



Process efficiency and greenhouse gas emissions in black soldier fly larvae composting of fruit and vegetable waste with and without pre-treatment

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ARTICLE INFO

Handling Editor: Cecilia Maria Villas Bôas de Almeida

Keywords:

Ammonia pre-treatment
Biological treatment
Hermetia illucens
Pre-treatment
Plant-based waste
Trichoderma reesei

ABSTRACT

One technology that implements circular economy principles is black soldier fly larvae (BSFL) composting. To assess the environmental impact of BSFL technology, more data on emissions of greenhouse gases (GHG) and ammonia are needed. This study investigated process efficiency and GHG emissions from BSFL composting of orange peels and a mix of broccoli and cauliflower trimmings, with and without pre-treatment. Two weeks of substrate pre-treatment with ammonia or fungi (*Trichoderma reesei*) were investigated, and direct emissions of GHG and ammonia from the process were evaluated. Process efficiency was evaluated by waste-to-biomass conversion efficiency (BCE) and material reduction. In BSFL composting of trimmings, BCE was not significantly improved by pre-treatment. However, larval volatile solids (VS) load in the fungi pre-treated treatment was very low, likely contributing to low BCE. BCE was low (6%) in the peel control and even lower in the pre-treatments, indicating that this substrate is unsuitable for BSFL composting. Material reduction was largest for trimmings (84%) and peels (60%) pre-treated with fungi. Emissions of methane (CH₄) and nitrous oxide (N₂O), expressed in CO₂-eq, were very low (0.04–1.57 g/kg initial wet weight (ww)) compared with direct CO₂ emissions (47–147 g/kg initial ww). Fungi pre-treatment appeared to make the trimmings more available to the larvae, while also drying out the substrate and removing a large proportion of available VS. Thus fungi pre-treatment could be used to increase waste treatment capacity. Ammonia pre-treatment reduced emissions of CH₄ and N₂O without affecting overall BCE, but significantly increased NH₃ emissions.

1. Introduction

Worldwide, municipal waste generation is expected to increase from 2 billion tonnes per year (average in 2016) to 3.4 billion tonnes by 2050 (Kaza et al., 2018). Globally, 70% of biodegradable waste is either dumped in the open or landfilled (Kaza et al., 2018). There, it degrades in an uncontrolled environment and can generate greenhouse gases (GHG), which contribute to climate change, and ammonia (NH₃), which contributes to acidification and eutrophication (Bernstad and Jansen, 2012; IPCC, 2014). Methane (CH₄) has a global warming potential of 34 carbon dioxide (CO₂)-equivalents with climate-carbon feedback over a 100-year period (GWP₁₀₀), while nitrous oxide (N₂O) has a GWP_{100v} of 298 CO₂-equivalents (IPCC, 2013). Globally, landfills are estimated to contribute 12% of total anthropogenic methane emissions (Saunois et al., 2020). In 2007, it was estimated that only 12% of methane emissions from landfills in that year were captured globally (Themelis and Ulloa, 2007).

The resources in biodegradable waste can be harvested if managed

adequately (Slorach et al., 2019). Efficient reuse of resources is in line with the resource efficiency strategy established in the European Commission's action plan for a circular economy (European Commission, 2020). According to the European Union (EU) waste hierarchy, methods allowing material re-use should be implemented with the highest priority (European Union, 2016). One such method is fly larvae composting, in which proteins, carbohydrates and lipids from biodegradable wastes are concentrated in the larval biomass and indirectly reused (Suckling et al., 2021). The most commonly used fly species for this purpose is black soldier fly (*Hermetia illucens* L. (Diptera: Stratiomyidae)) (Tomberlin et al., 2015). The black soldier fly larvae (BSFL) biomass produced can be used in animal feed, due to its high protein and lipid content of around 25–40% and 20–60% on a dry matter (dm) basis, respectively (Ewald et al., 2020; Meneguz et al., 2018), while the treatment residues (frass) can be used as a fertiliser (Song et al., 2021). Biodegradable streams available for conversion in fly larvae composting are limited in the EU under Regulation (EC) No. 1774/2002, allowing only plant-based biodegradable streams for use as feed for larvae. The

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<https://doi.org/10.1016/j.jclepro.2022.130552>

Received 29 December 2020; Received in revised form 20 December 2021; Accepted 14 January 2022

Available online 19 January 2022

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processed larvae protein can be used as feed for fish, poultry, pigs and non-production animals (EU 2021/1372) (European Union, 2021)).

Process efficiency, in terms of biomass conversion efficiency (BCE) and material reduction, in BSFL composting of fruit and vegetable waste is lower than for mixed food waste (Lalander et al., 2019). This is possibly a result of low protein content in relation to high content of carbon with low digestibility (lignin and hemicellulose) in fruit and vegetable substrates (Gold et al., 2018; Kumar et al., 2018; Meneguz et al., 2018; Nguyen et al., 2015; Nyakeri et al., 2017).

One way to tackle the lower substrate availability in BSFL composting of these substrates could be pre-treatment to improve their digestibility. Isibika et al. (2019) found that pre-treatment with fungi or ammonia solution increased BCE in BSFL composting of banana peels. The underlying concept when using ammonia is for microorganisms in the BSFL treatment system to convert non-protein nitrogen (NH₃) into proteins through nitrogen assimilation to generate amino acids. Ammonia has also been shown to help microorganisms break down complex molecules (Tadele and Amha, 2015). The underlying concept with fungal pre-treatment is to degrade complex molecules into forms more available to the larvae.

Emissions of GHG have been found to be considerably lower in BSFL composting than in conventional windrow composting (Ermolaev et al., 2019; Mertenat et al., 2019). Previous small-scale evaluations have shown that combined CH₄ and N₂O emissions are 0.03–8.6 g/kg initial wet weight (ww) and that NH₃ emissions are 0.05–0.5 g/kg initial ww (Chen et al., 2019; Ermolaev et al., 2019; Guo et al., 2021; Pang et al., 2020; Parodi et al., 2020). However, most studies published so far have focused on assessment of emissions from BSFL composting of food waste, agricultural waste and manure. To our knowledge, studies investigating GHG emissions from fruit and vegetable wastes are currently lacking. Treatment of fruit and vegetable wastes in BSFL composting may require pre-treatment to achieve reasonable BCE (Isibika et al., 2019). The impact of bacterial pre-treatment on emissions during BSFL composting has been examined previously in a study on food waste by Ermolaev et al. (2019), but GHG emissions during the pre-treatment were not measured in that study. It is thus important to investigate the direct emissions associated with pre-treatment and with BSFL composting itself, to enable an overall assessment of the suitability and performance of BSFL composting of plant-based waste.

This study investigated the process efficiency of BSFL composting of orange peels and a mix of broccoli and cauliflower trimmings, with and without pre-treatment, and evaluated the associated direct emissions of GHG and ammonia during the two steps of the process.

2. Materials and methods

2.1. Materials

The plant-based waste used in the experiments was provided by a fruit and vegetable wholesaler (Grönsakshallen Sorunda, Stockholm, Sweden). The two plant-based waste fractions studied, peels from organically grown oranges (OP) and trimmings from broccoli and cauliflower (BC), were delivered separately. Upon arrival at the BSFL composting facility, the orange peels were stored for up to 6 d at 15 °C and then milled (Robot-Coupe, model Blixer 4 V.V, France) directly before use in experiments. The broccoli and cauliflower trimmings were stored for up to 9 d at 15 °C before milling and then stored for up to a further 3 d at -7 ± 3 °C before use in experiments. The difference in storage time between orange peels and trimmings was caused by the delivery time of each substrate and the time when processing could occur. However, as the same within-substrate procedure was used, any difference in handling between substrates would not impact the results of the study.

Source-separated household food waste (FW) from Eskilstuna, Strängnäs and Örebro was collected and minced at the recycling plant Lilla Nyby in Eskilstuna (Eskilstuna & Strängnäs Energi och Miljö,

Eskilstuna, Sweden) and transported to the BSFL composting facility at the Swedish University of Agricultural Sciences (SLU, Uppsala, Sweden). The minced food waste was used in the experiments directly upon arrival.

The larvae used in the study weighed around 1 mg each and were provided by the black soldier fly colony at the Department of Energy and Technology, SLU, which has been run continuously since 2015 (Uppsala, Sweden).

2.2. Experimental set-up

The orange peels and mix of broccoli and cauliflower trimmings were used in three treatments: no pre-treatment control (-con), pre-treatment with the fungal species *Trichoderma reesei* (-tri) and pre-treatment with ammonia solution (-amm) (Table 1). The hypothesis in the *T. reesei* pre-treatment was that the fungi would assist in degradation of fibres unavailable to the larvae (Rehman et al., 2017) into shorter and more available carbohydrates, as *Trichoderma reesei* is known to produce cellulase and hemicellulase enzymes that break down high-fibre vegetable substrates (Kunamneni et al., 2014). The hypothesis for the ammonia pre-treatment was that it would improve the digestibility of fibres in the fruit and vegetable waste (Bals et al., 2010) and enhance the potential for ammonia assimilation by microorganisms (Wang et al., 2015).

2.2.1. Pre-treatments

Trichoderma reesei was pre-cultured on malt extract agar (MEA) at 28 °C for 7 d. The fungi were harvested by pouring sterile 0.9% NaCl solution onto the agar plate and scraping the fungi off with a 10- μ L inoculation loop. The fungi solution was transferred to 50-mL centrifuge tubes, concentrated by centrifugation to a density of 10⁷ g/mL and inoculated into the waste substrates to 1% (w/w) concentration. For ammonia pre-treatment, 24.5% ammonia solution (Nitor, Sweden) was added to the waste substrate to reach a total NH₃-N concentration of 1% (w/w), holding a pH of approximately 10. After the pre-treatment (Table 1), the pH of the pre-treated substrates was adjusted to pH 7.5 \pm 0.5 using stepwise addition of concentrated sulphuric acid (Fisher Chemicals, Uppsala, Sweden). The pre-treated substrate was sealed in plastic bags. The amount of substrate at the beginning of the pre-treatment was 15 kg and that remaining after completion of pre-treatment was divided into three equal feeding portions for BSFL composting.

All pre-treatments were performed in triplicate, in standard plastic

Table 1

List of substrates, pre-treatments used in each treatment, duration of pre-treatment and black soldier fly larvae (BSFL) composting, and total amount of substrate on a wet weight (ww) basis added in each treatment. Values presented are mean \pm SD (n = 3).

	Pre-treatment time [d]	BSFL composting time [d]	Total substrate [kg ww]
<i>Controls without pre-treatment</i>			
OP-con Orange peels	-	27	14.6 \pm 0.1
BC-con Broccoli and cauliflower trimmings	-	28	15.7 \pm 0.1
FW-con Food waste	-	17	15.4 \pm 0.2
<i>Trichoderma reesei pre-treatment</i>			
OP-tri Orange peels	16	31	15.4 \pm 0.1
BC-tri Broccoli and cauliflower trimmings	14	23	15.8 \pm 0.5
<i>Ammonia solution pre-treatment</i>			
OP-amm Orange peels	16	35	14.4 \pm 0.1
BC-amm Broccoli and cauliflower trimmings	16	30	15.9 \pm 0.6

crates (treatment boxes) with inner dimensions 36.5 cm × 56.2 cm × 11.5 cm (width x length x height). The pre-treatments were performed at 30 ± 3 °C for 14–16 d (Table 1).

2.2.2. BSFL composting

All experiments were performed in a greenhouse with the temperature regulated to 30 ± 3 °C and each composting treatment was performed in triplicate. The treatment boxes were kept on a rack, with 3 cm between the boxes. The larval feeding rate was calculated based on the total amount of material prior to the pre-treatment (Table 1), and thus the rate differed for each treatment (Table 2). In each treatment, approximately 15 × 10³ of 1-mg larvae were added, resulting in a density of 6.25 larvae/cm². The larvae were fed on days 0, 3 and 6 of the treatment, by adding new material without stirring. BSFL composting was stopped directly when the first prepupae were observed, but lasted for a minimum of 17 days (Table 1).

At the end of BSFL composting, larvae and residues were separated by sieving into two fractions, which were weighed separately. The average larval weight was estimated by weighing three sub-samples of 100 larvae each. The number of larvae was calculated by dividing the total weight of all larvae by the average larval weight and then estimating the larval survival. In two treatments, orange peel control (OP-con) and *T. reesei* pre-treated orange peels (OP-tri), larvae separation was too high, as the moisture content of the treatment residues was too high. In these treatments, samples of 100 larvae were handpicked randomly from the treatment boxes to get the average larval weight, and the survival rate was assumed to be 100% to give an estimate of the total larval biomass produced.

2.3. Sampling

The total amount of substrate was weighed before pre-treatment and before BSFL composting. Material in each replicate was sampled in triplicate for analyses of total solids (TS), total volatile solids (VS), total nitrogen and pH (Level One pH meter, Inolab, with a Sentix electrode, PHM210, MeterLab®, Radiometer, Copenhagen). Samples for determination of GHG emissions were taken during the pre-treatment and BSFL composting.

2.3.1. Sampling for physical and chemical analyses

Samples for TS, VS, pH and nitrogen analysis were taken before the start of pre-treatment, before the start of BSFL composting and after BSFL composting. Three TS and VS samples were taken from each replicate and each sample contained substrate from five randomly selected areas in the box (10–15 g per sample). The same procedure was followed for pH measurements and sampling for nitrogen analysis, but with only one sample from each box (10 g per sample). The material removed for sampling was taken into account when calculating the larval feeding rate.

2.3.2. Gas emissions sampling

During pre-treatment, gas measurements were performed as soon as the substrate was placed in the treatment box, then after 1 w and 2 w of pre-treatment. The last gas emission measurement of the pre-treatment coincided with the first measurement of the BSFL composting, during which gas measurements were performed four times in total: before each of the three feeding occasions and at the end of BSFL composting, which was 7–25 d after the third feeding depending on the BSFL composting duration (Table 1).

The gas emissions were measured using a chamber technique in which gas samples were collected over a period of approximately 1 h to obtain the gas emission rates (Ermolaev et al., 2019). The pre-treatment and treatment boxes were placed inside a plastic chamber (gas box, 51, 800 cm³) that was made airtight using a water lock with the container placed upside-down into a lid. The gas box was equipped with a rubber sampling port, from which gas samples were extracted with a syringe.

Table 2 Total solids (TS), total volatile solids (VS), pH and total nitrogen (N) content of all substrates measured at start (initial substrate), after pre-treatment, larvae and residue TS and VS content, and larval VS dose in the different treatments: OP (orange peels), BC (broccoli and cauliflower trimmings) and FW (food waste). Nitrogen content and pH values presented in brackets were measured after added ammonia solution at the start of pre-treatment. Values presented are mean ± SD (n = 3). Significant differences (p < 0.05) in TS, VS and pH of initial substrate, pre-treated substrate and BSFL-treated substrate are marked with *. Different superscript letters within columns indicate significant differences (p < 0.05) in TS and VS for larvae and in pH for residues.

	Initial substrate				After pre-treatment				Larval VS load ⁱ				Larvae				Residues			
	TS [%]	VS [%]	pH	N [g/kg TS]	TS [%]	VS [%]	pH	N [g/kg TS]	lg VS/larvae	TS [%]	VS [%]	TS [%]	VS [%]	TS [%]	VS [%]	TS [%]	VS [%]	TS [%]	VS [%]	pH
<i>Controls without pre-treatment</i>																				
OP-con	21.5 ± 0.4*	96.7 ± 0.4*	3.4 ± 0.1*	4.4 ± 0.4	-	-	-	-	0.20 ± 0.003 ^{ab}	25.4 ± 1.2 ^a	90.0 ± 0.2 ^a	31.8 ± 1.4 ^{ab}	95.0 ± 0.1 ^{ab}	3.7 ± 0.1 ^{ab}						
BC-con	8.2 ± 0.1*	87.4 ± 1.1*	5.7 ± 0.2*	14.1 ± 1.4	-	-	-	-	0.08 ± 0.0002 ^{abc}	19.5 ± 1.4 ^b	83.0 ± 1.6 ^b	29.7 ± 5.6 ^{ab}	70.5 ± 0.6 ^{ab}	9.8 ± 0.1 ^{ab}						
FW-con	24.7 ± 0.1*	89.3 ± 0.3*	4.1 ± 0.2*	25.5 ± 3.7	-	-	-	-	0.22 ± 0.004 ^b	40.6 ± 0.9 ^c	83.6 ± 0.1 ^{bc}	69.2 ± 3.0 ^{ab}	82.4 ± 0.2 ^{bc}	8.3 ± 0.1 ^{bc}						
<i>Trichoderma reesei pre-treatment</i>																				
OP-tri	21.1 ± 0.4*	96.6 ± 0.1*	4.3 ± 0.6*	-	29.8 ± 2.9*	94.0 ± 0.9*	3.5 ± 0.1*	19.0 ± 5.4	0.14 ± 0.02 ^{abc}	23.6 ± 1.0 ^{ab}	87.7 ± 1.7 ^a	37.5 ± 3.8 ^a	92.5 ± 0.2 ^d	3.9 ± 0.1 ^a						
BC-tri	8.2 ± 0.2	87.4 ± 1.5*	5.5 ± 0.1*	14.1 ± 1.4	11.3 ± 1.5*	70.8 ± 2.4*	8.1 ± 0.3*	-	0.03 ± 0.004 ^c	35.3 ± 2.6 ^d	77.3 ± 2.1 ^d	86.9 ± 2.0 ^{ac}	57.5 ± 1.2 ^{de}	10.1 ± 0.3 ^{ad}						
<i>Ammonia solution pre-treatment</i>																				
OP-amm	21.6 ± 0.2*	96.9 ± 0.1*	3.7 ± 0.6*	4.4 ± 0.4	19.2 ± 0.2*	96.4 ± 0.1*	6.4 ± 1.6*	19.5 ± 2.4	0.18 ± 0.003 ^{abc}	24.3 ± 2.0 ^{ab}	88.0 ± 0.8 ^a	52.8 ± 4.2 ^{cd}	94.2 ± 0.2 ^{abd}	7.6 ± 0.1 ^e						
BC-amm	8.6 ± 0.1*	89.5 ± 0.3*	5.8 ± 0.3*	-	7.3 ± 0.2*	88.0 ± 1.3*	8.1 ± 0.3*	(10 ± 0.4)	0.07 ± 0.004 ^{bc}	23.9 ± 2.7 ^{ab}	86.8 ± 0.9 ^{bc}	15.0 ± 5.1 ^e	76.9 ± 1.4 ^{ef}	8.2 ± 0.1 ^c						

The material reduction during pre-treatment of trimmings was significantly (p < 0.05) greater in *T. reesei* pre-treatment (65%) than in ammonia pre-treatment (15%) (Table 3). In general, the reduction was greater in the trimmings than in the peels in both pre-treatments.

ⁱ Not normally distributed, non-parametric test.

An air inlet consisting of a 1 m long and 3 mm diameter tube was connected, to avoid suppression. A portable fan (Rubicon mini fan, Kjell & Co Elektronik AB, Sweden) was placed in the gas box to disperse the gas concentrations evenly. For each emission measurement, gas samples were extracted on three occasions: directly upon sealing the box, after 20 min and after 1 h. Exact times and volumes removed from the chamber were recorded and later used together with measured concentrations to calculate the emission rates for each of the samples by linear regression. Dilution was accounted for following the method described in Ermolaev et al. (2019).

2.4. Physical and chemical analyses

The TS samples were weighed before drying and after drying for a minimum of 48 h, at 70 °C to avoid evaporation of volatile solids (Vahlberg et al., 2013). For VS analysis, the samples were combusted at 250 °C for 2 h and at 550 °C for 4 h (modified ISO 18122:2015). Total nitrogen was measured as described in Lalander et al. (2015). In brief, 0.5 g samples were boiled in 15 mL concentrated sulphuric acid in a 100 mL volumetric flask for 20 min and then cooled before being diluted 1:50 in deionised water. A Crack-test 10 (114544) was used to oxidise all forms of nitrogen to nitrate, after which the nitrate concentration was measured using Spectroquant® kit number 114764.

2.5. Gas analyses

Concentrations of CO₂ and NH₃ were measured during sampling by connecting a reagent tube from a pump (Gas Detector, Kitagawa, Japan) directly to the sampling port, withdrawing 50–300 mL of the gas and recording the concentration for each tube, as described in Ermolaev et al. (2019). For measurements of CH₄ and N₂O, the gas was extracted from the sampling port using a 60 mL syringe and directly flushed into a 20 mL evacuated injection flask (Perkin Elmer) pre-filled with N₂. Concentrations of CH₄ and N₂O were measured using a gas chromatograph (Perkin Elmer Clarus 500, USA) with flame ionisation detector (FID) and thermal conductivity detector (TCD), as described in Ermolaev et al. (2015).

2.6. Calculations

The percentage material reduction on a volatile solids basis (Red_{VS}) was calculated as:

$$\text{Red}_{\text{VS}} = \left(1 - \frac{m\text{VS}_{\text{res}}}{m\text{VS}_{\text{initial}}} \right) \times 100 \quad (1)$$

where $m\text{VS}_{\text{res}}$ and $m\text{VS}_{\text{initial}}$ is the total mass of volatile solids in residues and initial material, respectively. For material reduction after pre-treatment, $m\text{VS}_{\text{res}}$ and $m\text{VS}_{\text{initial}}$ is the total mass of volatile solids in pre-treatment residues and initial material, respectively. For material reduction after BSFL composting, $m\text{VS}_{\text{res}}$ and $m\text{VS}_{\text{initial}}$ is the total mass of volatile solids in residues and initial substrate, respectively.

The waste-to-biomass conversion efficiency on a volatile solids basis (BCE_{VS}) was calculated as:

$$\text{BCE}_{\text{VS}} = \left(\frac{m\text{VS}_{\text{larvae}}}{m\text{VS}_{\text{initial}}} \right) \times 100 \quad (2)$$

where $m\text{VS}_{\text{larvae}}$ and $m\text{VS}_{\text{initial}}$ is the total mass of volatile solids in larvae and initial substrate, respectively. The BCE on a wet weight basis (BCE_{WW}) was calculated in the same way, but using the total wet weight of larvae and initial substrate.

Total VS loss was calculated as:

$$\text{Tot loss}_{\text{VS}} = \left(\frac{m\text{VS}_{\text{initial}} - m\text{VS}_{\text{larvae}} - m\text{VS}_{\text{res}}}{m\text{VS}_{\text{initial}}} \right) \times 100 \quad (3)$$

where $m\text{VS}_{\text{initial}}$, $m\text{VS}_{\text{larvae}}$ and $m\text{VS}_{\text{res}}$ is the total mass of volatile solids in initial substrate, larvae and residues, respectively.

Percentage larval survival was calculated as:

$$\text{Survival} = \frac{n_{\text{lv},\text{end}}}{n_{\text{lv},\text{start}}} \times 100 \quad (4)$$

where $n_{\text{lv},\text{end}}$ and $n_{\text{lv},\text{start}}$ is total number of larvae at the end and at the start, respectively.

The total amount of gas emitted from pre-treatment and BSFL composting was calculated using the trapezoidal rule (Holman, 2011), in which the emission rates were plotted against time to calculate total emissions as described in Ermolaev et al. (2019).

2.7. Statistical analysis

For calculating gas emission rates, the Linest function in Excel (Microsoft version 16, USA) was used. Two-sided ANOVA (with 95% confidence interval) was used to evaluate whether the treatments were significantly different during pre-treatment and BSFL composting in terms of material reduction, BCE and gas emissions. Normality was verified in the model residuals (Shapiro-Wilk test). Where it was not verified, the non-parametric Kruskal-Wallis test was performed, followed by the Dunn test for multiple comparison (95% confidence interval) using the Benjamini and Hochberg correction method (Benjamini and Hochberg, 1995). General linear regression with 95% confidence interval was used to assess correlations between response variables and substrate properties. ANOVA, regression analyses, non-parametric tests and graphical representations were made in R (R Core Team, 2019).

3. Results

3.1. Process efficiency

All treatments with the same substrate had similar initial content of TS and VS (Table 2). In the treatments with orange peels and broccoli and cauliflower trimmings, the VS was reduced most by the *T. reesei* pre-treatment, which also significantly reduced the substrate moisture content, increasing the TS content of trimmings from 8.3 to 11%. Ammonia pre-treatment increased the moisture content of both peels and trimmings.

Larvae and residue TS varied significantly between the different treatments, ranging from 19.5 to 40.6%. Larvae fed on orange peels had similar TS and VS, regardless of whether the peels had been pre-treated or not, while the TS and VS of larvae reared on broccoli and cauliflower trimmings varied more between treatments. For all treatments, BSFL composting led to a reduction in moisture content (Table 2).

Neither of the pre-treatments gave a significant improvement in BCE_{VS} in BSFL composting. Similar BCE_{VS} was obtained for the broccoli and cauliflower trimmings and the control, while BCE_{VS} was significantly reduced for orange peels pre-treated with *T. reesei* (treatment OP-tri). BCE_{WW} for the entire process was not significantly different between the substrates within the same pre-treatments, but the *T. reesei* pre-treatment resulted in significantly lower BCE_{WW} than the ammonia pre-treatment (Table 3). Furthermore, the *T. reesei* pre-treated substrates had significantly lower BCE_{VS} compared with corresponding control.

Larval survival was lowest for *T. reesei* pre-treated broccoli and cauliflower trimmings (treatment BC-tri) (28%) and highest in the corresponding control (100%). Generally, larvae in all orange peel treatments had lower final larval weight (18–50 mg/larva) than in the other substrates, while larvae grown on food waste had the largest final weight (141 mg/larva), followed by the broccoli and cauliflower trimmings control (treatment BC-con) (108 mg/larva).

The material reduction on a VS basis for the entire process differed significantly ($p < 0.05$) between the trimmings treatments, with the material reduction of ammonia pre-treated trimmings (BC-amm) being

Table 3

Material reduction (Red) after pre-treatment; Red and biomass conversion efficiency (BCE) after black soldier fly larvae (BSFL) composting on a volatile solids (VS) basis and for the entire process on a VS and wet weight (ww) basis for the different substrates. and survival rate and final larval weight of the larvae; OP (orange peels), BC (broccoli and cauliflower trimmings) and FW (food waste). Values presented are mean \pm SD (n = 3). Different superscript letters within columns indicate significant differences (p < 0.05).

	Pre-treatment	BSFL composting				Entire process				
	Red _{VS} [%]	Red _{VS} [%]	BCE _{VS} [%]	Survival ⁱ [%]	Final larval weight [mg]	Red _{VS} [%]	BCE _{VS} [%]	Red _{ww} ⁱⁱⁱ [%]	BCE _{ww} [%]	Total VS loss [%]
<i>Controls without pre-treatment</i>										
OP-con		39 \pm 3 ^a	5.7 \pm 2 ^a	100 ⁱⁱ	50 \pm 12 ^{ac}	39 \pm 3 ^a	5.7 \pm 1.7 ^a	58 \pm 1 ^a	5.2 \pm 1.2 ^{ab}	33 \pm 5 ^a
BC-con		81 \pm 0.5 ^b	14 \pm 1 ^b	62.8 \pm 4.5 ^{ab}	108 \pm 8 ^{bc}	81 \pm 0.5 ^b	14 \pm 1 ^b	93 \pm 1 ^b	6.4 \pm 0.06 ^b	67 \pm 1 ^b
FW-con		64 \pm 1 ^c	23 \pm 1 ^c	100 \pm 6.3 ^c	141 \pm 5 ^b	64 \pm 1 ^{cd}	23 \pm 1 ^c	87 \pm 1 ^{ab}	14 \pm 1 ^c	41 \pm 2 ^a
<i>Trichoderma reesei pre-treatment</i>										
OP-tri	24 \pm 8 ^a	43 \pm 7 ^{ad}	2.6 \pm 0.7 ^d	100 ⁱⁱ	18 \pm 4 ^a	60 \pm 4 ^{cd}	1.8 \pm 0.4 ^d	82 \pm 0.5 ^{ab}	1.3 \pm 0.3 ^d	58 \pm 4 ^{bc}
BC-tri	65 \pm 5 ^b	52 \pm 9 ^{cd}	16 \pm 1 ^b	28.1 \pm 14.6 ^a	61 \pm 23 ^{cd}	84 \pm 1 ^b	5.4 \pm 0.8 ^a	98 \pm 0.1 ^b	1.4 \pm 0.3 ^d	79 \pm 1 ^d
<i>Ammonia solution pre-treatment</i>										
OP-amm	10 \pm 0.02 ^c	52 \pm 3 ^{ac}	5.0 \pm 0.6 ^{ad}	96.0 \pm 4.5 ^{cb}	43 \pm 0 ^{ac}	58 \pm 3 ^d	4.3 \pm 0.6 ^{ad}	83 \pm 1 ^{ab}	3.9 \pm 0.2 ^a	53 \pm 3 ^c
BC-amm	15 \pm 0.2 ^{ac}	59 \pm 3 ^c	15 \pm 0.5 ^b	55.7 \pm 25.0 ^{ab}	95 \pm 26 ^{de}	66 \pm 3 ^c	12 \pm 0.4 ^b	76 \pm 7 ^{ab}	4.6 \pm 0.5 ^a	53 \pm 4 ^c

ⁱ Statistical analysis excluded treatments OP-con and OP-tri.

ⁱⁱ Assumed survival.

ⁱⁱⁱ Not normally distributed, non-parametric test.

lower (66%) than that in the control and in trimmings pre-treated with *T. reesei* (BC-tri, 84%). Both peel pre-treatments resulted in higher Red_{VS} compared with the control over the entire process. Treatment BC-tri resulted in the highest total VS loss, while the orange peel control (treatment OP-con) resulted in the lowest.

In both pre-treatments with peels, BCE was lower than for pre-treated trimmings even though the larval VS load was greater, and hence no direct correlation between larval VS load and BCE was found (Fig. 1a). Removing the peel treatments from the model improved the fit from $R^2 < 0.1$ to an adjusted $R^2 = 0.74$ (Fig. 1b). An even stronger positive correlation (adjusted $R^2 = 0.93$), was found when the material reduction in pre-treatment was included as an explanatory variable (Fig. 1c). The final larval weight was found to correlate with the larval VS load that the surviving larvae would have received, assuming that any larvae which did not survive the treatment did not consume any VS (Fig. 1d).

3.2. Gas emissions

3.2.1. Emissions during pre-treatment

The CO₂ emissions were mainly associated with the type of pre-treatment. Emissions from ammonia pre-treatment of substrates reached 0.03 CO₂-C kg/kg initial VS, while *T. reesei* pre-treated substrates emitted 0.12 CO₂-C kg/kg initial VS (Fig. 2a). For CH₄ and N₂O emissions, the amount of emissions was associated with the substrate rather than the type of pre-treatment (Fig. 2b–c).

3.2.2. Emissions during BSFL composting

Concentrations of CO₂ increased linearly ($R^2 > 0.95$ n = 85, $0.95 > R^2 > 0.75$ n = 14) for all emission rate measurements in all replicates, confirming that the gas boxes remained airtight and that the gas measurements had high accuracy. The emission rates were calculated and plotted against time, and the cumulative emissions were calculated for all treatments (Fig. 3a). Cumulative CO₂ emissions in all treatments increased nearly linearly, except for the trimmings control (BC-con), and total emissions ranged from 0.10 to 0.17 kg CO₂-C/kg substrate VS during BSFL composting (Fig. 3a). The total cumulative CO₂ emissions in the trimmings control reached 0.23 kg CO₂-C/kg substrate VS. All the peel treatments except OP-tri had lower cumulative emissions than other treatments. The CO₂ accumulation rates were similar across the peel treatments.

In treatments OP-tri, ammonia pre-treated orange peels (OP-amm), BC-con, BC-tri and the food waste control (FW-con), cumulative CH₄ emissions were small and increased linearly, from 0.2 to 1.6 mg CH₄-C/

kg substrate VS (Fig. 3b). Ammonia pre-treated broccoli and cauliflower trimmings showed a sharper increase in accumulation rate after the initial period and reached total emissions of 23 mg CH₄-C/kg substrate VS (Fig. 3b). Total emissions from the peels control (OP-con) did not increase linearly and were 3.3 mg CH₄-C/kg initial VS. The cumulative CH₄ emissions from food waste were consistently lowest, but not significantly lower than in other treatments except for trimmings pre-treated with ammonia (BC-amm).

Cumulative N₂O emissions were close to zero for most treatments, with the exception of the trimmings control (BC-con) (Fig. 3c). Cumulative emissions increased gradually, from 0.1 to 1.9 mg N₂O-N/kg substrate VS in the peels and food waste treatments to 3.4 mg, 6.5 mg and 46 mg N₂O-N/kg substrate VS in BC-amm, BC-tri and BC-con, respectively (Fig. 3c).

3.2.3. Emissions during the entire process

Total CH₄ emissions varied more within treatment for treatments that had higher emissions (Fig. 4a). Total emissions of N₂O were higher in treatments with trimmings than with peels (Fig. 4b). Pre-treatment of trimmings reduced the total amount of N₂O emitted. Total N₂O emissions were not significantly different from zero in any of the peel treatments. The NH₃ pre-treatment increased total NH₃ emissions (Fig. 4c). Total CO₂ emissions from the entire process were within the same range for all assessed treatments (Fig. 4d).

Total emissions of CH₄ and N₂O, expressed in CO₂-equivalents over a 100-year period (IPCC, 2013) on a VS basis, were low compared with the direct CO₂ emissions, and were generally higher for trimmings as compared to the other substrates (Fig. 4e). The total emissions were similar between the treatments, with only the control trimmings and peels (BC-con, OP-con) being significantly different from each other (Fig. 4f).

4. Discussion

4.1. Impact of pre-treatment on biomass conversion efficiency and larval survival

In broccoli and cauliflower trimmings pre-treated with *T. reesei*, a large proportion of the initial VS was consumed in the pre-treatment (Table 3). This high VS reduction resulted in that treatment receiving the lowest larval VS feeding load (0.03 g VS/larva) in this study (Table 2), which could have caused the low larval survival rate (28%). However, BCE in BSFL composting of broccoli and cauliflower trimmings pre-treated with *T. reesei* (14%) was within the same range as in

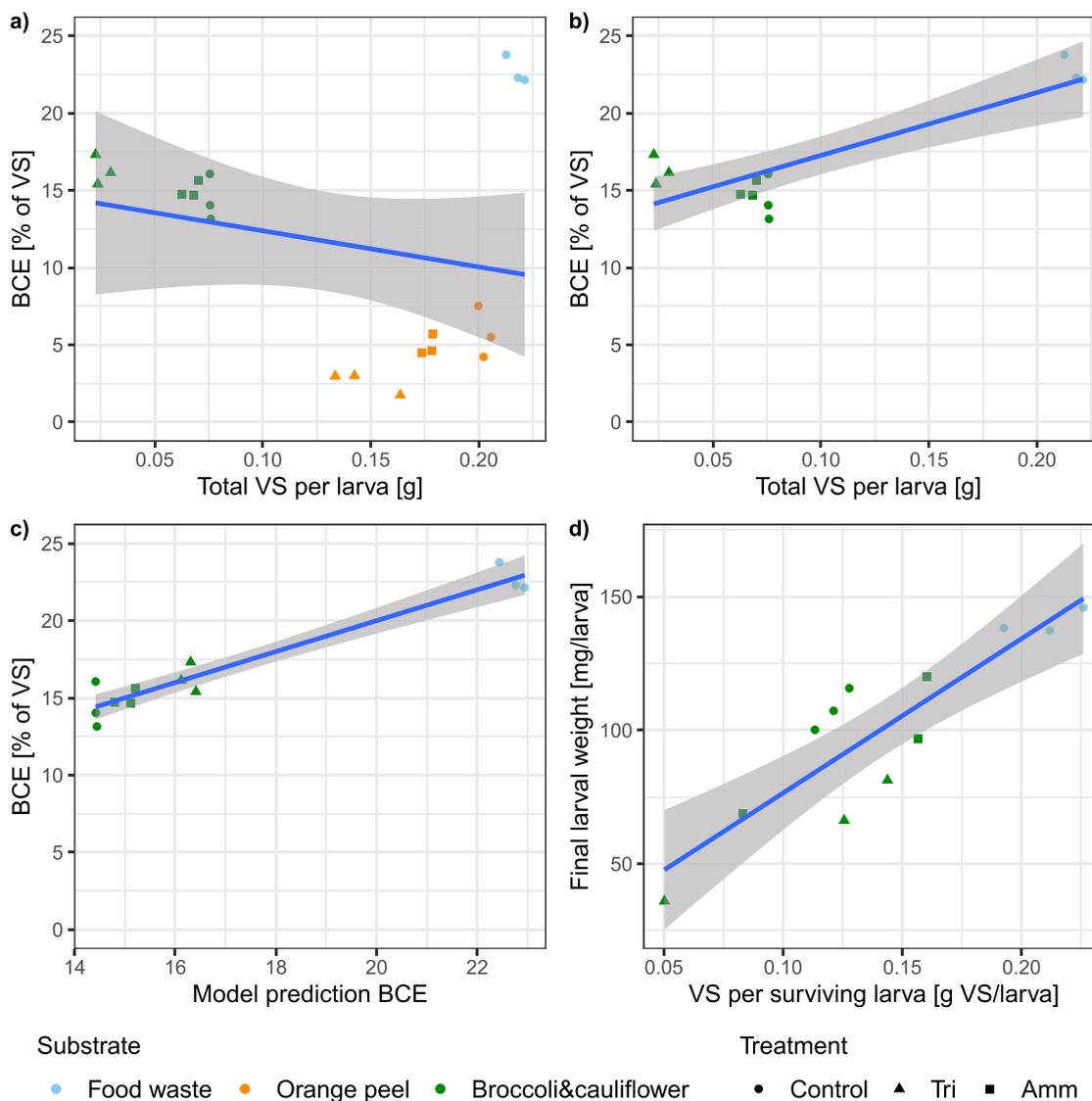


Fig. 1. Biomass conversion efficiency (BCE) in black soldier fly larvae (BSFL) composting in relation to a) total volatile solids (VS) per larva [g] ($R^2 < 0.1$), b) total VS per larva [g], when excluding the orange peel treatments (adjusted $R^2 = 0.74$; $p = 0.0003$), and c) predicted BCE for a model taking into account the total VS per larva and the reduction achieved in the pre-treatment (adjusted $R^2 = 0.93$, $p = 6 \times 10^{-6}$); and d) the final larval weight in relation to the total VS per surviving larva (adjusted $R^2 = 0.76$, $p = 0.0001$). Data shown for food waste (blue), orange peels (orange) and broccoli and cauliflower trimmings (green) without pre-treatment (control, ●) and pre-treated with *Trichoderma reesei* (tri, ▲) and ammonia solution (amm, ■). The grey areas represent the model fits with 95% confidence level.

the corresponding control (16%) (Table 3), suggesting that fungi pre-treatment made the trimmings more available to the larvae than in the control, which received a higher larval VS load (0.08 g VS/larva). A study by Lalander et al. (2019) demonstrated higher BCE with higher larval VS load, but in the present study no correlation between larval VS load and BCE was found when the full dataset was used (Fig. 1a). On excluding the orange peels, better model fit was found (Fig. 1b), suggesting that factors other than larval VS load played a role in the peel treatments. Including also the reduction during pre-treatment further improved the model fit (Fig. 1c), supporting the hypothesis that pre-treatment with *T. reesei* renders substrate more available to larvae, as this fungus has the ability to degrade complex molecules (Tadele and Amha, 2015). It is likely that a higher larval VS load for fungi pre-treated substrates would increase the BCE in the BSFL composting, however it would have to be investigated as to which point, since it has been suggested that there is a threshold at which increased larval feeding load no longer results in larger relative larval growth (Parra Paz et al., 2015).

It is likely that the low survival of larvae in broccoli and cauliflower

trimmings pre-treated with *T. reesei* was due to the low larval VS feeding load (30 mg/larva), and not due to any potential mycotoxins produced by the fungi, as no known mycotoxin is produced by *T. reesei* (Frisvad et al., 2018). Furthermore, Isibika et al. (2019) observed no impact on larval survival during BSFL composting of *T. reesei* pre-treated banana peels. An alternative, or complementary, reason for the low survival could be the low TS content of the broccoli and cauliflower trimmings (7–11%) (Table 2). A previous study by Lalander et al. (2020) using a similar set-up as in the present study observed decreased survival with higher substrate moisture content (around 80% survival at 90% moisture content, 57% survival at 95% moisture content) and high survival variation in BSFL treatment of substrates with high moisture content.

Final larval weight in broccoli and cauliflower trimmings control (100 mg/larva) was considerably greater than after *T. reesei* pre-treatment (around 60 mg/larva), most likely due to the low larval VS load in the latter treatment (Fig. 1d). Larval survival was generally lower for broccoli and cauliflower trimmings than for the other substrates (Table 3).

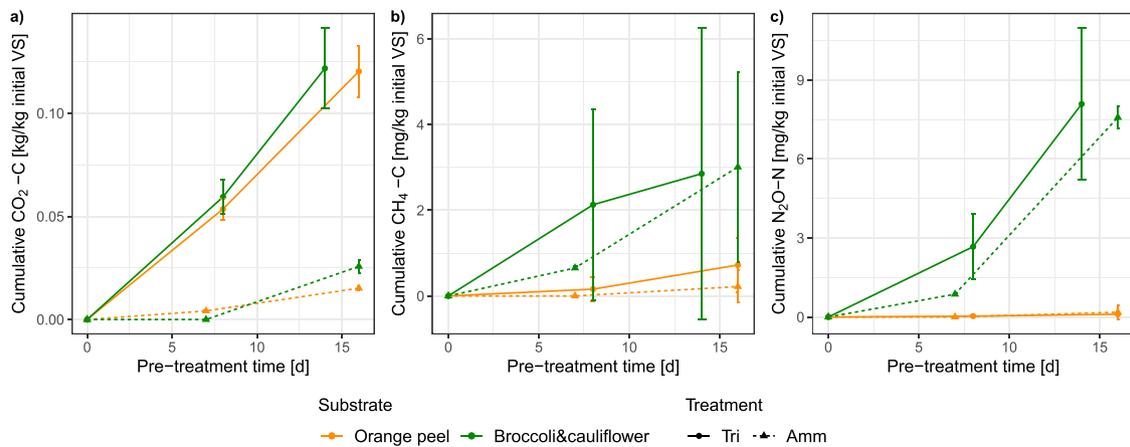


Fig. 2. Cumulative gaseous emissions of (a) CO₂, (b) CH₄ and (c) N₂O during pre-treatment of orange peels (orange) and broccoli and cauliflower trimmings (green) pre-treated with *Trichoderma reesei* (tri, ▲) or ammonia solution (amm, ■). The error bars represent the standard deviation between replicates (n = 3).

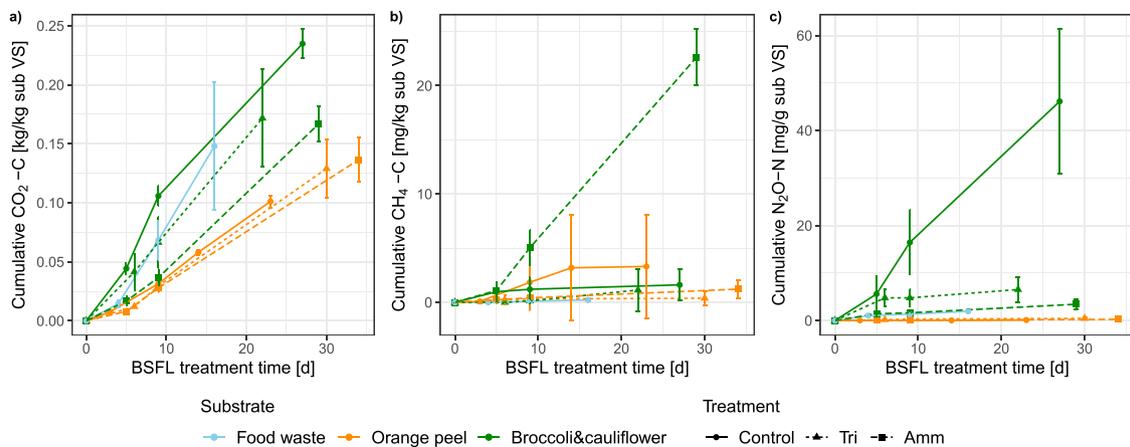


Fig. 3. Cumulative emissions of (a) CO₂, (b) CH₄ and (c) N₂O during black soldier fly larvae (BSFL) composting of food waste (blue), orange peels (orange) and broccoli and cauliflower trimmings (green) without pre-treatment (control, ●) and pre-treated with *Trichoderma reesei* (tri, ▲) and ammonia solution (amm, ■). The error bars represent the standard deviation between replicates (n = 3).

The orange peel control showed low BCE (<6%) and pre-treatment did not improve the BCE, in fact fungi pre-treatment (OC-tri) reduced it to >3. Orange peels have been found to have toxicity effects on insects (Amin et al., 2018; Bachi and Ahmed, 2017). Amin et al. (2018) found that orange peels caused 20–60% larval mortality among cotton leaf worms, while Bachi and Ahmed (2017) demonstrated toxicity in Mediterranean fruit flies, caused by the essential oils in the peels. The fungi pre-treatment decreased BCE, while the material reduction on a VS basis increased for both pre-treatments. This indicates that the pre-treatments did not result in complex molecules becoming more available to the fly larvae in the peel treatments, contradicting our initial hypothesis. However, the reduction during pre-treatment could have concentrated toxic compounds, resulting in decreased BCE compared with the control.

4.2. Impact of pre-treatment on material reduction

The VS reduction was lower during ammonia pre-treatment than in the *T. reesei* pre-treatment. The low reduction rate was correlated with low CO₂ emissions in the pre-treatments (Fig. 1a). In BSFL composting, the material reduction was lower for ammonia pre-treated broccoli and cauliflower trimmings compared with corresponding control, while BCE was not significantly different (Table 3). Furthermore, the total VS loss was lower for ammonia pre-treatment compared with the control and *T. reesei* pre-treatment, suggesting that the microbial community was

less active in the ammonia pre-treated substrate, as the VS loss represents a combination of larval and microbial respiration. In contrast, ammonia pre-treatment of orange peels resulted in a greater material reduction and total VS loss compared with the control. The added ammonia likely acted as a nutrition source in the low-nitrogen peels, and thus stimulated microbial degradation (Tadele and Amha, 2015). The BCE for ammonia pre-treated orange peel was not significantly different from the control (Table 3), and it is thus likely that the added nitrogen favoured microbial growth, rather than larval growth. This could be because substances in the peels were more toxic to the larvae, as discussed above, than to the microorganisms. In the case of the broccoli and cauliflower trimmings, the nitrogen concentration was considerably higher than in orange peels, and thus addition of ammonia could have made the environment less favourable for microorganisms, rather than favouring their growth. High NH₃ concentrations are known to be toxic to most microorganisms (Vinnerås et al., 2008). However, the larvae did not seem to be impacted by the increased ammonia concentration in the trimmings.

4.2. Gas emissions

The material reduction and total VS loss during treatments was reflected in the total CO₂ emissions. On calculating the amount of carbon lost in CO₂ emissions and converting it to the amount of corresponding

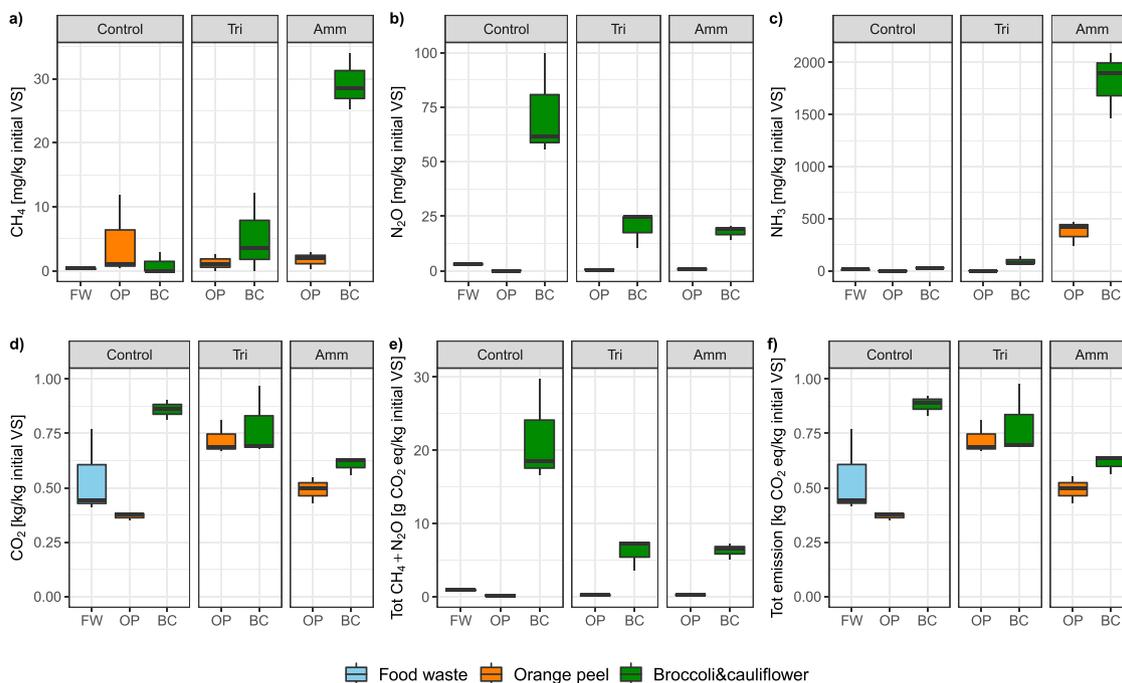


Fig. 4. Total emissions, i.e. emissions during black soldier fly larvae (BSFL) composting and during pre-treatment, calculated per kg of initial VS for food waste (FW, blue), orange peels (OP, orange) and broccoli and cauliflower trimmings (BC, green) without pre-treatment (control), pre-treated with *Trichoderma reesei* (tri) and ammonia solution (amm): a) CH₄ [mg/kg VS]; b) N₂O [mg/kg VS]; c) NH₃ [mg/kg VS]; d) total CO₂ [kg/kg VS]; e) CO₂ equivalents [g CO₂-eq/kg VS] of the CH₄ and N₂O emissions and f) CO₂ equivalents [kg CO₂-eq/kg VS] of all emissions.

VS, it was found that on average 48% of the degraded VS was lost via respiration, which closely matched the values calculated based on VS mass balance (Table 2). The remaining degraded VS were assumed to be transformed into larval biomass.

Total CO₂ emissions from the treatments ranged between 0.37 and 0.86 kg CO₂/kg VS (Fig. 3d). The trimmings control had significantly higher total emissions of CO₂ than the peels control, while the total emissions from food waste were not significantly different from either of the control treatments with peels and trimmings. There was a 67% loss of initial VS in the control treatment with trimmings, 41% in the food waste control and 33% in the peels control. This low biological activity in the peels control treatment corresponded to low BCE (Table 3). The VS loss did not correlate with high BCE, supporting the suggestion that a large proportion of material reduction was not due to biomass conversion into larvae; e.g. food waste had the highest BCE, while the emissions of CO₂ were the second lowest on average. This is in agreement with findings by Ermolaev et al. (2019). The CO₂ emissions generated per tonne of treated waste in BSFL composting were higher for trimmings than for food waste, which correlated well with the VS loss (Table 3, Fig. 4f).

The CH₄ emissions coincided with NH₃ emissions, while they correlated adversely with N₂O emissions in the trimmings treatments (Fig. 3a–c). Similarly, Chen et al. (2019) found that N₂O emissions were low while CH₄ emissions increased with moisture content in substrate and vice versa (Fig. 2b–c). In conventional composting, N₂O emissions can occur later in the process and can be associated with nitrate availability during the denitrification process (Feng et al., 2020). This is typically not seen in BSFL composting, as the process is considerably shorter. However, it has been reported that in substrates with initial nitrate availability, an early N₂O emissions peak can occur (Ermolaev et al., 2019), which declines as the nitrate is consumed and pH increases. This could explain the early N₂O emissions from treatments with trimmings and also the lower emissions in the ammonia pre-treatment, as pH

would be higher in the presence of NH₃ (Pang et al., 2020).

Emissions of CH₄ per ton ww initial material in this study were at the lower end of the range reported for conventional composting (Table 4). The highest CH₄ emissions level for BSFL composting detected in our study and in other studies (Mertenat et al., 2019; Pang et al., 2020) was around 0.1% of the highest reported level in conventional composting (Beck-Friis et al., 2000; Ermolaev et al., 2015). The degradation of the incoming material in BSFL composting was generally higher than in conventional composting, but when adding the mass of the larvae produced the emissions of CO₂ were within the same order of magnitude (Table 4). The BSFL composting process produces residues that do not represent a stabilised compost, leading to a larger proportion of the initial carbon in the substrate being retained and creating the potential for emissions after completion of treatment (Song et al., 2021).

Reported values for emissions of N₂O and NH₃ in conventional composting are up to 100-fold higher (Table 4). Compared with the other treatments in this study, the total ammonia emissions from BSFL composting were 0.12–10.0% of those reported for conventional composting (Table 4), which shows that overall less nitrogen is lost as NH₃ during fly larvae composting. In ammonia pre-treatment of broccoli and cauliflower, the ammonia emissions were higher and up to 50% of the ammonia added in the pre-treatment was lost. Other studies have reported higher losses of nitrogen in BSFL composting, of between 30 and 40% of the inflow nitrogen (Lalander et al., 2015; Lopes et al., 2021). The lower nitrogen emissions seen in this study may be due to lower temperatures and pH, and higher moisture content (Chen et al., 2019). A higher nitrogen content in the substrate likely also favours higher ammonia volatilisation, in particular as the pH shifts to more alkaline conditions due to microbial breakdown of organic nitrogen in the ammonification step, further pushing the ammonium-ammonia equilibrium towards volatile ammonia (Emerson et al., 1975). Thus Parodi et al. (2020) suggested stopping BSFL composting at the peak of CO₂ production, immediately before ammonia starts to be produced in the

Table 4

Summary of emissions per tonne waste on a wet weight (ww) basis and valuable products generated during black soldier fly larvae (BSFL) composting in treatments with orange peels (OP), broccoli and cauliflower trimmings (BC) and food waste (FW) and in other studies with fly larvae composting and conventional composting.

	Larvae [kg]	Residues [kg]	CO ₂ [kg]	CH ₄ [g]	N ₂ O [g]	NH ₃ [g]
<i>Current study</i>						
Orange peels	17–52	180–422	77–147	0.3–0.9	0.04–0.1	0.0–78
BC trimmings	14–64	23–243	47–62	0.07–2.2	1.4–5.2	5.4–342
FW control	142 ± 7	134 ± 7	115 ± 33	0.06 ± 0.01	0.6 ± 0.1	1.8 ± 1.3
<i>BSFL composting</i>						
Food waste ^a	120–190	570–640	92–100	1.0–3.0	0.19–1.0	Below detection limit
Biowaste ^b	220	320	-	0.4	8.6	-
Food waste ^c	19–136	-	26–48	0.06–0.79	0.06–0.50	0.05–0.50
<i>Composting</i>						
Food & garden waste ^d	-	330–750	16–270	23–520	4–400	Below detection limit
Household waste ^e	-	-	-	1700	78	-
Kitchen waste ^f	-	670–680	-	-	-	750–1540

^a Ermolaev et al. (2019).

^b Mertenat et al. (2019).

^c Pang et al. (2020).

^d Ermolaev et al. (2014).

^e Beck-Friis et al. (2000).

^f Zhang et al. (2016).

process. However, Guo et al. (2021) demonstrated that the largest contribution of emissions in BSFL treatment comes from post-treatment of the residues, indicating that more research is needed to determine how the treatment residues should be handled for lowest impact on the environment.

4.3. Feasibility of pre-treatment for BSFL composting

The moisture content of the broccoli and cauliflower trimmings was close to 92% at the start, but the residues were dry enough to allow larvae to be separated out at the end of the process. Cheng et al. (2017) concluded that the initial substrate moisture content should not exceed 80%, as dry separation would not be possible. However, Lalander et al. (2020) found that dry separation with initial moisture content 80–90% is possible if active ventilation is applied. The *T. reesei* and ammonia pre-treatments made a considerable impact on moisture content, with *T. reesei* pre-treatment decreasing the initial moisture content of trimmings in BSFL composting by 29%, while ammonia pre-treatment increased the moisture content of trimmings by 14%. The former led to a dry end product with a moisture content of 13%, compared with 70% in the control (Table 2). The peel treatments were considerably more difficult to separate, as they ended up with a moisture content of 68% and 63% for the control and fungi pre-treatment, respectively. Ammonia treatment of the peels resulted in a separable product with a moisture content of 47%. Comparing fungi pre-treatment of trimmings or not (assuming BCE as in the BSFL composting step, but a larval feeding load of 0.08 g VS in both treatments, and a total treatment time of 37 days for the fungi pre-treatment and 28 days for the control), the larvae production capacity was 0.4 and 2.2 g larvae/kg waste per day for the fungi pre-treatment and non-pre-treated substrate, respectively. The total treatment capacity, on the other hand, was 5.2 and 2.4 kg/m² and day, respectively, for the fungi pre-treatment and the non-pre-treated control. In cases where high waste throughput is of value due to waste fees, the fungi pre-treatment would add an extra benefit.

5. Conclusions

Pre-treatment of orange peels and broccoli and cauliflower trimmings with *T. reesei* or ammonia did not significantly improve biomass conversion efficiency during BSFL composting. However, both pre-treatments significantly increased the material reduction for the peels. BSFL composting of the orange peels resulted in low BCE and produced wet treatment residues (TS ~30%). Ammonia pre-treatment of peels resulted in drier (TS ~50%) treatment residues.

Both substrates performed less effectively than food waste in BSFL composting in terms of biomass conversion efficiency, total emissions of GHG and ammonia, and treatment time. However, higher waste treatment capacity was achieved on pre-treating the trimmings with *T. reesei*. Total CO₂-eq emissions (N₂O + CH₄) were almost four-fold higher for the control trimmings (no pre-treatment) than for ammonia pre-treated trimmings, with no negative impact on biomass conversion efficiency, but with increased NH₃ emissions.

CRediT authorship contribution statement

L. Lindberg: Conceptualization, Investigation, Formal analysis, Writing – original draft, Visualization. **E. Ermolaev:** Conceptualization, Investigation, Formal analysis, Supervision, Writing – review & editing. **B. Vinnerås:** Conceptualization, Resources, Supervision, Writing – review & editing. **C. Lalander:** Conceptualization, Investigation, Formal analysis, Resources, Supervision, Funding acquisition, Visualization, Writing – review & editing.

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.

Acknowledgements

We thank Viktoria Wiklicky for supplying the larvae used in the experiments, Giulio Zorzetto for assisting with experimental work and Grönsakshallen Sorunda for providing the substrates used in this study. The study was funded by Vinnova through the Phase 2 project *Five tonnes of green fish in the counter* (5TFID, 2019-00854).

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