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Research article

Recirculating frass from food waste bioconversion using black soldier fly larvae: Impacts on process efficiency and product quality

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ABSTRACT

Biowaste generation is increasing worldwide and inadequate disposal has strong negative impacts on food systems and ecosystems. Biodigestion of biowaste using black soldier fly (*Hermetia illucens*) larvae (BSFL) generates valuable by-products such as animal feed (larval biomass) and organic fertiliser (frass). However, the latter is typically unstable immediately after waste conversion and is thus unsafe for use as a fertilizer in terms of maturity. This study evaluated recirculation of frass within bioconversion of post-consumer food waste (FW) as a dietary component for BSFL to improve the quality of the subsequent frass obtained. Frass was introduced at increasing inclusion levels replacing food waste (2.5–100% on wet-weight basis) as part of the larvae's feeding substrate. Bioconversion efficiency and material reduction were significantly reduced by frass inclusion, while larval yield per experimental unit remained unchanged. When considering only the waste component in the larval diet, larval yield (dry-weight basis) ranged between 207 (0% frass inclusion) and 403 (40% frass inclusion) kg tonne FW^{-1}, thus increasing by up to 94% at higher frass inclusion. With increasing dietary inclusion rate of frass from 0% to 100%, crude protein content of larval biomass increased by 41%, while fat content was reduced by 32%. The recirculated frass (obtained after including frass in the larval diet) had elevated concentrations of P, K, S, Na and B and around 6% lower organic matter content, demonstrating a higher degree of decomposition. Frass inclusion in the larval diet generated recirculated frass that were more stable and mature, as indicated by self-heating capacity, CO_2 and NH_3 volatilisation, seed germination bioassays and other parameters. It was concluded that frass recirculation improves waste bioconversion efficiency in relation to food waste unit, as well as larval biomass and frass quality, ensuring safer use as a fertilizer.

1. Introduction

The global population continues to grow at a rapid pace and demand for food can be expected to increase significantly within coming decades in order to ensure global food security (van Dijk et al., 2021). Many food production systems have negative environmental impacts due to extensive land and water use, greenhouse emissions and loss of biodiversity (Li et al., 2022; Vermeulen et al., 2012). One major issue regarding food production is biowaste generation, which has strong negative impacts when biowaste is not properly managed, as is the case in many countries around the world (Kaza et al., 2018). According to the (United Nations Environment Programme, 2021), 931 million tons of food waste was generated in 2019, of which approximately 60% was composed of household post-consumer waste. Addressing the significant volume of food waste generated globally is crucial, as this waste poses severe environmental risks if not treated adequately. In the United Nations Environment Programme, 2024 it is stated that food waste generates 8–10 % of global greenhouse emissions, while utilizing 30% of agricultural land. This underscore the urgent need for not only prevention of food losses, but also the urgent need for sustainable management solutions within global food systems. Adequate management practices can help mitigating environmental damage and enable the re-integration of waste streams within a circular economy (Chen et al., 2020).

Among the biowaste management technologies currently available, treatment using black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae (BSFL) has the advantage of good potential for rapid bioconversion with low environmental impact (Bosch et al., 2019). It

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also generates two by-products of great interest: a larval biomass rich in protein and fat that can be used as a feed ingredient, and frass that can be used as a fertiliser (Surendra et al., 2020). Thus BSFL technology complies with the principles of a circular economy, in which the waste generated in a productive process (*e.g.* food production) is reintroduced into other processes as a resource, creating value (Velenturf and Purnell, 2021). Despite the known benefits of BSFL bioconversion in connecting waste management to food and feed production, a number of legal barriers in the EU prevent the use of some organic waste streams (*e.g.* food waste and animal manures) as feed substrate for the larvae, as thoroughly discussed by Lalander and Vinnerås (2022). Nevertheless, this technology is very promising and there is increasing interest worldwide in its adoption and in the use of the by-products generated in the process.

Frass derived from biowaste conversion using BSFL is a fertiliser product with high organic matter content and a nutrient profile that is comparable to that of animal manures and conventional compost commonly used in agriculture (Gärttling and Schulz, 2022). However, studies investigating frass as organic fertiliser/soil conditioner have obtained highly variable results, due to factors such as differences in composition of the initial feed substrate provided to the larvae, experiment sites and type of crop (Basri et al., 2022; Lopes et al., 2022). In fact, negative effects of frass application have been reported, such as stunted growth possibly caused by high electrical conductivity (EC), reflecting the presence of soluble salts (Setti et al., 2019), or phytotoxic characteristics due to high concentrations of ammonium-nitrogen (Bohm et al., 2023).

According to Lopes et al. (2022), one of the main limitations to using BSFL frass as a soil conditioner or fertiliser is lack of maturity and stability of the fresh product. The legal definition used by the European Union states that frass is "… a mixture of excrements derived from farmed insects, the feeding substrate, parts of farmed insects, dead eggs and with a content of dead farmed insects …" (European Commission, 2021). This suggests that it contains undigested substrate, which means that the organic matter content of frass is not stable and could still undergo further decomposition. The organic matter in larval feed substrates is partially consumed by BSFL, as demonstrated by previous studies using different techniques such as fluorescence and spectroscopy. For instance, Liu et al. (2020) reported reduced concentrations of low-molecular-weight molecules, but increased concentration of high-molecular-weight compounds, in the organic matter fraction of animal manures after BSFL bioconversion. Similarly, Wang et al. (2021) observed an increased degree of aromaticity of the organic matter in manures on bioconversion by BSFL, which they attributed to increasing stability.

The maturity and stability of soil amendments and organic fertilisers are generally attributed to specific parameters, such as low carbon-to-nitrogen (C/N) ratio, low ammonium-to-nitrate (NH_4^+/NO_3^-) ratio, low EC, high seed germination index, high humification degree of organic matter etc. (Wichuk and McCartney, 2010). Some of these parameters in BSFL frass are similar to those typically seen in mature and stable compost, suggesting that the frass itself may be mature and stable. For instance, Beesigamukama et al. (2022) evaluated the maturity status of frass fertilisers from several insect species and concluded that BSFL frass can be considered a mature fertiliser that does not require further processing, based on pH, EC, NH_4^+/NO_3^- ratio, C/N ratio and seed germination tests. However, their results were obtained using a single substrate blend as feed substrate for the larvae (brewery spent grains and potato peels) and they pointed out that the outcome could change upon using a different substrate.

Some studies have demonstrated that the negative impact of frass on plant growth can be minimised by stabilising the organic matter content. A study by Song et al. (2021) comparing the maturity of "raw" frass (obtained immediately after one bioconversion process) from BSFL bioconversion of a mixture of okara and wheat bran with that of thermophilically composted frass from the same source found that the raw frass was not mature and displayed phytotoxic traits. These traits were minimised after 5–6 weeks of composting, during which the organic matter in the frass was further stabilised (Song et al. (2021). Composting for 32 days has also shown to improve food waste-derived frass quality, which subsequently improved soil organic matter, nutrients availability and enzyme activity, as demonstrated by (Wu et al., 2023). Frass has been shown to contain a rather large proportion of organic compounds that could be further decomposed (*e.g.* carbohydrates, fat and protein). Even after composting for over three months, some of its characteristics may still not be within the expected range for mature compost, due to presence of undigested organic matter and other traits (Jasso et al., 2024). As suggested by Lopes et al. (2022), frass could be used for composting or even provided to other invertebrates (*e.g.* earthworms) for further decomposition and stabilisation. However, this represents an additional step in organic waste bioconversion and makes a major contribution to the global warming potential of the BSFL conversion process (Guo et al., 2021; Mertenat et al., 2019). Avoiding post-processing of the frass could thus lower the overall environmental impact of the BSFL bioconversion system.

The hypotheses tested in the present study were that frass can be further consumed by BSFL, allowing its organic matter to be further digested by the larvae, and that the recirculated frass obtained would have a more stable organic matter content. Specific objectives of the work were to evaluate the effects of frass inclusion in BSFL diets as feed substrate and to determine the effects of dietary inclusion of frass on process efficiency, larval biomass composition and on the composition, stability and maturity level of the subsequent recirculated frass.

2. Material and methods

2.1. Waste material and larvae

Food waste was collected in three restaurants located at the Swedish University of Agricultural Sciences (SLU) campus in Uppsala, Sweden. The waste from all restaurants was collected over a period of two weeks, during which time it was kept inside a cold chamber (4 °C) before being blended and ground in a food grinder (to particle size <5 mm) and then stored in a freezer (−18 °C) until use. Waste was thawed overnight at room temperature (20 °C) before use. The dry matter (DM) content of the food waste was 29.0 ± 1.6% and the total volatile solids (VS) content was 93.8 ± 0.7%. The proximate composition of the food waste was (in DM basis): crude protein 19.1 ± 0.9%; crude fat 26.7 ± 2.4%; and crude fibre 7.3 ± 0.7%.

Newly hatched larvae from a BSF colony that has been running at SLU since 2015 were kept on a substrate consisting of chicken feed (Granngården Hönsfoder Start, 80% DM) and water (achieving substrate moisture content of approximately 70%) for five days before being used in the experiments. Individual larval weight ranged between 1.4 and 1.7 mg at the start of the trials. Larvae were separated from their starter substrate by sieving (1-mm mesh), batch-counted and weighed, in order to achieve a known number of larvae at the beginning of each experiment.

2.2. Experimental set-up

Three bioconversion trials with BSFL were performed, with the first being carried out to produce the frass that was used in subsequent trials (Trials 2 and 3) investigating the impact of frass inclusion on process performance, larvae and frass quality. In this sense, in Trials 2 and 3 the frass produced in Trial 1 was used in distinct replacement levels of food waste, ranging from 2.5% to 100%. Considering that - to the authors' knowledge - this is the first study investigating frass dietary inclusion in BSF larvae bioconversion of food waste, the increasing intervals of every 2.5% inclusion were chosen in order to identify the effects of frass inclusion on process efficiency and product quality in a linear and accurate way. In Trial 2, the larval density was kept constant while the larval VS

feed dose was increased with increasing frass inclusion level. In Trial 3, the larval feed dose was kept constant while the larval density was increased with increasing frass inclusion level. These different trials were conducted in order to uncouple the impact of frass inclusion from that of larval density and larval VS dose, since these process parameters can have a significant impact on process performance (Lopes et al., 2023). The same food waste (see section 2.1) was used in all trials. The larvae were fed three times during each experiment, with the same feed dose at every feeding. All experiments were carried out inside a climate chamber with mean temperature 30.3 ± 0.5 °C and 27.3 ± 5.1% humidity.

2.2.1. Trial 1 - Frass production

In Trial 1, 12,000 larvae (each 1.7 ± 0.3 mg) were placed inside 60 cm × 40 cm x 12 cm plastic boxes (area 2400 cm^2), resulting in a larval density of 5 larvae cm^{-2}. Total feed load in each treatment unit (N = 10) was 11.5 kg (divided between three feeding events, on days 1, 4 and 7, a process repeated in Trials 2 and 3), resulting in a feed dose of 261 mg VS $larva^{-1}$. The trial was terminated on day 12 and larvae were separated from the frass by sieving using different mesh sizes (5–20 mm). The sieves used in this step were connected to a shaking table (40 × 60 cm) of a desired mesh size, and sieving took a maximum of 2 min per experimental box. Larvae and frass yield (wet weight) were 2.15 ± 0.06 kg box^{-1} and 1.55 ± 0.11 kg box^{-1}, respectively. Larvae and frass samples were collected for further analysis and the remaining frass was immediately frozen at −18 °C until further use in Trials 2 and 3.

2.2.2. Trial 2 - Frass inclusion at constant larval density

Since only a limited amount of frass was obtained in each treatment unit in Trial 1, smaller plastic boxes (16.5 cm × 14 cm x 14 cm, area 231 cm^2) were used in Trials 2 and 3. The same larval density as in Trial 1 was used in Trial 2, and thus 1155 individuals were placed inside each treatment unit (Table 1). As the frass had higher DM content (67.9 ± 3.5%) and lower VS content (86.7 ± 0.4%) than the food waste, it was not possible to exceed an inclusion level of 40%, as the total substrate depth exceeded the total depth of the treatment unit at higher frass inclusion rates. However, a treatment with 100% frass inclusion (limited to provision of around 67% of the total intended amount, due to the depth limitation) was established. The range of inclusion ratios was run in singlets and frass moisture content of the mixed feedstock was adjusted to 70%. Then, while the larval density was maintained, the feed dose was varied from 261 mg VS $larva^{-1}$ (0% inclusion of frass) to 385 mg VS $larvae^{-1}$ (40% inclusion). Trial 2 was terminated on day 12, the larvae were sieved from the so-called “recirculated frass” fertilisers (this term refers to the frass obtained after frass was used as a dietary component in Trial 1) as described in section 2.2.1 and samples were collected for further analysis.

2.2.3. Trial 3 - Frass inclusion at a constant larval feed dose

In Trial 3, the larval feed dose was kept constant at 263 mg VS $larva^{-1}$, and hence the larval density ranged between 5 larvae cm^{-2} (0% inclusion of frass) and 7.33 larvae cm^{-2} (40% inclusion) (Table 1). The differences in total number of larvae between treatment units were small (around 33 additional individuals in every treatment unit with

Table 1
Details of the experimental design adopted in Trial 2 (F- treatments) and Trial 3 (FD-treatments), in which food waste (FW) was replaced by frass, with increasing levels (0%–100%) of the frass obtained in Trial 1. Tot-N_{FW} refers to total nitrogen content (g) in the food waste/frass blend input in each experimental unit.

	Larvae (n)	Density (larvae cm^{-2})	FW (g)	Fresh frass (g)	Tot-N_{FW} in (g)	Larval feed dose (mg VS $larva^{-1}$)	VS FW (% of total)	VS frass (% of total)
Trial 2 (F treatments)								
F0%	1155	5	1115	0	10.0	263	100	0
F2.5%	1155	5	1087	29	10.4	270	94.7	5.3
F5%	1155	5	1059	56	10.9	278	89.8	10.2
F7.5%	1155	5	1030	84	11.3	286	85.1	14.9
F10%	1155	5	1003	111	11.8	293	80.6	19.4
F12.5%	1155	5	975	139	12.3	301	76.4	23.6
F15%	1155	5	947	167	12.7	309	72.4	27.6
F17.5%	1155	5	920	195	13.2	316	68.5	31.5
F20%	1155	5	892	223	13.6	324	64.9	35.1
F22.5%	1155	5	864	251	14.1	332	61.4	38.6
F25%	1155	5	836	279	14.5	339	58.1	41.9
F27.5%	1155	5	808	307	15.0	347	54.9	45.1
F30%	1155	5	780	334	15.5	355	51.9	48.1
F32.5%	1155	5	752	362	15.9	362	49.0	51.0
F35%	1155	5	724	390	16.4	370	46.2	53.8
F37.5%	1155	5	697	418	16.8	377	43.5	56.5
F40%	1155	5	669	446	17.3	385	40.9	59.1
F100%	1155	5	0	738	18.7	377	0	100
Trial 3 (FD treatments)								
FD0%	1155	5.0	1115	0	10.0	263	100	0
FD2.5%	1189	5.2	1087	28	10.4	263	94.7	5.3
FD5%	1222	5.3	1059	55	10.9	263	89.8	10.2
FD7.5%	1256	5.4	1031	84	11.3	263	85.1	14.9
FD10%	1290	5.6	1003	112	11.8	263	80.6	19.4
FD12.5%	1323	5.7	975	139	12.3	263	76.4	23.6
FD15%	1357	5.9	947	167	12.7	263	72.4	27.6
FD17.5%	1390	6.0	920	195	13.2	263	68.5	31.5
FD20%	1424	6.2	892	223	13.6	263	64.9	35.1
FD22.5%	1458	6.3	864	251	14.1	263	61.4	38.6
FD25%	1491	6.5	836	279	14.5	263	58.1	41.9
FD27.5%	1525	6.6	808	307	15.0	263	54.9	45.1
FD30%	1559	6.8	780	334	15.5	263	51.9	48.1
FD32.5%	1592	6.9	752	362	15.9	263	49.0	51.0
FD35%	1626	7.0	724	390	164	263	46.2	53.8
FD37.5%	1659	7.2	697	418	16.8	263	43.5	56.5
FD40%	1693	7.3	669	446	17.3	263	40.9	59.1
FD100%	1655	7.2	0	738	10.0	263	0	100

Trial 2 (*F*): varying larval volatile solids (VS) feed dose; Trial 3 (*FD*): varying larval density.

increasing frass level), and thus the larvae were counted manually. The frass provided to the larvae was adjusted to 70% moisture, following the same preparation procedure as used in Trial 2. Increasing frass inclusion level resulted in increasing substrate depth in the experimental units. Substrate depths at the third feeding event (day 7) ranged between 3.8 and 5.0 cm at 0–20% frass inclusion, 5.1–6.7 cm at 22.5–40% inclusion and reached 8.3 cm when 100% of frass was used as feed substrate. Trial 3 was terminated on day 12 when the larvae were sieved from the frass as described in section 2.2.1 and both larval biomass and recirculated frass were sampled for further analysis.

2.3. Sampling and analyses

2.3.1. Sampling

The feed substrate used in the trials was sampled for determination of DM and VS content. Food waste samples (N = 10) were taken after grinding and before freezing, while fresh frass from Trial 1 was taken from each experimental unit (N = 10) and immediately analysed for DM and VS. After harvest, a total larvae sample of 100 g was taken from each experimental unit in each trial for physico-chemical analysis. Throughout all trials, weight gain was monitored by subsampling 30 individual larvae before adding new feed substrate. Larvae were sampled once from each unit, collected from the corners and from the middle of the boxes, in order to acquire a representative sample in the treatment units. In addition, pH, EC and ammonium-N ($N–NH_4^+$) content of the substrate were monitored on feeding and harvest days.

2.3.2. Physico-chemical analysis and calculations

For pH, EC and $N–NH_4^+$ measurements, 5 g of substrate were dissolved in 20 mL deionised water in a 50-mL centrifuge tube, shaken and left to settle for 1 h at room temperature prior to analysis (Conductometer 912 and pH meter 913, Metrohm®). For determination of DM, samples were dried in an oven at 65 °C for 48 h. For VS determination, the dried samples were incinerated in a muffle oven, in which the temperature was increased to 250 °C during 30 min, maintained for 2 h and then increased to 550 °C for 4 h.

Larval biomass at the end of the process was analysed for crude protein using the Kjeldahl method for N analysis and a conversion factor of 4.76 as suggested by Janssen et al. (2017). Crude fat content was analysed according to 2009/152/EU and fibre content according to ISO 5498. Frass samples were analysed for a wide set of parameters, including amino acid composition and content of total organic carbon (TOC), organic matter, macronutrients (N, P, K, Ca, Mg, S), micronutrients (Cu, Mn, Zn, B) and humic substances (humic and fulvic acids). These analyses were performed at an accredited laboratory.

2.3.3. Calculation of the process efficiency parameters

Larvae survival (%) was calculated by dividing the number of larvae at harvest by the number of seed larvae added to each experimental unit at the beginning of the trials. Material reduction (Mat.Red) on a DM basis was calculated as:

$$\text{Mat.Red} = 1 - \frac{m\text{DM}_{frass}}{m\text{DM}_{feedstock}} \quad \text{(Eq. 1)}$$

where $m\text{DM}_{frass}$ and $m\text{DM}_{feedstock}$ is total dry mass of the final residue (frass) and the initial feedstock, respectively.

Bioconversion efficiency (BCE) of the feed substrate was calculated as:

$$\text{BCE} = 100 \times \frac{m\text{DM}_{larvae}}{m\text{DM}_{feedstock}} \quad \text{(Eq. 2)}$$

where $m\text{DM}_{larvae}$ and $m\text{DM}_{feedstock}$ is dry mass of larvae at the end of the experiment and of the initial feed substrate, respectively.

Yield of larval biomass (kg larvae tonne FW^{-1}) was also evaluated considering only the amount of food waste in the larval diets, thus disregarding the presence of frass in the diets. This was done in order to verify the effects of frass dietary inclusion on production of larvae per tonne of food waste.

2.3.4. Maturity and stability analysis

Frass maturity and stability was analysed using parameters developed for assessing compost maturation and stability, as previously suggested (Wichuk and McCartney, 2010). A self-heating capacity test was performed for each frass obtained in Trials 1–3, using Dewar vessels (1.5 L), according to the procedures described in Brinton et al. (1995). A pre-determined amount of frass (100 g DM) was adjusted to 60% moisture and placed inside a vessel containing a temperature probe connected to a monitor software and covered with a Styrofoam lid placed loosely on top to prevent excessive air exchange while maintaining adequate moisture. Temperature was monitored for seven days and frass was classified according to the proposed maturation degrees of I ("fresh compost") to V ("finished compost"). Additionally, frass was submitted to a Solvita® compost emissions test, which assesses emissions of CO_2 and NH_3. The test was conducted according to the manufacturer's instructions and the frass samples were classified according to the proposed maturity index calculator (I being "raw compost" and VIII being "finished compost").

Frass samples from Trials 1–3 were also evaluated in bioassays comprising a standardised seed germination test using watercress seeds (*Nasturtium officinale*), following the procedure described in Luo et al. (2018). In brief, frass extracts were prepared by dissolving 5 g of frass in 50 mL deionised water, shaking continuously for 1 h in a horizontal shaker, centrifuging at 4500 rotations per minute (rpm) and filtration through 0.45 μm filter paper. Extracts were analysed immediately after preparation. Two paper filters were placed inside 90-mm Petri dishes containing 5 mL of the extracts, in which 15 seeds were placed at 10 mm spacing. Triplicates were assembled for each extract, in addition to a control with deionised water, for which the same extract preparation procedure was performed. Seeds were germinated in a dark incubator at 25 °C for five days and then evaluated for germination and root elongation using recommended indices for assessing seed germination (SG) (Eq. (3)), relative seed germination (RSG) (Eq. (4)), relative radicle growth (RRG) (Eq. (5)) and germination index (GI) (Eq. (6)):

$$\text{SG}\ (\%) = 100 \times \frac{\text{number of germinated seeds}}{\text{number of total seeds}} \quad \text{(Eq. 3)}$$

$$\text{RSG}\ (\%) = 100 \times \frac{\text{number of germinated seeds in sample}}{\text{number of germinated seeds in control}} \quad \text{(Eq. 4)}$$

$$\text{RRG}\ (\%) = 100 \times \frac{\text{total radicle length of germinated seeds (sample)}}{\text{total radicle length of germinated seeds (control)}} \quad \text{(Eq. 5)}$$

$$\text{GI}\ (\%) = \text{RSG} \times \text{RRG} \times 100 \quad \text{(Eq. 6)}$$

2.4. Statistical analysis

The results obtained in Trial 1 were not evaluated statistically. The results obtained in Trials 2 and 3 (process parameters, larvae and frass composition) were evaluated by regression models, assuming equal dispersion around the regression line or homocedasticty and normal distribution of residuals, as verified by Breusch-Pagan test and Shapiro-Wilk test, respectively. As imbalanced intervals in frass inclusion rates were designed (*e.g.* from 40% to 100%), the data were log-transformed (log(dose+1)) and then evaluated by linear regression analysis. Maturity and stability-related parameters were evaluated by correlation analysis. A correlation matrix was built based on Spearman correlations among selected variables. All statistical analyses were carried out in RStudio (RStudio Team, 2020).

3. Results

3.1. Process parameters and larval quality

Increasing frass inclusion level significantly affected almost all process parameters investigated. Survival was not significantly affected in either Trial 2 or 3 ($p = 0.98$ and 0.46, respectively). However, larval survival on average was around 23% lower in Trial 2 (68.7 ± 6.7%) than in Trial 3 (89.4 ± 3.5%). In both trials, BCE, Mat.Red and DM loss (respired solids) were significantly reduced ($p < 0.05$) with increasing frass inclusion levels (Fig. 1). In Trial 2, BCE ($\%_{DM}$) ranged between 9% (F100, only frass) and 20% (F0, no frass), while in Trial 3 BCE ranged between 11% (FD100%, only frass) and 25% (FD0, no frass). Nonetheless, larval yield (g of larvae obtained per treatment unit) was not significantly affected ($p > 0.05$) by frass inclusion level in either trial, averaging 168.0 ± 12.7 g unit^{-1} in Trial 2 and 199.9 ± 15.8 g unit^{-1} in Trial 3 (Tables S1 and S2). On calculating larval yield per tonne of food waste (kg BSFL tonne^{-1} FW) at the different frass inclusion levels, it was found that larval yield increased ($p < 0.05$) with increasing frass inclusion (Fig. 1E and J), from 207 kg (DM basis) BSFL at 0% fresh frass inclusion to 360–403 kg BSFL per tonne FW at 40% frass inclusion. On a wet basis, 47–56% less food waste was needed in treatments F40 and FD40, respectively, to obtain the same amount of larval biomass as in the treatments without frass inclusion (F0 and FD0) (Tables S1 and S2).

Larval proximate composition was significantly impacted by increased frass inclusion in the diet. There was a significant ($p < 0.05$) linear increase in larval crude protein content (DM basis) from around 27.5% at 0% inclusion of frass to 31.6–33.5% at 40% inclusion in both trials. Larval crude fibre content also increased linearly ($p < 0.01$) with frass inclusion level in Trial 2, but not in Trial 3. Hence, when larvae density was not adjusted (Trial 2, F treatments), no significant difference in larval crude fibre content was observed. Larval crude fibre (DM basis) increased linearly by 58% in Trial 3 ($p = 0.019$), from 7.5% at 0% frass inclusion to 11.9% at 100% frass inclusion. Crude fat content of larval biomass was significantly reduced ($p = 0.0043$) by 48% in Trial 3, from 31.1% at 0% frass to 16.3% at 100% frass (Table 2).

3.2. Frass composition

Increased inclusion levels of frass resulted in similar changes in the composition of the frass fertilisers generated in both Trials 2 and 3. Organic matter and TOC content of the fertilisers were significantly reduced ($p < 0.001$) with increasing levels of frass in the diet of BSFL, while total N levels remained unchanged ($p > 0.05$). Concentrations of most macro- and micronutrients analysed were significantly increased in the resulting recirculated frass, especially in Trial 3 (FD treatments) (Table 3). Higher concentrations of P, K, S and B were detected in the recirculated frass in both trials, Ca and Zn concentrations remained unchanged and there was a small but significant increase in Mg concentration in Trial 3, with increasing frass inclusion level.

3.3. Frass maturity and stability

With increasing frass inclusion levels in the diet of BSFL in both trials, significant reductions in EC ($p < 0.02$), OM ($p < 0.001$) and humic extract ($p < 0.001$) were found, while C/N ratio was also significantly reduced ($p = 0.018$) in Trial 2. The Solvita® test revealed a linear relationship between increasing level of frass in the diet of BSFL and increased maturity of the recirculated frass ($p_F = 0.0157$; $p_{FD} = 0.0061$). Likewise, the self-heating capacity test, which classifies composts from

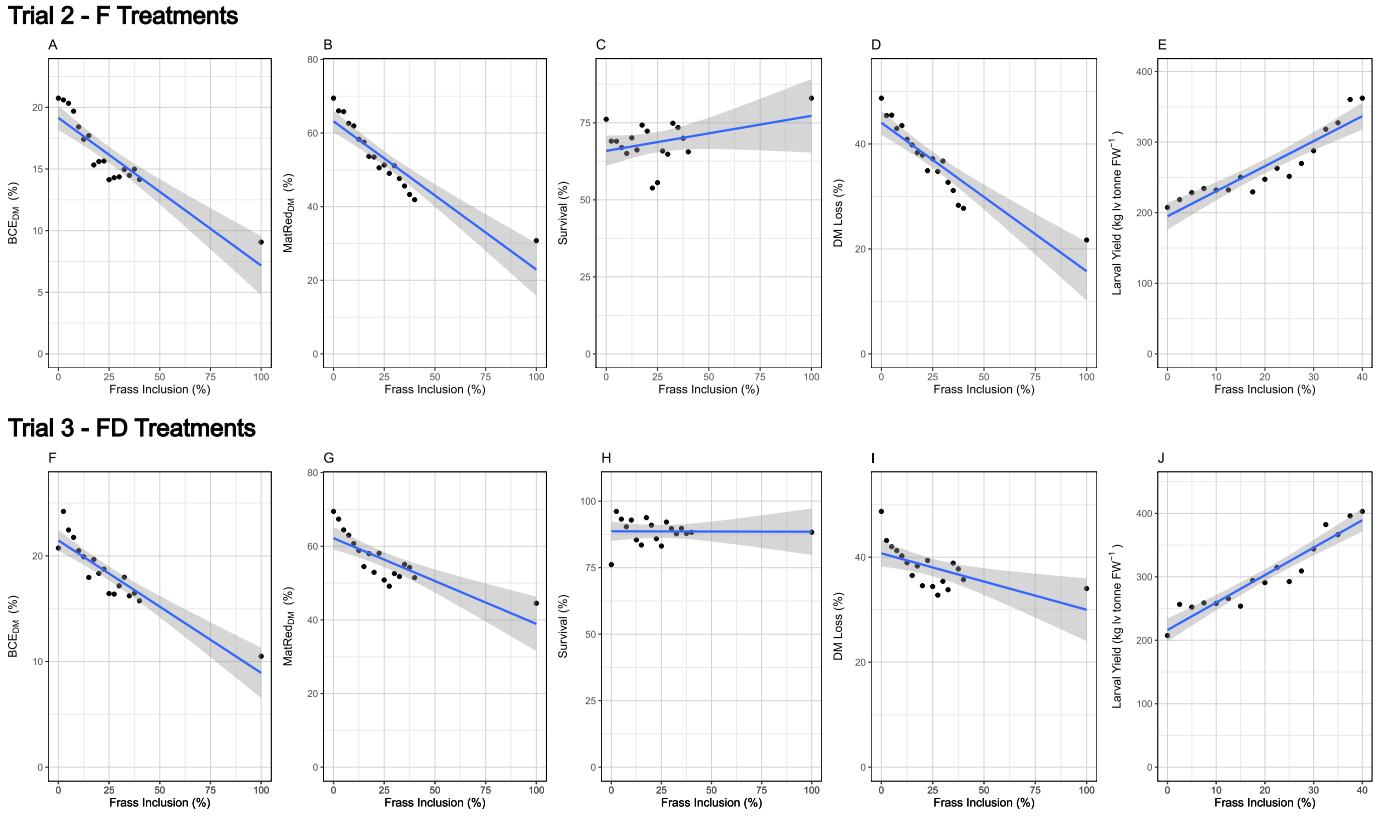

Fig. 1. Process efficiency parameters during black soldier fly larvae bioconversion of food waste with different inclusion levels of frass. (A and F) biomass conversion efficiency (BCE_{DM}) on dry matter (DM) basis; (B and G) material reduction ($MatRed_{DM}$); (C and H) larval (lv) survival (%); (D and I) total loss of DM (DM Loss); and (E and J) yield of larval biomass per tonne of food waste on DM basis, disregarding the presence of frass in the larval diets. Panels A to E show data from Trial 2 and panels F to J data from Trial 3. Significant effects ($p < 0.01$) were observed for BCE_{DM}, DM loss $Mat.Red_{DM}$ and larval yield, but not for survival ($p_F = 0.98$, $p_{FD} = 0.46$).

Table 2
Composition of larval biomass obtained in Trial 2 (F treatments) and Trial 3 (FD treatments), in which food waste was replaced with increasing levels of the frass obtained in Trial 1. Values expressed as percentage of dry matter ($\%_{DM}$).

	Crude protein ($\%_{DM}$)	Crude fat ($\%_{DM}$)	Crude fibre ($\%_{DM}$)	Ash ($\%_{DM}$)
Trial 2 (F treatments)				
F0%	27.5	31.1	7.5	8.3
F2.5%	29.7	32.5	8.5	8.4
F10%	30.9	18.7	9.4	8.8
F17.5%	30.5	19.6	10.3	8.9
F25%	30.3	23.5	7.6	9.2
F32.5%	33.6	20.8	9.4	9.2
F40%	31.6	26.4	7.3	7.1
F100%	38.9	21.1	8.4	9.9
Regression *p* value	0.0136*	0.0663^{NS}	0.732^{NS}	0.398^{NS}
Trial 3 (FD treatments)				
FD0%	27.5	31.1	7.5	8.3
FD2.5%	29.5	36.4	7.4	7.8
FD10%	30.3	25.4	8.8	7.6
FD17.5%	31.3	25.0	9.4	8.5
FD25%	31.5	26.3	9.6	6.7
FD32.5%	31.6	22.6	8.2	8.8
FD40%	33.5	26.5	9.0	9.0
FD100%	38.8	16.3	11.9	11.4
Regression *p* value	0.0046**	0.0043**	0.0196*	0.191^{NS}

Trial 2 (*F*): varying larval VS feed dose; Trial 3 (*FD*): varying larval density. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS: not significant.

level I to V, indicated greater stability with increased frass inclusion ($p < 0.01$), from level II at 0–10% frass dietary inclusion to level IV at 32.5–100% inclusion in both trials (Table 4).

The amino acid composition of the recirculated frass obtained in Trials 2 and 3 did not differ significantly between inclusion levels, with the single exception of proline, which decreased slightly ($p = 0.0133$) with increased frass inclusion level. However, the sum of all amino acids present in frass was significantly reduced in both trials on inclusion of frass in the larval diet ($p_F = 0.026$; $p_{FD} = 0.021$) (Table 5).

In addition to the stability- and maturity-related variables presented above, the bioassays performed using seed germination parameters (SG, RSG, RRG and GI), which indirectly measure compost phytotoxicity, revealed opposing trends in Trials 2 and 3. When the larval density was not adjusted and the larval VS feed dose was higher in the treatments with higher frass inclusion levels (Trial 2), the resulting recirculated frass did not increase the seed germination rate ($p > 0.05$ for all parameters). When the larval VS feed dose was adjusted to 263 g VS $larva^{-1}$ (Trial 3), there was a positive correlation ($p < 0.02$ for all parameters) between frass inclusion level and germination-related parameters, with GI increasing from <2% at 0–2.5% frass inclusion to 42.9% at 100% inclusion (Table 6).

Strong positive correlations were found between organic matter and humic extracts in the recirculated frass (both declined significantly with increasing frass inclusion level) in both trials ($R^2_F = 0.93$, $R^2_{FD} = 0.99$) (Fig. 2). There was also a strong positive correlation between increasing frass inclusion level (represented by "Dose" in Fig. 2) and seed germination parameters (SG, RSG, RRG and GI), frass fertiliser stability (Solvita® stability test) and frass fertiliser maturity (self-heating capacity). A negative correlation between inclusion level (dose) and EC ($R^2_F = -0.90$ and $R^2_{FD} = -0.93$) was observed in both trials.

4. Discussion

4.1. Frass recirculation affects process efficiency and larval quality

Including frass as a dietary component for BSFL when bioconverting food waste significantly affected process performance and composition of the larval biomass obtained (Fig. 1, Table 2). There were similar effects in both Trial 2, where an increase in frass inclusion was achieved by increasing larval VS feed dose while the larval density was kept constant at 5 larvae cm^{-2}, and in Trial 3, where increasing frass inclusion was achieved while maintaining the larval VS feed dose at 263 mg VS $larva^{-1}$, by varying the larval density. Survival (Fig. 1C and H) was not affected by inclusion of frass in the larval diet. However, overall higher survival was observed in larvae from Trial 3, in which the VS feed dose was kept constant. The seed larvae used in Trial 2 were somewhat lighter than those used in Trial 3 (1.44 and 1.72 mg $larva^{-1}$, respectively). This could be one of the reasons for the lower survival in Trial 2. However, similar yields were observed at harvest, which corroborates findings by Lopes et al. (2023) that total mass yield of larvae in a treatment unit was maintained at different larval densities, with the individual larvae growing larger at lower densities. When the feed substrate was provided

Table 3
Composition of the frass fertilizers obtained in Trial 2 (F treatments) and Trial 3 (FD treatments) when rearing black soldier fly larvae on food waste in combination with increasing levels (0%–100%) of frass as part of the feed substrate in terms of organic matter (OM), total organic carbon (TOC), total nitrogen (N_T), organic nitrogen (N_{org}), phosphorus (P), potassium (K), sulphur (S), calcium (Ca), magnesium (Mg), sodium (Na), zinc (Zn) and boron (B).

	OM	TOC	N_T	N_{org}	P	K	S	Ca	Mg	Na	Zn	B
	$\%_{DM}$										$mg\ kg^{-1}_{DM}$	
Trial 2 (F treatments)												
F0%	86.7	45.1	3.77	2.75	0.42	2.09	0.47	0.75	0.10	3.7	31	14.6
F2.5%	86.4	43.2	4.08	2.64	0.47	2.29	0.49	0.50	0.11	3.9	35	15.7
F10%	85.4	41.3	4.26	3.33	0.49	2.30	0.50	0.64	0.11	4.0	34	15.9
F17.5%	85.3	41.5	4.44	3.52	0.45	2.32	0.49	0.72	0.11	4.1	33	16.2
F25%	84.6	41.3	4.45	3.59	0.47	2.34	0.52	0.75	0.11	4.4	35	16.8
F32.5%	84.2	41.2	4.66	3.68	0.52	2.40	0.56	0.57	0.12	4.7	36	18.1
F40%	83.8	40.0	4.21	3.18	0.52	2.50	0.59	0.69	0.12	4.8	63	19.1
F100%	82.5	39.9	3.99	2.89	0.53	2.48	0.62	0.65	0.11	5.1	40	20.0
Regression *p* value	0.000***	0.000***	0.201^{NS}	0.308^{NS}	0.007**	0.001***	0.007**	0.982^{NS}	0.07^{NS}	0.001**	0.247^{NS}	0.002**
Trial 3 (FD treatments)												
FD0%	87.1	45.6	3.78	2.75	0.42	2.06	0.47	0.74	0.10	3.7	31	14.4
FD2.5%	86.8	42.1	4.23	3.42	0.38	1.93	0.46	0.77	0.10	3.7	29	16.7
FD10%	86.2	41.4	4.31	3.35	0.39	1.98	0.46	1.36	0.09	3.8	41	15.9
FD17.5%	85.2	40.9	3.83	2.85	0.43	2.20	0.53	0.65	0.10	4.3	35	18.3
FD25%	84.8	41.3	3.92	3.00	0.45	2.29	0.59	0.63	0.11	4.3	33	18.1
FD32.5%	84.1	40.8	3.90	2.88	0.48	2.42	0.55	0.53	0.11	4.7	35	19.4
FD40%	83.8	40.2	3.71	2.72	0.46	2.43	0.6	0.64	0.11	4.9	36	19.9
FD100%	82.0	40.0	3.86	3.02	0.52	2.71	0.66	0.54	0.11	5.4	39	20.7
Regression *p* value	0.000***	0.000***	0.549^{NS}	0.483^{NS}	0.023*	0.010*	0.005**	0.392^{NS}	0.02*	0.004**	0.112^{NS}	0.000***

Trial 2 (*F*): varying larval volatile solids (VS) feed dose; Trial 3 (*FD*): varying larval density. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS: not significant.

Table 4
Maturity-related properties of the frass fertilizers obtained in Trial 2 (F treatments) and Trial 3 (FD treatments) when rearing black soldier fly larvae on food waste in combination with increasing levels (0%–100%) of frass as part of the feed substrate, indicated by carbon-to-nitrogen ratio (C/N ratio), pH, electrical conductivity (EC), humic extract (Humic Ext), maturity index calculated based on CO_2 and NH_3 emissions analysed in the Solvita® test (Solvita) and stability as verified by the self-heating capacity in Dewar vessels.

	C/N ratio	pH	EC (mS cm^{-2})	Humic Ext (%)	Solvita®	Stability
Trial 2 (F treatments)						
F0%	11.96	7.10	26.86	26.5	1	2
F2.5%	10.59	6.93	23.76	25.2	1	2
F10%	9.69	7.36	21.24	15.4	1	2
F17.5%	9.35	7.19	21.79	11.8	1	3
F25%	9.28	7.07	20.74	10.1	3	3
F32.5%	8.84	7.43	20.86	9.1	4	4
F40%	9.50	7.61	15.98	9.1	5	4
F100%	10.00	8.07	19.10	6.0	6	4
Regression p value	0.0186*	0.035*	0.0043**	0.0000***	0.0157*	0.0064**
Trial 3 (FD treatments)						
FD0%	12.06	7.10	26.86	29.4	1	2
FD2.5%	9.95	6.71	24.88	24.7	1	2
FD10%	9.61	7.07	28.10	20.3	1	2
FD17.5%	10.68	7.59	22.60	11.9	2	3
FD25%	10.54	7.64	22.73	8.8	4	3
FD32.5%	10.46	7.95	20.31	11.3	5	4
FD40%	10.84	8.12	20.27	10.3	5	4
FD100%	10.36	8.32	19.99	8.7	6	4
Regression p value	0.314NS	0.005**	0.0176*	0.0002***	0.0061**	0.0064**

Trial 2 (*F*): varying larval volatile solids (VS) feed dose; Trial 3 (*FD*): varying larval density. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS: not significant.

in depths exceeding 6.5 cm in that study, a greater proportion of the VS provided was not converted into frass, but remained wet and visually unprocessed. Similarly, Dortmans et al., 2017 concluded that substrate depth should not exceed 5 cm, otherwise larvae could not gain access to the substrate. In the present study, visual observations showed that the larvae at all frass inclusion levels in Trials 2 and 3 were able to access the whole substrate throughout the 12 days of bioconversion and the recirculated frass fertilisers obtained after biodigestion in both trials were dry and easily sievable using dry separation methods (active sieving with different mesh sizes). Additionally, as larval yield was not significantly different at any frass inclusion level in both trials, it is feasible to assume that at 0–100% inclusion of dietary frass, larvae were able to digest the available nutrients in their diet to the same degree. It was hypothesized that lower yields would be obtained at intermediate and high frass inclusion levels (*e.g.* >30%), due to a theoretical lower nutrient availability in frass in comparison to food waste. However, this was not found in neither Trial 2 (with increasing feed doses) nor Trial 3 (with a stable feed dose and increasing larval density). Therefore, it is feasible to assume that frass from food waste still contain significant concentration of nutrients that can be digested by the larvae. For other feed substrates, particularly lignin-rich ones with already low amounts of digestible nutrients, this response may not be seen. Future research should address this in order to validate frass recirculation with distinct substrates.

Increased inclusion levels of frass in the diet of BSFL correlated negatively with BCE and Mat.Red (Fig. 1A–B, 1F-1G). It has been established that several characteristics of BSFL diet substrates can reduce BCE, such as nutritional imbalances (Barragan-Fonseca et al., 2018), high moisture content (Bekker et al., 2021) and high proportion of lignocellulosic substances (Peguero et al., 2022). Thus, the linear reduction observed in BCE of food waste and frass blends (Fig. 1A and 1F) might have been due to lower utilisation of the available nutrients in the diets, due to the presence of partly digested dietary components in that frass. It is feasible to assume that the use of nutrients in frass by BSFL changes with frass type, with *e.g.* fibre-derived frass having lower nutritional content than frass deriving from food waste.

A major scientific contribution by this study was the significant larval yield increase per tonne of food waste from 207 kg at 0% frass inclusion to 362–403 kg at 40% inclusion (DM basis). These observations highlighted that the same larval mass yield on a wet basis could be obtained by using up to 56% less food waste (Fig. 1; Tables S1 and S2). The large increase in larval mass was achieved simply by recirculating frass from the production back into the bioconversion process. This innovative strategy delivered a very significant gain in efficiency. Moreover, frass is a dry material and, when reintroduced into production, could possibly reduce the moisture content of wet substrates, an important requirement in the BSF industry. In another waste management technology, thermophilic composting, reintroducing mature compost into newly established piles is common practice to improve process efficiency in multiple ways, including duration and microbial activity in the pile, physico-chemical characteristics of the final compost and its degree of phytotoxicity (Ma et al., 2019; Yang and Zhang, 2022). As shown in the present study, the frass generated in a frass-recirculation process had higher stability and maturity. However, it is feasible to assume that a dietary inclusion of already recirculated frass could give lower larval yield per tonne of food waste than found in this study. On the other hand, the improvement in substrate texture brought about by frass inclusion, as demonstrated by the reduced impact of substrate depth, could have additional benefits beyond nutritional aspects. Further investigations on this issue are needed.

Although larval yield remained unchanged with increasing frass inclusion levels, the composition of the larvae changed (Table 2). Protein and fat build-up in BSFL is highly dependent on diet composition, with protein build-up in the larvae being positively correlated with protein present in the substrate (Barragan-Fonseca et al., 2021). This corroborates findings in the present study that higher inclusion level of frass in their diets (from 0 to 100%) meant that more N was present in the substrate (*i.e.* more protein). The higher N content lead to increased protein accumulation in the larval biomass, to almost 39% on a DM basis with 40% frass inclusion, while the fat content in larval biomass was reduced with such inclusion, reaching values as low as 16% (Table 2). Such changes in the crude protein and crude fat content of the larval biomass could have interesting implications regarding the use of this larval biomass as animal feed. Generally, the most costly ingredient in animal feeds is protein, which is frequently obtained from unsustainable sources, such as fishmeal and soybean meal (Hua et al., 2019). Great efforts have been made for reducing the use of such ingredients and increasing the use of more sustainable ones, such as insect meal (Quang Tran et al., 2022). Therefore, it may be possible to tailor larvae meals by incorporation of frass as a dietary component for BSF larvae at various

Table 5
Amino acid composition (mg $100g^{-1}$) of the recirculated frass generated in Trial 2 (F treatments) and Trial 3 (FD treatments) when rearing black soldier fly larvae on food waste in combination with increasing levels (0%–100%) of frass as part of the feed substrate. **Ala**: alanine, **Arg**: arginine, **Asn**: asparagine, **Cys + Cyst**: cysteine + cystine, **Gln**: glutamine, **Gly**: glycine, **His**: histidine, **Ile**: isoleucine, **Leu**: leucine, **Lys**: lysine, **Met**: methionine, **Phe**: phenylalanine, **Pro**: proline, **Ser**: serine, **Thr**: threonine, **Trp**: tryptophan, **Tyr**: tyrosine, **Val**: valine, **Sum_{aa}**: sum of all amino acids analysed.

	Ala	Arg	Asn	Cys + Cyst	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	Sum_{aa}
Trial 2 (F treatments)																			
F0%	601	281	826	125	1620	542	167	311	557	409	135	348	603	341	431	115	259	438	8109
F2.5%	638	355	900	157	1460	608	189	295	543	373	134	322	368	387	493	120	283	428	8053
F10%	804	399	983	149	1760	631	195	387	702	487	152	412	478	441	550	141	321	553	9545
F17.5%	889	359	1080	153	1900	684	225	433	802	521	171	461	598	425	546	156	313	603	10,319
F25%	766	316	982	161	1550	651	202	339	593	392	120	370	447	404	507	112	325	501	8738
F32.5%	563	330	764	116	1510	511	176	327	589	412	140	348	340	304	357	116	279	471	7653
F40%	577	200	722	80	1160	464	157	285	550	323	95	335	298	283	360	100	219	390	6598
F100%	667	249	626	80	1190	446	161	286	543	278	89	268	336	225	250	103	224	392	6413
Regression *p* value	0.83	0.44	0.39	0.223	0.27	0.39	0.81	0.9	0.92	0.38	0.26	0.66	0.11	0.26	0.22	0.58	0.58	0.85	0.02*
Trial 3 (FD treatments)																			
FD0%	633	306	889	129	1580	554	182	332	597	392	143	363	548	349	463	122	282	476	8340
FD2.5%	959	432	1260	219	2490	797	275	461	864	641	211	492	673	495	603	172	365	678	12,087
FD10%	886	454	1110	184	2280	744	242	452	840	556	185	429	595	481	589	161	319	649	11,156
FD17.5%	751	363	968	149	1760	602	206	392	691	499	160	425	488	400	490	145	330	546	9365
FD25%	713	390	886	149	1610	555	198	348	604	446	153	318	365	367	440	144	265	458	8409
FD32.5%	686	203	738	121	1400	530	182	306	561	307	118	347	397	316	361	111	236	419	7339
FD40%	613	244	659	103	1230	456	156	253	489	320	108	294	276	272	305	106	237	353	6474
FD100%	447	152	596	82	923	409	130	232	437	216	70	293	217	251	291	94	219	314	5373
Regression *p* value	0.21	0.17	0.08	0.14	0.11	0.12	0.14	0.14	0.15	0.13	0.08	0.12	0.01*	0.14	0.07	0.19	0.09	0.10	0.02*

Trial 2 (*F*): varying larval volatile solids (VS) feed dose; Trial 3 (*FD*): varying larval density. *$p < 0.05$.

Table 6
Phytotoxicity in seed germination bioassays of frass fertilisers generated in Trial 1 and 2, obtained when rearing black soldier fly larvae on food waste in combination with increasing levels (0–100%) of frass as part of the feed substrate. Seed germination was evaluated based on water cress (*Nasturtium officinale*) seed germination rate (SG), relative seed germination in relation to a control treatment (RSG), relative radicle growth in relation to a control treatment (RRG) and germination index (GI).

	SG (%)	RSG (%)	RRG (%)	GI (%)
Trial 2 (F treatments)				
F0%	4.4	4.7	17.5	0.8
F2.5%	2.2	2.3	10.3	0.2
F10%	2.2	2.3	12.3	0.3
F17.5%	2.2	2.3	26.7	0.6
F25%	15.6	16.3	23.9	3.9
F32.5%	17.8	18.6	32.5	6.
F40%	13.3	17.7	28.9	5.1
F100%	4.4	5.9	23.9	1.4
Regression *p* value	0.29^{NS}	0.228^{NS}	0.078^{NS}	0.199^{NS}
Trial 3 (FD treatments)				
FD0%	4.4	4.6	17.4	0.8
FD2.5%	6.7	7.0	18.6	1.3
FD10%	22.2	29.4	26.7	7.9
FD17.5%	20.0	20.9	38.2	7.8
FD25%	26.7	27.9	21.2	5.9
FD32.5%	48.9	64.7	34.6	22.
FD40%	60.0	79.4	34.2	27.2
FD100%	77.8	102.9	42.8	42.9
Regression *p* value	0.0039**	0.0072**	0.0125*	0.0131*

Trial 2 (*F* treatments): varying larval VS feed dose; Trial 3 (*FD* treatments): varying larval density. *$p < 0.05$; **$p < 0.01$; NS: not significant.

inclusion levels that could be of interest to the livestock production sector. This is a topic that future studies should address: how to balance protein and fat levels of larval biomass using frass as a dietary component.

Similar trends for fat accumulation in BSF larvae were observed by Ewald et al. (2020) on feeding BSFL with bread and increasing level of mussels (0–50% on a wet-weight basis), with fat accumulation (DM basis) declining from 20.4 ± 0.7% at 10% mussel inclusion to 16.1 ± 2.3% at 50% mussel inclusion and with a major shift in larval fatty acid content with increasing mussel inclusion level. These results demonstrate that inclusion of frass in BSFL diets can alter the composition of BSFL biomass. For a better understanding of how the composition of BSFL changes with frass inclusion, the amino acid and fatty acid composition of frass-fed larvae should be investigated.

4.2. Frass recirculation affects nutrient concentration of subsequent frass

Increasing frass inclusion in the BSFL diet resulted in a higher degree of organic matter decomposition throughout the bioconversion process, indicating that frass derived from bioconversion of food waste still contained easily degradable organic matter. The organic matter and TOC content in frass from BSFL fed 100% frass in both Trials 2 and 3 was around 5% and 12% lower, respectively, than when the BSFL were fed only food waste (0% frass, F0 and FD0) (Table 3). Song et al. (2021) composted frass derived from a mixture of okara and wheat bran under forced aeration for approximately six weeks and found that the total C content in the composted frass was approximately 17% lower than in raw frass, which indicates that the recirculation process might resemble a composting process.

The concentrations of macro- and micronutrients in the recirculated frass fertilisers obtained with inclusion of frass as a dietary component for BSFL were affected slightly differently in Trials 2 and 3, but were within the expected ranges based on other studies (Gärttling and Schulz, 2022; Lopes et al., 2022). While total N and organic N content were not altered by increasing frass inclusion level, while P, K and S concentrations increased significantly with increasing amount of fresh frass in the diet (Table 3). Including frass in BSFL diets may lead to a higher

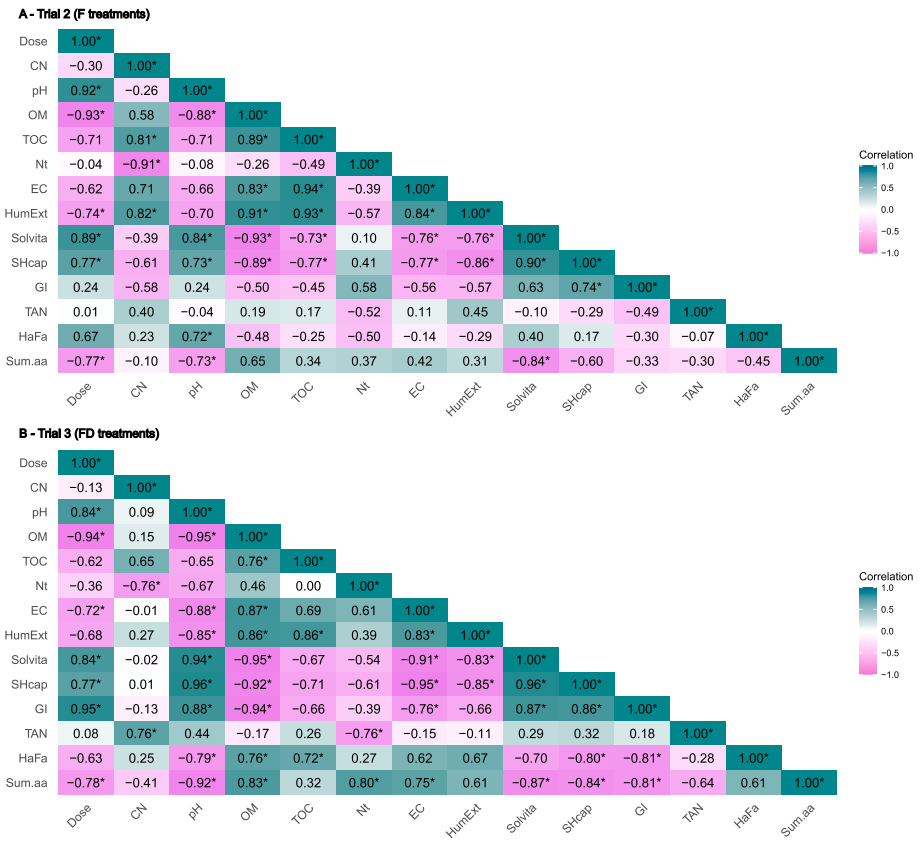

Fig. 2. Correlation matrix showing all variables of interest regarding frass fertiliser maturity and stability in (A) Trial 2 (F treatments) with varying larval VS feed dose, and (B) Trial 3 (FD treatments) with varying larval density. The frass fertilizers from Trials 1 and 2 were obtained when rearing black soldier fly larvae on food waste in combination with increasing levels (0%–100%) of frass as part of the feed substrate. **Dose:** level of fresh frass included in BSFL diets; **CN**: carbon-to-nitrogen ratio; pH: pH of frass fertiliser; **OM**: organic matter; **TOC**: total organic carbon; **N_t**: total nitrogen; **EC**: electrical conductivity; **HumcExt**: humic extract calculated as sum of fulvic and humic acids; **Solvita**: numerical result of Solvita® test; **SHcap**: self-heating capacity; **GI**: germination index; **TAN**: total ammonium-nitrogen; **HaFa**: humic acid/fulvic acid ratio; **Sum.aa**: sum of all amino acids analysed in frass fertilisers. * indicates significant correlations between two variables ($p < 0.05$) based on Pearson correlation.

concentration of plant nutrients in the frass fertiliser generated. However, Ca, Mg and Zn concentrations generally remained unchanged. These differences in the accumulation pattern of different minerals were unexpected, especially for minerals that do not volatilise. However, it is possible that larval uptake of nutrients differed between the frass inclusion levels tested.

With increasing frass inclusion, sodium was further concentrated in the recirculated frass to a highly significant extent, from 3.73% to 5.11–5.45% on a DM basis. The high concentration of Na was possibly due to the fact that the food waste substrate used was post-consumer food waste collected from Swedish restaurants, and many people add a lot of salt to their food. However, the sodium concentrations in the frass were much higher than expected, *e.g.* a previous study using food waste-derived compost reported a salt concentration of 2.7% (Yang et al., 2021a). Therefore, accumulation of this soluble salt in frass needs to be monitored closely in further studies, in order to understand its dynamics in BSFL bioconversion of food waste. The presence of soluble salts such as sodium in compost can result in plant growth inhibition when the compost is applied to soil, through increased soil salinity and effects on organic matter aggregates by altering binding site competition through negative charges on soil colloids (Leogrande and Vitti, 2019). The build-up of sodium from including fresh frass in the BSFL conversion process should be monitored in frass fertilisers before their application to soil.

Regardless of the level of frass included in the larval diet, there were no significant differences in amino acid composition of the frass fertilisers generated except for proline. However, the total sum of amino acids in the recirculated frass was reduced with higher inclusion level of dietary fresh frass (Table 5). In relation to soil fertility and plant physiology, amino acids are considered biostimulants. In general, biostimulants are naturally occurring substances that stimulate plant physiological processes (nutrition efficiency, abiotic stress tolerance), thereby modulating crop quality and yield, regardless of its nutrient content (Calvo et al., 2014; Du Jardin, 2015). Regarding amino acids, these can be divided into three main groups: growth-promoting (aspartate, glutamate, glutamine, serine, tryptophan and alanine), salt-resistant (tyrosine, phenylalanine, leucine, lysine, threonine, isoleucine, methionine and arginine) and heavy metal-resistant (asparagine, glycine, valine, histidine, cysteine and proline) (Yao et al., 2021). Amino acids are also precursors for formation of humic substances throughout composting and are related to microbial enzymatic activity in the soil (Wu et al., 2017: Olaetxea et al., 2018). Yao et al. (2021) reported increasing concentrations of all three groups of amino acids throughout a 75-day composting time when using maize straw and pig manure as raw materials. As mentioned above, the opposite trend was seen in the present study, suggesting that the BSFL might have consumed some of these amino acids during the 12 days of bioconversion.

Nitrogen is the most important nutrient that plants acquire from the soil. Specifically, plants take up organic nitrogen compounds of low molecular mass, including amino acids, peptides, and proteins, via membrane transporters into the roots (Paungfoo-Lonhienne et al., 2008; Rentsch et al., 2007; Tegeder and Rentsch, 2010; Näsholm et al., 2000). Amino acids are a nitrogen source for plants in natural ecosystems and agricultural systems, but peptides and proteins have received less

attention as potential nitrogen sources for plants. Amino acids in soil are usually present at nanogram scale (Gonzalez Perez et al., 2015; Tegeder and Rentsch, 2010), while organic materials such as the frass obtained in this study can contain amino acids at milligram scale (Table 5). Interactions between specific amino acids are known to impact soils in different ways. For instance, alanine can have metabolic priming effects on soil microorganisms, resulting in degradation of complex soil organic matter (Hamer and Marschner, 2005), while leucine stimulates enzyme production of selected bacterial groups (Kuzyakov, 2002). According to Tan et al. (2023), a compost rich in amino acids stimulates N absorption by plants due to increased abundance of distinct groups of microorganisms (*e.g. Acidobacteria*, *Firmicutes* and *Ascomycota*), benefiting soil as a whole. Amino acids and proteins comprise a major group of growth-promoting substances and are classified as biostimulants, i.e. substances that when applied to plants in very small concentrations enhance growth, plant nutrient use efficiency, abiotic stress tolerance and crop quality, regardless of their nutrient content (du Jardin, 2015; Jolayemi et al., 2023). The amino acid composition of a commercial BSFL frass was evaluated by Yildirim-Aksoy et al. (2020), who used frass as a dietary component for Pacific white shrimp (*Litopenaeus vannamei*) and concluded that up to 30% of standard protein ingredients could be replaced by BSFL frass without compromising the health of shrimp. Interestingly, the concentration of almost all amino acids in the recirculated frass obtained in the present study was higher than that in the abovementioned shrimp study, and also greater than that in other materials, such as mature compost prepared from maize straw and pig manure (Yao et al., 2021). Future studies should investigate the fate of amino acids from a mass balance perspective, identifying the fate of these compounds in the larvae and frass. Similarly, profiling the phytohormonal biostimulants in the frass would be interesting as humic materials are generally known to contain phytohormones (Pizzeghello et al., 2013;Nardi et al., 2018; Wong et al., 2020; Zhang et al., 2015).

4.3. Frass recirculation improves maturity and stability of subsequent frass

A small reduction in C/N ratio was observed in Trial 2 when no frass was included (C/N ratio = 11.96) in comparison with 40% (9.5) or 100% (10.0) inclusion. These values are within the recommended range (<30) for compost application to soil without soil microorganisms immobilising N, which is a typical sign of immaturity (Chen et al., 2014). Thus, based on the results obtained, C/N ratio alone does notseem be a good predictor of frass readiness for soil application, even though the frass fertilisers obtained have C/N ratios much below 30 and contained more than 3% N. However, up to 82% of the N present in frass was found to be organic (Table 3), meaning that a low C/N ratio would possibly not result in prompt availability of N for plants when applied to soil, as the organic fraction of N needs to be mineralised over time before plants can absorb it, a process that might take more than 60 days (Beesigamukama et al., 2021). Including frass as a dietary component could further exacerbate this, as the N concentration did not increase, while the P concentration increased, with increased frass inclusion, making the frass fertiliser more P-dominated. Gärttling et al. (2020) found higher N concentrations in the soil when using dried adult BSF as fertiliser than when using frass, even though a majority of N in the adults was in organic form and the concentration of total ammonium-nitrogen in the frass was considerably higher. Those authors attributed this to the frass being P-dominated, while the BSFL adults were N-dominated.

A usual change occurring during maturation of compost is the reduction of EC, which can be either a result of leaching of salts (not applicable in this study, as frass was produced in a closed container) or by decomposition of organic acids in the compost (Wichuk and McCartney, 2010). This trend was verified by the increasing levels of frass in both Trial 2 and 3, even though the sodium concentration increased with higher frass inclusion (Table 4), suggesting that the recirculated frass fertilisers obtained with higher dietary inclusion levels were more mature than those generated at low frass inclusion levels.

Significant positive correlations were seen between EC, organic matter and TOC (Fig. 2). These parameters were also strongly correlated with humic extract content (humic plus fulvic acids) in the frass, which was considerably lower in both trials when no frass was added than when 100% frass was added. The process responsible for the formation of humic substances during thermophilic composting is still under debate, with no clear consensus. It is generally assumed that humic substances derive from a combination of two main processes that occur concomitantly: breakdown of lignin into smaller compounds that act as precursors of humic substances (*e.g.* phenolic compounds) and through the formation of complexes of other molecules such as polysaccharides and proteins, with both processes being mediated by a succession of distinct groups of microorganisms (Guo et al., 2019). Zhao et al. (2017) describe two processes related to humic and fulvic acids in the composting processes: reduced concentration of fulvic acid (low molecular weight) at the start of the process (thermophilic stage) and increased concentration of humic acids (complex group of molecules of high molecular weight) later in the process (mesophilic stage). Many studies evaluating frass as a soil amendment or fertiliser in different soils and crops have reported limitations relating to its use, especially in terms of plant growth, as discussed by Lopes et al. (2022). Their study suggested that such problems derive mainly from the fact that organic waste bioconversion with BSFL is a very rapid process (<20 days) that does not allow sufficient time for the resulting frass to stabilise and mature. A typical composting process can take from two to 12 months depending on the size of the pile, composting conditions and type of material, to allow all stages of organic matter decomposition and further complexation, ensuring stabilisation and maturation (Fialho et al., 2010). A typical and successful BSFL bioconversion process in which organic waste is rapidly and completely transformed into biomass and frass should not exceed 15–20 days (Surendra et al., 2020). However, more than one feeding occasion is often implemented, meaning that a proportion of the material in the frass could have spent as little as seven days in the process. Considering the short BSFL bioconversion time, it is reasonable to assume that humic substances would not have sufficient time to form if this occurs through similar pathways in BSFL bioconversion as in composting.

In contrast to the findings in this study, several studies have demonstrated stabilisation of organic matter with humic extract formation in BSFL bioconversion. For example, Liu et al. (2020) observed an approximately 50% reduction in fulvic acid concentration in different manures (chicken, cow, pig) and 3-fold increase in humic acid concentration over a period of 10 days. However, food waste composition and its decomposition dynamics are not comparable to those of animal manures, which are already products of decomposition of other molecules. Similarly, Wang et al. (2021) evaluated different manures (chicken, cow, pig) as feed substrate for BSFL and observed that BSFL efficiently altered the quality of the substrate organic matter, with simple structure organic components (*e.g.* sugars, carbohydrates and non-recalcitrant fibres) in manure decreasing, and aromatic degree and molecular weight increasing, throughout BSFL bioconversion. While humic substances were reduced in the recirculated frass obtained at higher frass inclusion rates in the present study, other maturity and stability metrics suggested that frass maturity and stability had increased, to a score of 6 in the Solvita® test and IV in the self-heating capacity test in the treatments with 100% frass (Table 6). In both tests, the frass fertiliser (especially at inclusion levels of 25% and more) was categorised as “active curing compost”, meaning that it was moderately stable but still in the curing stage. Further studies should investigate the transformation of organic matter in frass deriving from substrates of different origin and degrees of decomposition. In addition, continuous recirculation of frass in a long-term waste bioconversion setting should be investigated, in order to verify if several rounds of bioconversion would result in differential organic matter decomposition degrees.

In order to further assess the maturity level of the frass fertilisers, germination tests were conducted using water cress seeds, since water cress is highly sensitive to a number of compounds that causes phytotoxicity, such as heavy metals, salts and ammonia (Luo et al., 2018). A significant increase in seed germination rate and GI was observed for the frass fertilisers obtained in Trial 3, where the larval density was adjusted while the larval VS feed dose was maintained at 265 mg VS larva^{-1}. The larvae in Trial 2 received a higher larval VS feed dose (from 0.26 at 0% frass to around 0.38 g VS larvae^{-1} at 40%/100% inclusion) and did not process it as well as in Trial 3. However, there was a significant increase in GI, a common trend in compost of increasing maturity over time (Gavilanes-Terán et al., 2016; Yang et al., 2021b).

While compost maturity and stability can be measured using multiple parameters, determining whether it is stable and/or mature and safe for use, is certainly not straightforward and dependent on factors such as compost composition, type of biological process used etc., as thoroughly discussed byKomilis and Tziouvaras (2009). The findings of the present study suggested that the maturity and stability of frass fertilisers can be improved by replacing the larval feed substrate with frass, with a minimum inclusion level of 25% being recommended. Conversely, no correlation was found between material degradation and C/N ratio, pH or NH_4^+/NO_3^- ratio in BSFL bioconversion, although these parameters were correlated with stability in thermophilic composting processes (Wichuk and McCartney, 2010), suggesting that some commonly used stability parameters may be more strongly related to frass stability than others.

5. Implications of frass inclusion as a dietary component for BSFL and future research directions

This study demonstrated that recirculating frass back into the bioconversion process affects the composition of both the larval biomass and the recirculated frass obtained. Specifically, larval biomass tended to have higher protein and lower fat content than biomass from larvae fed exclusively on food waste, which could impact further processing into a protein and oil fraction. Bioconversion efficiency was reduced when BSFL were fed blends of food waste and frass. However, a striking finding in this study was that larval yield per tonne of food waste increased with increasing frass inclusion level. This means that it is possible to produce significantly higher quantities of larvae per unit with less food waste.

The frass fertilisers obtained from recirculation of frass into the process contained higher levels of specific macro- and micronutrients and also demonstrated higher degree stability/maturity for frass inclusion rates >5%. It is not yet known whether the recirculated frass fertiliser obtained could be used for another round of bioconversion, as the most easily available compounds (such as short-chain carbohydrates) were likely degraded. Whether or not the frass could be used for subsequent bioconversion cycles is likely to depend on the initial substrate used to produce the first-cycle frass and on the degree of organic matter decomposition in the substrate after the second bioconversion step. Further research is needed to find the optimal recycling protocol.

The BSFL industry is currently highly dependent on use of dry materials to adjust the moisture content of waste substrates (which usually have low DM content), aiming to achieve consistently high throughput. World market commodities (*e.g.* wheat bran and soybean meal) are currently used for this purpose, meaning that the insect industry needs to buy feedstock instead of being paid for processing waste, thus reducing the environmental benefits of the process as a whole. Recirculating frass (which is expected to have low moisture content at harvest) into the BSFL bioconversion process could completely avoid use of world market commodities as a drying agent for high-moisture waste substrates. In addition, frass fertilisers with increased stability could be generated, as demonstrated in this study.

A possible barrier to adoption of this approach is that frass contains larval faeces, and thus legally falls within the group 'animal manures' and is currently not permitted for use as a feed substrate for rearing BSFL in some places. In the EU, for instance, insect rearing falls under the General Food Law (European Commission, 2002) and the Hygiene Package (European Commission, 2004), which allow only materials of vegetable origin (with some exceptions) to be used as feed substrate for larvae. Additionally, the animal by-product (ABP) regulation (European Commission, 2009) restricts consumption of animal by-products by livestock. The larvae in the present study (considered livestock) consumed frass (manure) from other batches of larvae (ABP category 2) within the same process unit, so the legislation is unclear. However, recirculating frass (manure) within BSFL production with plant-origin waste streams could certainly represent the first step towards a more circular industry. Having an internal recycling flow of microorganisms in BSFL treatment has been shown to result in a more stable biological process (Lundgren, 2019). The two primary challenges associated with establishing a circular frass flow are the risk of accumulating unwanted substances (*e.g.* heavy metals) and disease management. With a high substrate retention time, recalcitrant materials might accumulate and could affect the larvae treatment process if the concentrations become too high. Keeping the concentrations of recalcitrant materials such as metals at low levels should be possible by avoiding high substrate retention time in the process (Haug, 1993). The risk of disease transmission could be reduced by maintaining a linear system without substrate recycling and implementing regular sanitisation of the process, implementing stronger barriers, such as pasteurisation of all incoming material, and enhancing protection against wild insects or other organisms that can enter the system and introduce diseases. Recycling frass back into the process would then lead to more stable performance of the treatment and higher productivity of larvae biomass in relation to incoming substrate.

Based on the aforementioned observations, it is suggested that future research on frass recirculation should investigate the effect of frass recirculation from waste streams other than food waste and the potential effects of recirculating frass constantly, in long-term investigations, in order to evaluate how many times, and at which inclusion levels, the recirculation of frass could be made continuously. The risks associated with a long-term recirculation, including disease transmission, heavy metals accumulation and others should be assessed. In addition, environmental impact assessments should be conducted to determine if frass recirculation could reduce the environmental impacts of BSF rearing, by enabling the use of less optimal feed substrates and eliminating the need for commodity drying agents (*e.g.* wheat bran), while still ensuring predictable and consistent product a quality, a challenge faced in multiple regions around the world, including the European insect sector (Lalander and Lopes, 2024).

6. Conclusions

Inclusion of frass in the diet of BSFL reared on post-consumer food waste resulted in larvae with up to 40% higher protein content and 47% lower fat content. Bioconversion efficiency was reduced with increasing levels of frass incorporation in the larval diet, while the yield of larval biomass per tonne of food waste (DM basis) increased by up to 96%. Increased inclusion of frass also resulted in a higher recirculated frass production rate, indicating decreased substrate digestibility. The frass fertilisers generated from frass-amended substrate had elevated concentrations of relevant plant nutrients (P, K, S, B); but also of Na, which could potentially hamper their use in agriculture. Overall, frass inclusion in BSFL bioconversion of food waste resulted in more stable and mature frass fertilisers, as indicated by the positive correlations with some parameters used to assess compost stability (organic matter decomposition, CO_2 evolution, N volatilisation, self-heating capacity, germination index), but not others (C/N ratio, ammonium/nitrate ratio, pH, humic substance formation). These observations revealed that some widely used compost maturity and stability indicators might not be suitable for assessing BSFL frass stability. An additional benefit of recirculating frass is its ability to act as a drying agent for the wet

substrates and therefore ressolving a major concern hinderingthe insect rearing industry globally.

CRediT authorship contribution statement

Ivã Guidini Lopes: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Viktoria Wiklicky:** Writing – review & editing, Visualization, Validation, Investigation, Data curation, Conceptualization. **Björn Vinnerås:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization. **Jean Wan Hong Yong:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Cecilia Lalander:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, 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

Data will be made available on request.

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Appendix A. Supplementary data

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References

Barragan-Fonseca, K.B., Dicke, M., van Loon, J.J.A., 2018. Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (*Hermetia illucens*). Entomol. Exp. Appl. 166, 761–770.

Barragan-Fonseca, K.B., Gort, G., Dicke, M., van Loon, J.J.A., 2021. Nutritional plasticity of the black soldier fly (*Hermetia illucens*) in response to artificial diets varying in protein and carbohydrate concentrations. Journal of Insects as Food and Feed 7, 51–61. https://doi.org/10.3920/JIFF2020.0034.

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

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.

Beesigamukama, D., Subramanian, S., Tanga, C.M., 2022. Nutrient quality and maturity status of frass fertilizer from nine edible insects. Sci. Rep. 12, 7182. https://doi.org/10.1038/s41598-022-11336-z.

Bekker, N.S., Heidelbach, S., Vestergaard, S.Z., Nielsen, M.E., Riisgaard-Jensen, M., Zeuner, E.J., et al., 2021. Impact of substrate moisture content on growth and metabolic performance of black soldier fly larvae. Waste Manag. 127, 73–79. https://www.sciencedirect.com/science/article/pii/S0956053X21002269.

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.

Bosch, G., van Zanten, H.H.E., Zamprogna, A., Veenenbos, M., Meijer, N.P., van der Fels-Klerx, H.J., et al., 2019. Conversion of organic resources by black soldier fly larvae: legislation, efficiency and environmental impact. J. Clean. Prod. 222, 355–363. https://www.sciencedirect.com/science/article/pii/S0959652619306791.

Chen, B., Liu, E., Tian, Q., Yan, C., Zhang, Y., 2014. Soil nitrogen dynamics and crop residues. A review. Agronomy for Sustainable Development 34, 429–442. https://doi.org/10.1007/s13593-014-0207-8.

Brinton, W., Evans, E., Droffner, M., Brinton, R.B., 1995. A standardized Dewar test for evaluation of compost self-heating. BioCycle 36, 1–16.

Calvo, P., Nelson, L., Kloepper, J.W., 2014. Agricultural uses of plant biostimulants. Plant Soil 383, 3–41. https://doi.org/10.1007/s11104-014-2131-8.

Chen, D.M.-C., Bodirsky, B.L., Krueger, T., Mishra, A., Popp, A., 2020. The world's growing municipal solid waste: trends and impacts. Environ. Res. Lett. 15, 074021 https://doi.org/10.1088/1748-9326/ab8659.

Dortmans, B., Diener, S., Verstappen, B., Zurbrügg, C., 2017. Black soldier fly biowaste processing - a step-by-step guide. In: Donahue, P. (Ed.), Department of Sanitation, Water and Solid Waste for Development (Sandec). Dübendorf, Switzerland.

du Jardin, P., 2015. Plant biostimulants: definition, concept, main categories and regulation. Sci. Hortic. 196, 3–14. https://www.sciencedirect.com/science/article/pii/S0304423815301850.

European Commission, 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. In: Official Journal of the European Union L 31, pp. 1–24. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2002:031:TOC.

European Commission, 2004. Regulation (EC) No 852/2004 of the European parliament and of the council of 29 April 2004 on the hygiene of foodstuffs. Off. J. Eur. Union L 139, 1–54. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2004:139:TOC.

European Commission, 2009. Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). Off. J. Eur. Union 1–33. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2009:300:TOC.

European Commission, 2021. Commission Regulation (EU) 2021/1925 of 5 November 2021 Amending Certain Annexes to Regulation (EU) No 142/2011 as Regards the Requirements for Placing on the Market of Certain Insect Products and the Adaptation of a Containment Method, L393. Official Journal of the European Union, pp. 4–8. https://eur-lex.europa.eu/oj/direct-access.html.

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S., Lalander, C., 2020. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) – possibilities and limitations for modification through diet. Waste Manag. 102, 40–47. http://www.sciencedirect.com/science/article/pii/S0956053X19306440.

Fialho, L.L., Lopes da Silva, W.T., Milori, D.M.B.P., Simões, M.L., Martin-Neto, L., 2010. Characterization of organic matter from composting of different residues by physicochemical and spectroscopic methods. Bioresour. Technol. 101, 1927–1934. https://www.sciencedirect.com/science/article/pii/S0960852409014151.

Gavilanes-Terán, I., Jara-Samaniego, J., Idrovo-Novillo, J., Bustamante, M.A., Moral, R., Paredes, C., 2016. Windrow composting as horticultural waste management strategy – a case study in Ecuador. Waste Manag. 48, 127–134. https://www.sciencedirect.com/science/article/pii/S0956053X15302105.

Gonzalez Perez, P., Zhang, R., Wang, X., Ye, J., Huang, D., 2015. Characterization of the amino acid composition of soils under organic and conventional management after addition of different fertilizers. J. Soils Sediments 15, 890–901. https://doi.org/10.1007/s11368-014-1049-3.

Guo, H., Jiang, C., Zhang, Z., Lu, W., Wang, H., 2021. Material flow analysis and life cycle assessment of food waste bioconversion by black soldier fly larvae (*Hermetia illucens* L.). Sci. Total Environ. 750, 141656. https://www.sciencedirect.com/science/article/pii/S0048969720351858.

Guo, X-x, Liu, H-t, Wu, S-b, 2019. Humic substances developed during organic waste composting: formation mechanisms, structural properties, and agronomic functions. Sci. Total Environ. 662, 501–510. https://www.sciencedirect.com/science/article/pii/S0048969719301548.

Gärttling, D., Kirchner, S.M., Schulz, H., 2020. Assessment of the N- and P-fertilization effect of black soldier fly (Diptera: Stratiomyidae) by-products on maize. J. Insect Sci. 20 https://doi.org/10.1093/jisesa/ieaa089.

Gärttling, D., Schulz, H., 2022. Compilation of black soldier fly frass analyses. J. Soil Sci. Plant Nutr. 22, 937–943. https://doi.org/10.1007/s42729-021-00703-w.

Hamer, U., Marschner, B., 2005. Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. Soil Biol. Biochem. 37, 445–454. https://www.sciencedirect.com/science/article/pii/S0038071704002901.

Haug, R.T., 1993. Chapter 11: process dynamics I - development of simulation models. The Pratical Handbook of Compost Engineering. Lewis Publisher, Boca Raton, USA.

Hua, K., Cobcroft, J.M., Cole, A., Condon, K., Jerry, D.R., Mangott, A., et al., 2019. The future of aquatic protein: implications for protein sources in aquaculture diets. One Earth 1, 316–329. https://www.sciencedirect.com/science/article/pii/S2590332219301320.

Janssen, R.H., Vincken, J.-P., van den Broek, L.A.M., Fogliano, V., Lakemond, C.M.M., 2017. Nitrogen-to-Protein conversion factors for three edible insects: *Tenebrio molitor, Alphitobius diaperinus*, and *Hermetia illucens*. J. Agric. Food Chem. 65, 2275–2278. https://www.ncbi.nlm.nih.gov/pubmed/28252948.

Jasso, B., Quinchia, L., Waliczek, T.M., Drewery, M.L., 2024. Black soldier fly larvae (Hermetia illucens) frass and sheddings as a compost ingredient. Front. Sustain. Food Syst. 7. https://www.frontiersin.org/articles/10.3389/fsufs.2023.1297858.

Jolayemi, O.L., Malik, A.H., Vetukuri, R.R., Saripella, G.V., Kalyandurg, P.B., Ekblad, T., Yong, J.W.H., Olsson, M.E., Johansson, E., 2023. Impact of protein-based biostimulants on agronomic traits, protein components and metabolic processes of

sugar beet. International Journal of Molecular Sciences 24, 9720. https://doi.org/10.3390/ijms24119720.
Kaza, S., Yao, L., Bhada-Tata, P., Van Woerden, F., 2018. What a Waste 2.0: A Global Snapshot of Solid Waste Management to 2050. The world bank, Washington, USA.
Komilis, D.P., Tziouvaras, I.S., 2009. A statistical analysis to assess the maturity and stability of six composts. Waste Manag. 29, 1504–1513. https://www.sciencedirect.com/science/article/pii/S0956053X08003668.
Kuzyakov, Y., 2002. Review: factors affecting rhizosphere priming effects. J. Plant Nutr. Soil Sci. 165, 382–396. https://doi.org/10.1002/1522-2624(200208)165:4<382::AID-JPLN382>3.0.CO;2.
Lalander, C., Lopes, I.G., 2024. Advances in Substrate Source Composition for Rearing Black Soldier Fly Larvae as a Protein Source. Insects as Alternative Sources of Protein for Food and Feed. Burleigh Dodds Science Publishing.
Lalander, C., Vinnerås, B., 2022. Actions needed before insects can contribute to a real closed-loop circular economy in the EU. Journal of Insects as Food and Feed 8, 337–342. https://doi.org/10.3920/JIFF2022.x003.
Leogrande, R., Vitti, C., 2019. Use of organic amendments to reclaim saline and sodic soils: a review. Arid Land Res. Manag. 33, 1–21. https://doi.org/10.1080/15324982.2018.1498038.
Li, M., Jia, N., Lenzen, M., Malik, A., Wei, L., Jin, Y., et al., 2022. Global food-miles account for nearly 20% of total food-systems emissions. Nature Food 3, 445–453. https://doi.org/10.1038/s43016-022-00531-w.
Liu, T., Awasthi, M.K., Awasthi, S.K., Zhang, Y., Zhang, Z., 2020. Impact of the addition of black soldier fly larvae on humification and speciation of trace elements during manure composting. Ind. Crop. Prod. 154, 112657. https://www.sciencedirect.com/science/article/pii/S0926669020305732.
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://www.sciencedirect.com/science/article/pii/S0956053X23005615.
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://www.sciencedirect.com/science/article/pii/S0956053X22000666.
Lundgren, K., 2019. Impact of Fly Specific Bacteria on Fly Larvae Composting. MSc. Swedish University of Agricultural Sciences & Uppsala University. http://www.diva-portal.org/smash/record.jsf?pid=diva2%3A1291251&dswid=-3373.
Luo, Y., Liang, J., Zeng, G., Chen, M., Mo, D., Li, G., et al., 2018. Seed germination test for toxicity evaluation of compost: its roles, problems and prospects. Waste Manag. 71, 109–114. https://www.sciencedirect.com/science/article/pii/S0956053X17306918.
Ma, C., Hu, B., Wei, M.-B., Zhao, J.-H., Zhang, H.-Z., 2019. Influence of matured compost inoculation on sewage sludge composting: enzyme activity, bacterial and fungal community succession. Bioresour. Technol. 294, 122165. https://www.sciencedirect.com/science/article/pii/S0960852419313951.
Mertenat, A., Diener, S., Zurbrügg, C., 2019. Black Soldier Fly biowaste treatment – assessment of global warming potential. Waste Manag. 84, 173–181. http://www.sciencedirect.com/science/article/pii/S0956053X18307293.
Näsholm, T., Huss-Danell, K., Högberg, P., 2000. Uptake of organic nitrogen in the field by four agriculturally important plant species. Ecology 81, 1155–1161.
Nardi, S., Pizzeghello, D., Ertani, A., 2018. Hormone-like activity of the soil organic matter. Appl. Soil Ecol. 123, 517–520. https://doi.org/10.1016/j.apsoil.2017.04.020.
Olaetxea, M., de Hita, D., Garcia, C.A., Fuentes, M., Baigorri, R., Mora, V., Garnica, M., Urrutia, O., Erro, J., Zamarreño, A.M., et al., 2018. Hypothetical framework integrating the main mechanisms involved in the promoting action of rhizospheric humic substances on plant root and shoot-growth. Appl. Soil Ecol. 123, 521–537. https://doi.org/10.1016/j.apsoil.2017.06.007.
Paungfoo-Lonhienne, C., Lonhienne, T.G.A., Rentsch, D., Robinson, N., Christie, M., Webb, R.I., Gamage, H.K., Carroll, B.J., Schenk, P.M., Schmidt, S., 2008. Plants can use protein as a nitrogen source without assistance from other organisms. Proc. Natl. Acad. Sci. USA 105, 4524–4529. https://doi.org/10.1073/pnas.0712078105.
Peguero, D.A., Gold, M., Vandeweyer, D., Zurbrügg, C., Mathys, A., 2022. A review of pretreatment methods to improve agri-food waste bioconversion by black soldier fly larvae. Front. Sustain. Food Syst. 5. https://www.frontiersin.org/articles/10.3389/fsufs.2021.745894.
Pizzeghello, D., Francioso, O., Ertani, A., Muscolo, A., Nardi, S., 2013. Isopentenyladenosine and cytokinin-like activity of different humic substances. J. Geochem. Explor. 129, 70–75.
Quang Tran, H., Van Doan, H., Stejskal, V., 2022. Environmental consequences of using insect meal as an ingredient in aquafeeds: a systematic view. Rev. Aquacult. 14, 237–251. https://onlinelibrary.wiley.com/doi/abs/10.1111/raq.12595.
Rentsch, D, Schmidt, S, Tegeder, M, 2007. Transporters for uptake and allocation of organic nitrogen compounds in plants. FEBS Lett 581, 2281–2289. https://doi.org/10.1016/j.febslet.2007.04.013.
RStudio Team, 2020. RStudio: Integrated Development for R. RStudio. RStudio, Inc., Boston, MA.
Setti, L., Francia, E., Pulvirenti, A., Gigliano, S., Zaccardelli, M., Pane, C., et al., 2019. Use of black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae processing residue in peat-based growing media. Waste Manag. 95, 278–288. http://www.sciencedirect.com/science/article/pii/S0956053X19303952.
Song, S., Ee, A.W.L., Tan, J.K.N., Cheong, J.C., Chiam, Z., Arora, S., et al., 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://www.sciencedirect.com/science/article/pii/S0959652620357103.
Surendra, K.C., Tomberlin, J.K., van Huis, A., Cammack, J.A., Heckmann, L.-H.L., Khanal, S.K., 2020. Rethinking organic wastes bioconversion: evaluating the potential of the black soldier fly (*Hermetia illucens* (L.)) (Diptera: Stratiomyidae) (BSF). Waste Manag. 117, 58–80. http://www.sciencedirect.com/science/article/pii/S0956053X20304281.
Tan, Y., Wang, J., He, Y., Yu, X., Chen, S., Penttinen, P., et al., 2023. Organic fertilizers shape soil microbial communities and increase soil amino acid metabolites content in a blueberry orchard. Microb. Ecol. 85, 232–246. https://doi.org/10.1007/s00248-022-01960-7.
Tegeder, M., Rentsch, D., 2010. Uptake and partitioning of amino acids and peptides. Mol. Plant 3, 997–1011. https://doi.org/10.1093/mp/ssq047.
United Nations Environment Programme, UNEP DTU Partnership, 2021. Reducing Consumer Food Waste Using Green and Digital Technologies. Copenhagen and, Nairobi.
United Nations Environment Programme, 2024. Food Waste Index Report 2024. Think Eat Save: Tracking Progress to Halve Global Food Waste. Nairobi, Kenya.
van Dijk, M., Morley, T., Rau, M.L., Saghai, Y., 2021. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. Nature Food 2, 494–501. https://doi.org/10.1038/s43016-021-00322-9.
Velenturf, A.P.M., Purnell, P., 2021. Principles for a sustainable circular economy. Sustain. Prod. Consum. 27, 1437–1457. https://www.sciencedirect.com/science/article/pii/S2352550921000567.
Vermeulen, S.J., Campbell, B.M., Ingram, J.S.I., 2012. Climate change and food systems. Annu. Rev. Environ. Resour. 37, 195–222. https://doi.org/10.1146/annurev-environ-020411-130608.
Wang, Q., Ren, X., Sun, Y., Zhao, J., Awasthi, M.K., Liu, T., et al., 2021. Improvement of the composition and humification of different animal manures by black soldier fly bioconversion. J. Clean. Prod. 278, 123397. https://www.sciencedirect.com/science/article/pii/S0959652620334429.
Wichuk, K.M., McCartney, D., 2010. Compost stability and maturity evaluation — a literature reviewA paper submitted to the Journal of Environmental Engineering and Science. Can. J. Civ. Eng. 37, 1505–1523. https://doi.org/10.1139/L10-101.
Wu, J., Zhao, Y., Zhao, W., Yang, T., Zhang, X., Xie, X., et al., 2017. Effect of precursors combined with bacteria communities on the formation of humic substances during different materials composting. Bioresour. Technol. 226, 191–199. https://www.sciencedirect.com/science/article/pii/S096085241631690X.
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. Wiley, pp. 155–180. https://doi.org/10.1002/9781119357254.ch6.
Wu, N., Yu, X., Liang, J., Mao, Z., Ma, Y., Wang, Z., et al., 2023. A full recycling chain of food waste with straw addition mediated by black soldier fly larvae: focus on fresh frass quality, secondary composting, and its fertilizing effect on maize. Sci. Total Environ. 885, 163386. https://www.sciencedirect.com/science/article/pii/S0048969723020053.
Yang, J.-W., Luyima, D., Park, S.-J., Kim, S.-H., Oh, T.-K., 2021a. Mixing sodium-chloride-rich food waste compost with livestock manure composts enhanced the agronomic performance of leaf lettuce. Sustainability 13.
Yang, W., Zhang, L., 2022. Addition of mature compost improves the composting of green waste. Bioresour. Technol. 350, 126927. https://www.sciencedirect.com/science/article/pii/S0960852422002565.
Yang, Y., Wang, G., Li, G., Ma, R., Kong, Y., Yuan, J., 2021b. Selection of sensitive seeds for evaluation of compost maturity with the seed germination index. Waste Manag. 136, 238–243. https://www.sciencedirect.com/science/article/pii/S0956053X21005262.
Yao, X., Zhou, H., Meng, H., Ding, J., Shen, Y., Cheng, H., et al., 2021. Amino acid profile characterization during the co-composting of a livestock manure and maize straw mixture. J. Clean. Prod. 278, 123494. https://www.sciencedirect.com/science/article/pii/S0959652620335393.
Yildirim-Aksoy, M., Eljack, R., Beck, B.H., 2020. Nutritional value of frass from black soldier fly larvae, *Hermetia illucens*, in a channel catfish, *Ictalurus punctatus*, diet. Aquacult. Nutr. 26, 812–819. https://onlinelibrary.wiley.com/doi/abs/10.1111/anu.13040.
Zhang, H., Tan, S.N., Teo, C.H., Yew, Y.R., Ge, L., Chen, X., Yong, J.W.H., 2015. Analysis of phytohormones in vermicompost using a novel combinative sample preparation strategy of ultrasound-assisted extraction and solid-phase extraction coupled with LCMS. Talanta 139, 189–197. https://doi.org/10.1016/j.talanta.2015.02.052.
Zhao, Y., Zhao, Y., Zhang, Z., Wei, Y., Wang, H., Lu, Q., et al., 2017. Effect of thermo-tolerant actinomycetes inoculation on cellulose degradation and the formation of humic substances during composting. Waste Manag. 68, 64–73. https://www.sciencedirect.com/science/article/pii/S0956053X17304634.