significantly differed across the three insect species in terms of observed species (fresh: $F_{2,6} = 31.9$, $p < 0.001$; heated: $F_{2,6} = 118$, $p < 0.001$) and

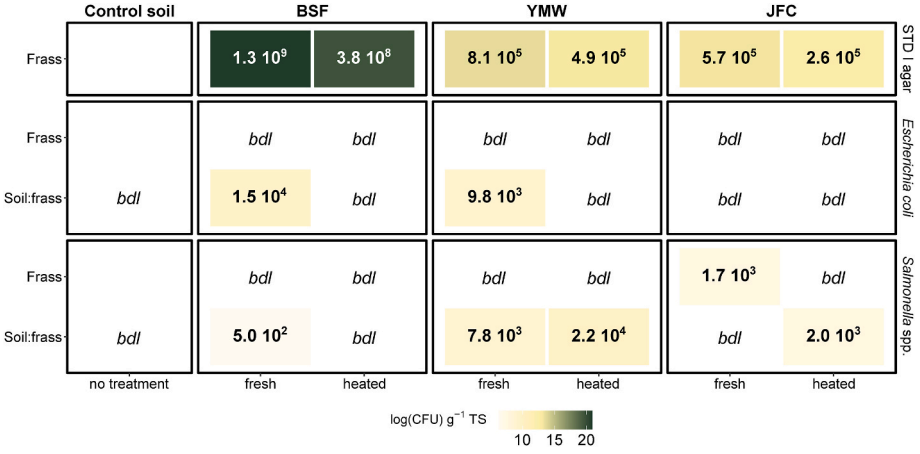

Fig. 3. Average counts of colony forming units g^{-1} TS frass and soil:frass on Standard I (STD I), TBX ChromoCult™ (selective for *Escherichia coli*), and XLT4 (selective for *Salmonella* spp.) medium after 48 h of incubation (n = 3) under fresh and heated conditions. Counts have been log-transformed for the gradient fill scale. Total viable counts of aerobic microorganisms (STD I agar) were only assessed for frass samples. CFU = colony forming unit, TS = total solids, bdl = below detection limit.

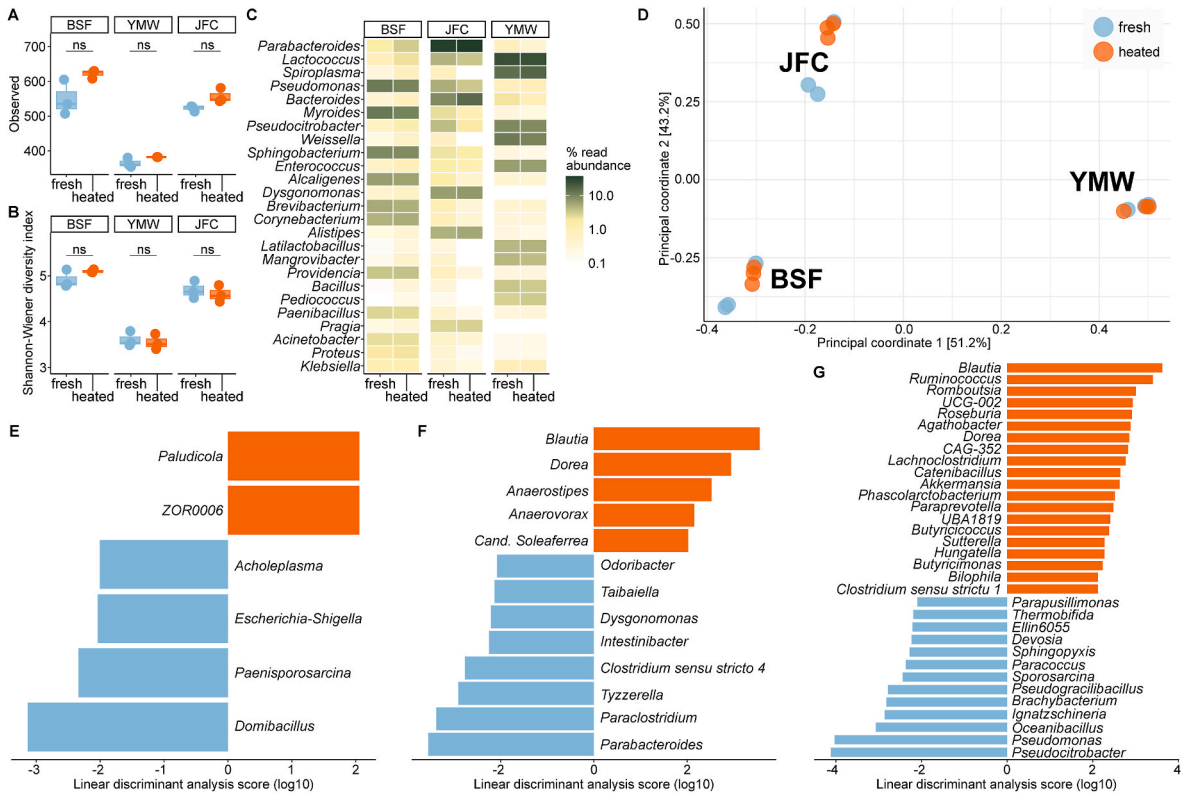

Fig. 4. Analysis of microbial communities in fresh (blue) and heat-treated (orange) frass samples of black soldier fly (BSF), yellow mealworm (YMW), and Jamaican field cricket (JFC) (n = 3). Alpha diversity explained by (**A**) observed species and (**B**) Shannon-Wiener diversity index. **C.** The 25 most abundant genera based on relative abundance. Amplicon sequence variants without classification to the genus level were removed from the list. **D.** Principal coordinates analysis showing the species- and treatment-derived (dis)similarity between samples based on Bray-Curtis dissimilarity. Differentially abundant bacterial genera in BSF (**E**), YMW (**F**), and JFC (**G**) samples as determined via linear discriminant analysis of effect size. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the Shannon-Wiener diversity index (fresh: $F_{2,6} = 43.7$, $p < 0.001$; heated: $F_{2,6} = 90.1$, $p < 0.001$). Significant differences ($p < 0.05$, Tukey HSD test) between JFC and BSF were evident only after heating in terms of both observed species and the Shannon-Wiener diversity index.

The differences in the frass microbiome composition among the three insect species were further illustrated by the diverging insect species-specific patterns in the relative abundances of the top 25 bacterial genera (Fig. 4C) and the distance between sample aggregates visualized by principal coordinates analysis (Fig. 4D). Permutational multivariate analysis of variance validated the presence of significant differences among the frass microbiomes of the three insect species, both in their fresh state ($F_{2,6} = 51.82$, $p < 0.01$) or after heat treatment ($F_{2,6} = 51.82$, $p < 0.01$). The most dominant genera in BSF, JFC, and YWM frass were *Pseudomonas*, *Parabacteroides*, and *Lactococcus*, respectively.

Little overlap was found in biomarker genera characterizing the fresh and heat-treated samples of BSF (Fig. 4E), YMW (Fig. 4F), and JFC (Fig. 4G). The most differentially abundant genera explaining the divergence in microbiome composition between fresh and heated frass were found in JFC samples. Only two genera (*Blautia* sp. and *Dorea* sp.) characteristic for heat-treated JFC frass were also found in heat-treated YMW samples. The least characteristic genera were found in BSF samples. The bacterial genera explaining the overall differences in frass microbiomes among the three insect species are shown in Fig. S2. The redundancy analysis revealed that the first two axes account for 96% of the variance in the data (Fig. S3). This underscores the species-specific clustering of samples, marked by distinct bacterial genera, and highlights the main drivers responsible for shaping the frass' features.

3.4. Physicochemical characterization of frass-supplemented soils and control soils

To assess the effect of frass supplementation on the soil, the soil:frass mixtures were analyzed after a two-week greenhouse incubation period and compared both with each other and with the control soil. Consequently, at the end of the incubation period, the water content and TS in the frass-treated soil samples were comparable to those in the control soil (Table 3). Although YMW and JFC frass led to a slight increase in soil pH to a max. of 8.59 after treatment, the addition of BSF frass caused a slight decrease in pH from 8.06 to 7.56. Electrical conductivity and total dissolved solids increased in all frass-treated soils, with maxima of 1201.00 μS cm^{-1} and 480.00 mg L^{-1} measured after the supplementation of heat-treated JFC frass.

At approx. 6%, the VS content in the control soil was approx. 1% lower than that in soils treated with BSF frass and 3–5% lower than that in soils treated with JFC and YMW frass, respectively. Supplementation with YMW and JFC frass resulted in a significant increase in the soil C and N contents by up to 2.8% and 0.3%, respectively, leading to a decrease in the C:N ratio from 15.49 to a minimum of 11.82 in soil treated with fresh JFC frass.

Although NH_4^+-N concentrations were initially highest in samples of fresh and heated BSF frass (Table 2), they were lowest in soils supplemented with BSF frass, given the recommended dosage used. The JFC frass application (12.5% fresh or 11.6% heated, w/w) resulted in the highest soil NH_4^+-N concentration of 1723.90 μg g^{-1} TS and, thus, to an increase of 900% of the original soil concentration after two weeks and a one-time amendment. The YMW frass, which had the highest NO_2–NO_3^--N content, increased the soil NO_2–NO_3^--N levels by ca. 350 μg g^{-1} TS compared to the control soil. The plant-available P reached an average

Table 3
Physicochemical parameters (mean ± standard deviation) measured in the control soil and the soil:frass mixtures after two-week incubation in a greenhouse at 20 °C. Statistical differences were calculated via analysis of variance followed by Tukey HSD posthoc tests for pairwise comparison of sample groups (n = 4). TS = total solids, bdl = below detection limit.

	Control soil	Soil:Frass (BSF)		Soil:Frass (YMW)		Soil:Frass (JFC)		*F-value*
		Fresh	Heated	Fresh	Heated	Fresh	Heated	
Water content [%]	24.76 ± 0.35^{c}	25.03 ± 0.44^{bc}	24.27 ± 0.21^{c}	26.24 ± 0.44^{a}	26.13 ± 0.40^{a}	26.07 ± 0.76^{ab}	24.18 ± 0.42^{c}	*F = 15.33****
TS [%]	75.24 ± 0.35^{a}	74.97 ± 0.44^{ab}	75.73 ± 0.21^{a}	73.75 ± 0.44^{c}	73.86 ± 0.40^{c}	73.93 ± 0.76^{bc}	75.81 ± 0.42^{a}	*F = 15.33****
Volatile solids [%]	6.23 ± 0.13^{e}	7.14 ± 0.32^{d}	7.07 ± 0.15^{d}	11.09 ± 0.25^{a}	10.97 ± 0.49^{ab}	10.21 ± 0.36^{b}	9.19 ± 0.56^{c}	*F = 128.08****
Ash [%]	69.01 ± 0.40^{a}	67.83 ± 0.53^{ab}	68.70 ± 0.36^{a}	62.67 ± 0.29^{c}	62.90 ± 0.75^{c}	63.72 ± 0.97^{c}	66.63 ± 0.50^{b}	*F = 91.23****
C [%]	4.80 ± 0.27^{b}	5.55 ± 0.29^{b}	5.51 ± 0.16^{b}	7.59 ± 0.54^{a}	7.43 ± 0.30^{a}	7.19 ± 0.23^{a}	7.46 ± 0.63^{a}	*F = 33.49****
N [%]	0.31 ± 0.02^{b}	0.38 ± 0.01^{b}	0.38 ± 0.01^{b}	0.59 ± 0.04^{a}	0.59 ± 0.02^{a}	0.61 ± 0.04^{a}	0.63 ± 0.05^{a}	*F = 62.13****
C:N ratio	15.49 ± 1.96^{a}	14.61 ± 0.78^{ab}	14.47 ± 0.71^{ab}	12.88 ± 0.76^{bc}	12.53 ± 0.21^{bc}	11.82 ± 0.99^{c}	11.93 ± 0.56^{c}	*F = 8.59****
NH_4^+-N [µg g^{-1} TS]	1.92 ± 0.38^{d}	12.41 ± 1.75^{c}	12.61 ± 0.64^{c}	821.31 ± 41.82^{b}	788.60 ± 41.41^{b}	1690.53 ± 37.40^{a}	1757.28 ± 73.24^{a}	*F = 5553.25****
NO_2–NO_3^--N [µg g^{-1} TS]	34.14 ± 4.54^{c}	243.29 ± 38.09^{b}	231.81 ± 15.66^{b}	386.26 ± 15.74^{a}	377.23 ± 41.20^{a}	8.88 ± 0.82^{d}	4.94 ± 1.23^{d}	*F = 706.73****
$P_{plant\ available}$ [µg g^{-1} TS]	8.99 ± 0.17^{d}	72.12 ± 21.28^{c}	64.70 ± 9.93^{c}	788.60 ± 66.53^{a}	861.57 ± 40.07^{a}	583.23 ± 49.03^{b}	613.47 ± 69.06^{b}	*F = 571.75****
pH	8.06 ± 0.05^{c}	7.62 ± 0.03^{d}	7.56 ± 0.01^{d}	8.18 ± 0.1^{bc}	8.26 ± 0.03^{b}	8.59 ± 0.08^{a}	8.54 ± 0.05^{a}	*F = 179.66****
Electrical conductivity [µS cm^{-1}]	84.08 ± 4.16^{d}	225.25 ± 36.31^{c}	222.50 ± 10.34^{cd}	882.25 ± 45.07^{b}	820.75 ± 50.61^{b}	1099.25 ± 113.48^{a}	1201.00 ± 84.67^{a}	*F = 227.60****
Salinity	bdl^{c}	bdl^{c}	bdl^{c}	0.20 ± 0.00^{b}	0.18 ± 0.05^{b}	0.33 ± 0.05^{a}	0.38 ± 0.05^{a}	*F = 94.44****
Total dissolved solids [mg L^{-1}]	33.75 ± 1.71^{c}	89.75 ± 14.15^{c}	89.00 ± 4.55^{c}	353.00 ± 18.13^{b}	328.25 ± 19.92^{b}	440.25 ± 45.49^{a}	480.00 ± 33.71^{a}	*F = 228.64****

of 11.69 mg g^{-1} TS (YMW, JFC) and 20.60 mg g^{-1} TS in raw BSF frass. Taking the concentration of the amended 2% (BSF), an average of 12% (YMW and JFC), and the original soil concentration into account, the plant-available P concentrations ranged from 15% to 38% and 64% of the initially applied P in the BSF, JFC, and YMW treatments, respectively. Thus, significantly higher levels of available P in soils supplemented with YMW frass (788.60 and 861.57 µg P g^{-1} TS for fresh and heated frass, respectively) were established compared to soils supplemented with BSF (72.12 and 64.70 µg P g^{-1} TS for fresh and heated frass, respectively) and JFC frass (583.23 and 613.47 µg P g^{-1} TS for fresh and heated frass, respectively). The ratios of plant-available N (NH_4^+-N and NO_2–NO_3^--N) to P (N:P) in frass-amended soils reached 1.4 (YMW), 2.9 (JFC), and 3.6 (BSF).

3.5. Microbiological characterization of frass-supplemented soils and control soils

Although enriched in C_{mic}, BSF frass application to soil did not significantly promote microbial activity (MR) when compared to the control soil (Table 4). However, supplementation with YMW and JFC frass led to significantly higher soil microbial activity. While MR was comparable in soils supplemented with both fresh and heat-treated YMW and JFC frass, microbial biomass was remarkably higher in soils mixed with fresh and heat-treated YMW frass. Comparable MR rates but lower C_{mic} in soils containing JFC frass, in turn, resulted in higher MQ values.

Besides microbial activity and biomass, no colonies identified as *E. coli* were detected in any of the raw frass samples; however, abundances of 1.5 10^4 and 9.8 10^3 CFU g^{-1} TS soil of *E. coli* were found in soils mixed with fresh BSF and YMW frass, respectively (Fig. 3). Neither the control soil nor the soils supplemented with JFC frass showed *E. coli* growth. Initially, *Salmonella* spp. was exclusively detected in fresh JFC frass but not in any other frass samples. However, following the two-week soil incubation period, *Salmonella* spp. were found in soils mixed with fresh BSF, fresh or heat-treated YMW, and heat-treated JFC in numbers ranging from 5.0 10^2 to 2.2 10^4 CFU g^{-1} TS soil. *Salmonella* spp. was not detected in the control soil.

4. Discussion

4.1. Frass exhibits multi-level variations depending on the insect species

The physicochemical and microbiological characterization of black soldier fly (BSF), yellow mealworm (YMW), and Jamaican field cricket (JFC) frass have shown that frass properties are intrinsically related to the insect species (Fig. 2 and Table 1), which goes in line with our first research hypothesis. While C, H, and S content among the three frass types did not differ significantly, total N contents varied insect-

Table 4
Microbiological parameters (mean ± standard deviation) of the control soil mixed with either fresh or heated frass (treating the frass at 70 °C for 1 h). Statistical differences were calculated via analysis of variance followed by Tukey HSD posthoc tests for pairwise comparison of sample groups (n = 4). TS = total solids, MR = microbial respiration, C_{mic} = microbial biomass carbon, MQ = metabolic quotient.

	Control soil	Soil:Frass (BSF)		Soil:Frass (YMW)		Soil:Frass (JFC)		*F-value*
		Fresh	Heated	Fresh	Heated	Fresh	Heated	
MR [µg CO_2 g^{-1} TS soil h^{-1}]	6.13 ± 0.54^{c}	8.54 ± 1.20^{c}	7.54 ± 1.24^{c}	133.84 ± 23.17^{ab}	117.69 ± 14.56^{b}	118.89 ± 9.59^{b}	149.66 ± 16.48^{a}	*F = 269.99****
C_{mic} [µg C g^{-1} TS soil]	678.99 ± 27.55^{f}	1178.07 ± 275.71^{e}	1340.70 ± 331.23^{e}	22,773.21 ± 729.75^{a}	19,446.90 ± 333.39^{b}	11,344.60 ± 919.54^{d}	14,777.33 ± 1109.62^{c}	*F = 1007.83****
MQ [µg CO_2–C h^{-1} mg^{-1} C_{mic}]	9.03 ± 0.49^{ab}	7.45 ± 1.59^{bc}	5.71 ± 0.63^{c}	5.86 ± 0.92^{c}	6.05 ± 0.76^{c}	10.51 ± 0.82^{a}	10.21 ± 1.65^{a}	*F = 14.93****

specifically, with JFC frass containing up to twice as much total N as compared to BSF and YMW frass. The plant-available ammonium content was highest in BSF, while plant-available nitrate was 3 times higher in YMW frass compared to JFC and 50 times higher than in BSF, highlighting the shifted availability of the plant-available N fractions. BSF frass samples had more than 20 mg g^{-1} TS of plant-available phosphorus, exceeding concentrations in the other two frass types by a factor of 2. Variations in nutrient concentrations of frass are consistent with prior studies showing that differences in feed requirements and rearing conditions are reflected in the properties of the frass (Beesigamukama et al., 2022). In our study and at the physicochemical level, the significantly higher moisture content in BSF frass can be explained by the faster development of the larvae compared to YMW and JFC, which typically take three times longer before reaching a harvest-ready stage, thus leaving less time for the substrate to dry. The frass moisture content at the time of harvest is a crucial parameter that influences the separability of insects and rearing residues through sieving (Gärttling and Schulz, 2022). Operators face the challenge of finely adjusting the initial substrate moisture to establish suitable conditions throughout the rearing process while simultaneously considering water loss through evaporation, uptake from insects, and metabolization by microorganisms, as this affects the successive processing steps.

At the microbiological level, fresh BSF frass showed significantly higher microbial activity along with CFU counts of viable aerobic microorganisms that were up to four orders of magnitude higher than those in fresh YMW and JFC frass. This disparity in microbial growth and metabolism is likely to be sustained by the higher moisture content in BSF frass. At the microbiome level, the dominant genera did not overlap among insect species, indicating that the different types of frass exhibited unique microbial signatures (Fig. 4C). While NH_4^+-N, P, C, and H profiles as well as high microbial activity drive the spread in bacterial beta diversity in BSF frass, NO_2–NO_3-N was found a strong driver for JFC frass while total N, salinity, and electrical conductivity affected microbiome composition in YMW frass (Fig. S3).

4.2. Heat treatment has limited impact on frass nutrients but reduces microbial activity and viable counts of pathogenic microbes

The primary rationale for heat-treating insect frass is to guarantee its safety by removing any potential microbial pathogens present within these residues. Notably, in the EU, frass has recently been classified in the same category as processed manure, thus requiring further treatment (Regulation (EU) 2021/1925, 2021). The selection of substrates authorized for insect rearing remains considerably constrained when compared with the extensive range of organic wastes deemed suitable for this purpose, but microbes residing in insect guts are inevitably transferred into the frass via excretion. However, the prescribed heat treatment (70 °C for 1 h) seems to be appropriate for reducing the load of pathogenic microbes below the detection limit of all tested frass types, which cover a broad range of species that are currently mass reared in Europe for feed and food purposes. Our study further highlights the insect-specific variability in the presence of specific pathogens and confirms the respective positive impact of heat treatment and thus our second hypothesis. Also, in line with the second hypothesis, total cultivable microbial load, microbial activity, and microbial biomass were significantly reduced by heat treatment, irrespective of frass type, still acknowledging that two insect frass types, YMF and JFC, exhibited such a reduced water availability that microbial activity or biomass were below the detection limit. Nevertheless, it is noteworthy that these suppressed microbial dynamics could be re-activated upon water supply, as indicated by our soil incubation trial (see 4.3). Besides the direct microbial aspect, the nutritional load (N, P) was not significantly reduced due to heat treatment, except for reduced plant-available nitrate content in heated JFC frass and reduced plant-available ammonium in heated BSF frass (the latter still providing high concentrations of NH_4^+-N).

Although heat treatment may be efficient in hygienizing frass from a microbiological perspective, the effect of temperature on other potential pollutants in frass should be considered. Organic wastes suitable as rearing substrates are prone to microplastic contamination. While previous studies have suggested that the development of farmed insects is not significantly affected by microplastics, these particles are excreted in their original form after passing through the larvae's digestive tract and accumulate in the frass (Heussler et al., 2023; Lievens et al., 2023). Temperatures exceeding 60 °C have been shown to melt or clump specific microplastics, and temperatures near 100 °C may even lead to their elimination (Munno et al., 2018). Nonetheless, the potential impacts on frass production have yet to be explored.

4.3. Frass supplementation improves plant nutrient content and microbial activity in soils

Frass, as the main byproduct of insect rearing, has the potential to be used as a soil improver and plant fertilizer by supplying soil particularly with nitrogen (N), phosphorus (P) and potassium (K) (Fuertes-Mendizábal et al., 2023; Klammsteiner et al., 2020a). The recent EU regulation (Regulation (EU) 2021/1925, 2021) foresees the heat treatment of frass to guarantee safety upon the consumption of crops and plants fertilized by frass. Nutrient conditions in soils amended with fresh and heat-treated frass were significantly improved in both cases, and heat treatment did not significantly alter the improvement, irrespective of the frass type. To the best of our knowledge, this is the first report of using heat-treated frass as soil amendment, demonstrating that the fertilizer capacity of frass remains unaltered after a one-time heating.

Generally, the nutrient load of frass is comparable to that of other organic fertilizers such as compost (Poletschny, 1994). Thus, fertilization with frass exhibits highly favorable attributes with regard to all essential nutrients, including carbon (C), N, and P. In terms of C and N content, frass aligns with concentrations established in other organic fertilizers (Poletschny, 1994). The utilization of frass is scalable, and like other organic fertilizers, it has a sustainable impact on soil, in contrast to mineral fertilizers. This sustainability arises from the fact that nutrients are mobilized by microorganisms and/or incorporated into microbial biomass. This was particularly pronounced in the case of the N fractions, where an average amendment of 12% (w/w) of YMW and JFC frass to soil led to strong mineralization, resulting in the transfer of a relevant fraction of total N to NH_4^+-N. Consequently, the soil:frass mixture contained up to six times more NH_4^+-N than the initial addition. Similarly important for plant nutrition, albeit less pronounced here, this pattern was observed for available P as well. In terrestrial ecosystems, nitrogen loading can enhance phosphorus limitation with associated effects on soil organic carbon stabilization and decomposition (Luo et al., 2022). In detail, the addition of nitrogen can lead to soil acidification, reducing phosphorus availability, and aggravating typical P limitations found in soils. In alkaline soils, N loading has variable effects on phosphorus limitation, depending on factors like aluminum mobilization or increased extractable phosphorus (Luo et al., 2022). In case of frass, particularly promising is the high amount of plant-available P in all three frass types, as after nitrogen, P is the second most limited nutrient and is not available for the plant despite abundant phosphorus reserves (Illmer and Schinner, 1992).

In the soil, phosphate is usually present as insoluble aluminum, iron, and calcium phosphate (Kooijman et al., 2002). Owing to its insoluble form, P fertilizers are commonly used in agriculture to increase crop productivity (Ros et al., 2020). In fact, the available P content in the frass exceeded the concentrations by approximately 4-fold when compared to the typical total P levels found in organic fertilizers. This high amount of P was also reflected in the available P present in the soil after fertilization. Two weeks after a single application, P levels reached up to 860 μg g^{-1} TS, increasing soil phosphorus by 100–1000 times compared to the current control and available P in soils globally (McDowell et al., 2023). P fertilization associated with frass is

consistently beneficial in all cases and supports the replacement or reduction of chemical P fertilizers, which is a major goal in sustainable agriculture. Furthermore, the increased concentration of phosphorus established in frass-amended soils could positively affect soil N pools and processes (Wang et al., 2022). However, whether elevated P levels are ecologically feasible should be considered.

In addition to the added nutrients during the single frass application, the supplementation of frass boosted soil microbial activity, especially for YMW and JFC treatments. The lower performance of the BSF frass can be associated with the lower dosage recommended by producers. The recommended high-dosage applications of YMW and JFC refer to small-scale garden practices; thus, large-scale applications will be lower, as a linear upscaling of the necessary amount of frass will lead to unfeasible amounts. Nutrient support by frass amendment and autochthonous microbes in the frass significantly increased microbial biomass in the soils amended with JFC and YMW frass. Despite the absence of detectable physiologically active microbial biomass in the case of raw YMW and JFC frass, the addition to the soil significantly boosted microbial activity and biomass, which can be traced back to the associated increase in water availability for the frass microbes and the high nutrient input, indicating that not only did the frass provide nutrients but also benefited the soil's resident microbes. Heat treatment led to contrasting and frass-type specific effects, still providing very similar positive effects on soil quality compared with untreated fresh frass, thus contradicting our third research hypothesis. In light of these observations, we acknowledge that exploring the effects of (heat-treated) frass on various types of soil and long-term soil fertilization trials would be a highly interesting avenue for future experiments, expanding our understanding of the broader implications and potential applications of frass in different soil environments.

5. Conclusion

Variations among frass samples were primarily attributed to insect species, with minimal influence of heat treatment. While the BSF frass demonstrated the highest NH_4^+-N concentrations, the levels of NO_2–NO_3^--N were significantly elevated in the YMW frass. Interestingly, BSF frass also exhibited the highest plant-available P content even after heat treatment, and it was the only frass type displaying microbial activity that significantly decreased following heat treatment. Supplementing the soil with frass led to distinct shifts in soil properties, with YMW frass having the most wide-ranging effects on nutrient concentrations. Collectively, these findings provide substantial insights into the intricate interactions between insect frass, heat treatment, and soil dynamics, with potential implications for sustainable agricultural practices. However, it is important to acknowledge the limitations of this study, which was conducted at a laboratory scale and over a limited period of time. Future research should explore additional factors influencing frass properties and their interactions with soil ecosystems, with a particular focus on the long-term effects and safety considerations of the fertilization process in an agricultural setting. Addressing these aspects will advance our understanding of frass as a fertilizer and facilitate the development of effective strategies for its use in sustainable agriculture.

CRediT authorship contribution statement

Nadine Praeg: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Thomas Klammsteiner:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Data availability

We have provided a link to the data in the manuscript

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.120622.

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