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Black soldier fly reared on pig manure: Bioconversion efficiencies, nutrients in the residual material, greenhouse gas and ammonia emissions

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A B S T R A C T

There is an increased interest for using insects, such as the black soldier fly, to treat surplus manure and upcycle nutrients into the food system. Understanding the influence that BSFL have on nutrient flows and nutrient losses during manure bioconversion is key for sustainability assessments. Here we quantified and compared nutrient balances, nutrient levels in residual materials and emissions of greenhouse gases and ammonia between manure incubated with black soldier fly larvae (BSFL) and manure without BSFL, during a 9-day experimental period. We obtained high analytical recoveries, ranging between 95 and 103%. We found that of the pig manure supplied, 12.5% of dry matter (DM), 13% of carbon, 25% of nitrogen, 14% of energy, 8.5% of phosphorus and 9% of potassium was stored in BSFL body mass. When BSFL were present, more carbon dioxide (247 vs 148 g/kg of DM manure) and ammonia-nitrogen (7 vs 4.5 g/kg of DM manure) emitted than when larvae were absent. Methane, which was the main contributor to greenhouse gas emissions, was produced at the same levels (1.3 vs 1.1 g/kg of DM manure) in both treatments, indicating the main role that manure microbial methane emissions play. Nitrous oxide was negligible in both treatments. The uptake of nutrients by the larvae and the higher carbon dioxide and ammonia emissions modified the nutrient composition of the residual material substantially relative to the fresh manure. Our study provides a reliable basis to quantify the environmental impact of using BSFL in future life cycle assessments.

1. Introduction

Insect farming for feed and food purposes is considered a new emerging agricultural sector (van Huis, 2020). Factors such as the development of accessible technologies to upscale insect farming and legislation changes allowing the use of mass-produced insects as human food and animal feed have contributed to the sector's growth over the last years (van Huis, 2020). The increased interest in farmed insects as an alternative food and feed source, however, is rooted in their potential to improve the sustainability of food systems. By feeding farmed insects with organic residual streams, insects can recover part of the nutrients contained in these streams, and the insect biomass obtained can be used in the food system either as human food or animal feed. This innovation is expected to reduce the environmental impact of the food system as wasted resources are reconverted and reused. To efficiently use insects as recyclers in our food systems, these should be produced using the same principles proposed for livestock in circular food systems (Van Zanten et al., 2019). Thus, insects envisioned for food should be only fed with organic streams inedible to humans such as by-products from the food industry, food waste, crop residues (van Hal et al., 2019b, 2019a), while insects envisioned for feed should be fed with streams that cannot be directly consumed by humans nor by livestock or fish. An abundant organic stream that is not directly consumed by livestock and fish, and that in addition is considered as an environmental burden when not properly managed (Strokal et al., 2016), is animal manure.

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Surplus manure produced in livestock-dense regions where animals are intensively produced and where adjacent croplands are saturated with nutrients, is a source of environmental pollution (Gerber et al., 2013; Leip et al., 2015; Strokal et al., 2016; Wang et al., 2013; Yang et al., 2017) and a public health threat (Venglovsky et al., 2009; Xie et al., 2018; Zhu et al., 2013). Regional surpluses of manure are often transported to other regions. In addition, diverse manure processing technologies have been developed to modify the physical, chemical and/or biological properties of manure and hence reduce its environmental impact or facilitate its transport. However, despite the wide range of manure processing techniques available (Flotats et al., 2011) and the potential that some of these have to reduce some of the environmental impacts caused by manure (Hou et al., 2017; Zhang et al., 2019), these technologies have not been widely adopted in global livestock chains (Cai et al., 2019; Foged et al., 2011; Ministry of Agriculture, 2016). High investment and operational costs with low returns, logistical complexity for implementation at the farm level, low social acceptance and environmental concerns due to the emission of malodorous volatile organic compounds and greenhouse gas (GHG) emissions, have constrained the large scale adoption of these technologies (Cai et al., 2019; Chadwick et al., 2015; Martinez et al., 2009). Although policies to reduce animal numbers in livestock-dense regions would directly reduce the environmental and health problems caused by surplus manure, simultaneously new short-term approaches for surplus manure management are needed to guarantee the transition towards more sustainable food systems.

Insects, specifically larvae of fly species (Diptera), are being considered promising candidates to treat surplus manure and upcycle it into the food system (van Huis, 2019). Manure treatment with black soldier fly larvae (BSFL) is an innovation that could bring multiple benefits at once. BSFL can modify the physical, chemical and biological properties of manure in one to two weeks and thus modify the initial moisture and nutrient levels (Sanchez Matos et al., 2020). When safe (Charlton et al., 2015; van Raamsdonk et al., 2017), the larvae could be used as feed for aquaculture or livestock (Moula et al., 2018) and hence decrease the dependency on imported high-protein feeds with high environmental impact such as soybean and fish meal (Heuel et al., 2021; Van Zanten et al., 2015). Alternatively, the fat contained in the larvae could be used for biofuel production (Li et al., 2011). The residual material obtained from the process (i.e., insect excreta, uneaten manure and larval exuviae) can be used as a soil amendment (Bortolini et al., 2020). In addition, manure bioconversion with BSFL can strongly reduce the emission of malodorous compounds present in fresh manure (Beskin et al., 2018), reduce the loads of pathogens (Erickson et al., 2004; Liu et al., 2008) and mitigate the risk of antibiotic resistance genes in manure by degrading antibiotics (Cai et al., 2018a, 2018b). Despite the increasing evidence supporting the benefits of using BSFL as a manure management strategy, primary data on complete nutrient balances and emission of GHG during manure bioconversion with BSFL are scarce. This information is crucial to assess the transformational potential of manure-fed BSFL systems towards sustainable food systems.

Complete nutrient balances and quantification of gaseous emissions during the rearing of BSFL have been mainly reported for systems in which BSFL were reared on food waste (Mertenat et al., 2019; Pang et al., 2020) and by-products from the food industry (Parodi et al., 2020). Only recently, Chen et al. (2019) quantified the GHG and ammonia (NH_3) emissions of BSFL reared on pig manure at different moisture levels. Although Chen et al. (2019) provided valuable estimations of the emissions occurring when BSFL was reared on pig manure, the nitrogen balance reported was not complete, indicating that not all outputs were accurately quantified (i.e., larvae, residual material and gaseous emissions). Moreover, a missing link in the literature is the quantification of nutrient losses from manure itself (i.e., without larvae) during the same time and under climatic conditions equal to those for manure bioconversion by larvae. Manure, being a material with high microbial activity, is expected to have nutrient losses via gaseous emissions caused by microbial fermentation. Quantifying such fermentation-related losses and comparing these with those occurring when manure is incubated with BSFL, is key to accurately understand the effect that BSFL have on the final nutrient levels in the residual material and the emission of GHG and NH_3. Therefore, the aims of this study were to 1) construct a complete balance of nutrient inputs and outputs used to quantify the nutrient and energy bioconversion efficiency of pig manure by BSFL, 2) quantify the levels of DM content, carbon, nitrogen, ammonia-nitrogen, energy, phosphorus and potassium in the residual material of manure incubated with BSFL and without BSFL, and 3) determine the time course and cumulative gaseous emissions of CO_2, CH_4, N_2O and NH_3 in both treatments.

2. Materials and methods

2.1. Manure collection

Pig manure was collected from a pig farm located in the surroundings of Rhenen, the Netherlands. The manure was produced by fattening pigs (Topigs 50 × PIC 408) fed with a commercial feed produced by Kamphuis Mengvoeders (see SI.1 for the nutrient composition of the feed). The pigs were 35 weeks old when the first batch of manure was collected and 39 weeks old when the last batch of manure was collected (i.e., 4th batch). Two days before the manure collection day, the pig farmer stopped cleaning the slopping concrete floors on which the pigs were kept. The manure samples consisted mainly of fresh faeces (i.e., maximum 2-day old) that were in contact with the urine present on the floor. In every batch, approximately 50 kg of manure was collected with a shovel, stored in three sealed plastic buckets of 20 L and transported to the facilities of Wageningen University & Research. The same day, a small (i.e., less than 80 g) but representative sample of manure was dried with a moisture analyser Ohaus MB-90 (Parsipany, United States) to determine its DM content (see SI.2 for details). The buckets with manure were kept at 21 °C for the subsequent 5 h until the start of the experiment. In total, four batches of manure were collected over a period of 31 days following the procedure described above.

2.2. Insect sourcing and experimental set-up

Just-hatched larvae of the Texas strain (Zhou et al., 2013) of BSF (*Hermetia illucens* L.; Diptera: Stratiomyidae; 100 generations; 38 days egg to egg cycle) were fed with a substrate containing 30% wheat bran and wheat flour, and 70% water for 7 days at the facilities of Bestico B.V., the Netherlands. Once larvae were 7 days old (hereafter called starter larvae), they were sieved, packaged at 10–15 °C and shipped to the facilities of Wageningen University & Research. Upon arrival, one plastic crate (100 × 50 × 15 cm) was filled with fresh manure in an amount equivalent to 4500 g of DM and 20,000–25,000 starter larvae. The height of the feed layer in the crate was around 5 cm high, the density of larvae was 4.7 ± 0.5 per cm^2, 0.9 ± 0.1 per cm^3 and the amount dry manure provided per larvae was 22 ± 2 mg DM manure $larvae^{-1}$ day^{-1} (mean ± std. deviation). The ratio between DM manure and larvae was selected based on pilot studies (see SI.3 for details). Another identical crate was filled with the same amount of manure without starter larvae added. Each crate was placed inside an open-circuit climate respiration chamber of 1800 L (1.00 × 0.8 × 1.1 m) and kept inside for a 208-hour experimental period (i.e., start time day 1–17:00 and end time day 10–09:00). Both climate respiration chambers were designed and built at Wageningen University & Research (Heetkamp et al., 2015). No manure was added during the experimental period. Inside the chambers, air temperature was maintained at 27 ± 0.5 °C, relative humidity at 70 ± 5% and L:D (light:dark) periods were set to 12:12. Ventilation air flow through the respiration chambers was set to 27 L/min and internal fans were used to ensure proper mixing of air. The experiment was repeated four times, here referred as four trials. Since two identical respiration chambers were available in parallel, every trial was made with a different batch of manure and starter larvae. Overall, we had four trials, four batches of manure and hence four replicates for both manure incubated with BSFL and for manure incubated without BSFL (Fig. S1).

2.3. Material sampling and nutrient analyses

Homogeneous samples of fresh manure and starter larvae were collected in 1 L plastic containers prior to the start of every trial. At the end of every trial, chambers were opened and samples of mature larvae and residual material from both treatments were collected in 1 L plastic containers. For manure incubated with BSFL, the residual material consisted on a mixture of larval excreta, larval exuviae and dry uneaten manure (i.e., crust layer on top). For manure incubated without BSFL, the residual material consisted of pig manure, partly decomposed by microbial activity. All samples were stored at −20 °C for subsequent nutrient analyses. Condensate from the heat exchanger of the climate respiration chamber and 25% sulphuric acid solution containing the NH_3 trapped from the outgoing air stream, were collected and stored at 5 °C for subsequent nitrogen analyses (see Parodi et al., 2020 for details).

Prior to freeze drying all solid samples, subsamples of manure and residual material were collected for the colorimetric determination of ammonium nitrogen. Freeze dried samples of manure and residual material were ground to pass a 1 mm screen (Retsch ZM200), while samples of starter and mature larvae, due to their high fat content, were ground three times with the same mill, but without a screen. Nutrient analyses were performed in duplicate at the Animal Nutrition Laboratory of Wageningen University & Research, except for potassium which was analysed at Nutricontrol Laboratories, Veghel, the Netherlands. Samples of fresh manure, residual material, starter and mature larvae were analysed for contents of DM (ISO 6496, 1999), nitrogen and carbon (Dumas method, ISO 1634-1, 2008), gross energy (oxygen bomb method, ISO 9831, 1998), phosphorus (spectrophotometry method, ISO 6941, 1998), potassium (ICP-OES method, ISO 21033, 2016), and crude fat (hydrolysis method, ISO 6492, 1999). Samples of condensed water and acid were analysed for nitrogen (Kjeldahl method, ISO 5983-2, 2005). The results of these analyses are presented in Table 1.

2.4. Gas measurements

The consumption of O_2, the emissions of CO_2, CH_4, NH_3, N_2O, the production of heat, and the elaboration of the nutrient balances were calculated following the same procedures described in Parodi et al. (2020). As a complete system validation, two CO_2 recovery tests per chamber were performed prior to the start of the experiment (see Heetkamp et al., 2015 for details). Measured CO_2 recoveries were 99.7% and 100.1% in chamber 1, and 99.4% and 99.6% in chamber 2. For NH_3 we used two methods as described in Parodi et al. (2020). In short, with the acid washing bottle method, we measured the NH_3 lost by exhaust air, and added the ammonium in the condensed water that was produced from the cooling of the recirculating air (see Parodi et al. 2020 for details). To obtain the time course emissions of NH_3, we measured every 18 min the NH_3 concentrations in the outgoing air stream using a calibrated NH_3 sensor (Dräger Polytron® 8100 EC with sensor type NH_3-FL range 0–300 ppm NH_3, Lübeck, Germany). N_2O loss by air was quantified and calculated as described in Parodi et al. (2020). Air samples were collected once per day with a syringe (BD Plastipak, Drogheda, Ireland) from the ingoing and outgoing air streams of the climate respiration chambers and subsequently analysed in a gas chromatograph (Trace1300 GC, Waltham, United States), using a Haysep Q80-100 mesh 3 × 1/8″ SS column at temperature 60 °C and with an injection volume of 1 ml.

2.5. Nutrient balances and recoveries

To quantify how much of the initial nutrients and energy provided in the pig manure were recovered as larval biomass (i.e., also known as BSF bioconversion efficiency as described by Bosch et al., 2020), residual material and gaseous emissions, we used equations (1), (2), (3), respectively.

$$BE_n = \frac{(\mathrm{ML}_n - \mathrm{SL}_n)}{\mathrm{I}_n} \tag{1}$$

where BE_n is the BSFL bioconversion efficiency (%) of nutrient n, ML_n is amount of nutrient n contained in mature larvae (in g), SL_n is the amount of nutrient n contained in the starter larvae (in g) and I_n is the amount of nutrient n contained and provided in the manure (in g).

$$\mathrm{RM}_n = \frac{\mathrm{R}_n}{\mathrm{I}_n} \times 100 \tag{2}$$

where RM_n is the percentage of nutrient n recovered in the residual material and $\mathrm{R}_n$is the amount of nutrient n found in the residual material (in g).

$$\mathrm{GE}_n = \frac{E_n}{\mathrm{I}_n} \times 100 \tag{3}$$

where GE_n is the percentage of nutrient n recovered as gaseous emissions and E_n is the amount of nutrient n lost as emissions or heat (in g or kJ). E was only quantified analytically for carbon, energy and nitrogen. Gaseous emissions of phosphorus and potassium were not quantified given that these two elements and compounds in which they occur are not volatile. DM lost via emissions (E_{DM}) was calculated by difference (equation (4)).

$$E_{DM} = 100 - (BE_{drymatter} + RM_{drymatter}) \tag{4}$$

Table 1
Nutrient composition of the fresh pig manure, starter larvae, 16-day old larvae and residual materials (mean ± standard deviation, n = 4). Except for dry DM, all values are expressed per 100 g of DM. Nitrogen values include both ammonium and non-ammonium nitrogen.

Component	Dry matter (%)	Carbon (%)	Nitrogen (%)	Energy (kJ/100 g)	Ammonium- nitrogen (%)	Potassium (%)	Phosphorus (%)
Fresh manure	23.9 ± 1.0	44.8 ± 0.4	3.4 ± 0.0	1860 ± 17	2.1 ± 0.2	2.2 ± 0.1	1.8 ± 0.1
Starter larvae	26.3 ± 0.3	52.3 ± 1.1	9.5 ± 0.4	2373 ± 59	–	1.8 ± 0.1	1.9 ± 0.1
16-day old larvae	27.6 ± 0.4	46.1 ± 0.4	6.9 ± 0.2	2042 ± 33	–	1.6 ± 0.1	1.2 ± 0.1
Residues manure with BSFL	32.5 ± 1.2	41.9 ± 0.4	2.4 ± 0.0	1682 ± 15	1.6 ± 0.1	2.6 ± 0.2	2.1 ± 0.2
Residues manure without BSFL	29.4 ± 1.2	43.5 ± 0.4	3.2 ± 0.0	1787 ± 23	2.0 ± 0.2	2.4 ± 0.1	2.0 ± 0.1

To verify if we had a matching balance in which all inputs provided were recovered in the outputs, we calculated the total recovery (RE) (in %) for each nutrient *n* using equation (5). For manure incubated without BSFL, *BE* was zero because larvae were absent. As E_{DM} was calculated by difference (equation (4)), the total recovery of DM was 100%.

$$\mathrm{RE}_n = \sum (\mathrm{GE}_n + \mathrm{RM}_n + \mathrm{BE}_n) \tag{5}$$

2.6. Data analysis

Experimental data were analysed using R (R Core Team, 2019). All analyses and visualizations are reproducible and accessible at https://doi.org/10.4121/14318780. Results were expressed as mean ± standard error (n = 4). To test whether the production of N_2O was not different from zero, we performed a two-sided *t*-test. To test for differences between treatments in the recovery of nutrients in residual materials and gaseous emissions, nutrient ratios and production of GHG, we used ANOVA with treatment and trial as fixed effects. We were not able to robustly test for normality and homogeneity of variance due to the small sample size (n = 4), and therefore assumed that data was normal and variances homogeneous.

3. Results

In both treatments, the recovery of the initial quantity of nutrients present in the manure at the start of the incubation were close to 100%. For manure incubated with BSFL, the recovery was 100 ± 2% for carbon, 98 ± 6% for nitrogen, 100 ± 2% for energy, 103 ± 1% for potassium and 100 ± 1% for phosphorus. For manure incubated without BSFL, the recovery was 95 ± 4% for carbon, 96 ± 8% for nitrogen, 95 ± 4% for energy, 97 ± 5% for potassium and 97 ± 9% for phosphorus. Considering that a recovery of 100% indicates that all inputs were recovered in the outputs, we were able to successfully quantify the outputs of the system in both treatments.

3.1. Bioconversion efficiency

The bioconversion efficiency of manure by BSFL was 13 ± 0.3% for DM (mean ± std. error), 25 ± 0.6% for nitrogen, 13 ± 0.4% for carbon, 14 ± 0.5% for energy, 9 ± 0.5% for phosphorus and 9 ± 0.7% for potassium (Fig. 1). The fresh larvae yield per kg of fresh manure was 110 ± 3 g, while the dry larvae yield per kg of dry manure was 127 ± 3 g. The final larval individual fresh weight was 84 ± 9 mg. Additional parameters of the bioconversion process are presented in Table S1.

3.2. Nutrients in the residual material

Incubating pig manure with BSFL substantially decreased the levels of total nitrogen, carbon, energy, phosphorus and potassium in the residual material (Fig. 1). The residual material of manure incubated with BSFL had 12% less DM, 37% less nitrogen, 20% less carbon, and 9% less phosphorus and potassium than the residual material of manure incubated without BSFL. Moreover, the presence of BSFL decreased substantially the fractions of ammonia and non-ammonia nitrogen found in fresh manure (Fig. 2). In contrast, in the absence of BSFL, both nitrogen fractions were within the confidence interval found in fresh manure (Fig. 2).

The presence of BSFL also modified markedly the C:N, N:P and N:K ratios in the residual material. The C:N ratio of the residual material of manure incubated with BSFL was higher than the ratio found in fresh pig manure, while the N:P and N:K ratios were lower (Fig. 3). The nutrient ratios of the residual material of manure without BSFL did not differ from those initially present in the fresh pig manure, and the P:K ratio did not change from the initial levels as a result of the treatment (Fig. 3). Overall, these results confirm that when manure is incubated with BSFL, there are substantial changes in the nutrient composition of the residual material relative to the original manure. Compared to the original manure, the residual material contains a C:N ratio which is closer to the 24:1 ratio that soil microbes need, while still ensuring nitrogen mineralization. In addition, the reduced N:P and N:K ratios are beneficial for the fertilization of soils with high levels of nitrogen and potassium.

3.3. Nutrient losses in the form of gaseous emissions

Incubating manure with BSFL increased carbon emissions and heat loss compared with incubating manure without BSFL (Fig. 1). Although nitrogen emissions between treatments were not significantly different (Fig. 1), nitrogen emissions varied largely between trials. In two trials nitrogen emissions were only 5% higher when BSFL was present, but in the other two, nitrogen emissions were 50% higher (Fig. S1). Volatilization of DM was similar for both treatments (Fig. 1).

In both treatments, carbon losses occurred mainly as CO_2, although losses of CH_4 were also detected (Table 2). The CO_2 emissions produced when manure was incubated with BSFL nearly doubled those emitted in the absence of BSFL (Table 2). The development of CO_2 emissions over time started on the same time and were similar in both treatments until the second day. After the second day, CO_2 emissions were always higher in the treatment with BSFL (Fig. 4A) and reached its peak on day 7. The CO_2 emissions of treatment without BSFL did not show any peak and remained constant until the end of the experiment. CO_2 emissions were 1956 ± 105 g per kg of DM larvae (mean ± standard error, see Table 3).

Emissions of CH_4 did not differ between the two treatments (Table 2). During the first four days both treatments had similar time dynamics with peaks of CH_4 production (Fig. 4B and Fig. S3). However, after day 4, CH_4 emissions in the treatment with BSFL declined. Such decline was not observed in the treatment without BSFL. CH_4 emissions were 10.1 ± 1.33 g per kg of dry larvae.

While statistical tests did not show significant differences, on average, nitrogen emissions were higher when manure was incubated with BSFL (Fig. 1, Table 2). The intensity of the nitrogen emissions, however, varied between trials (Fig. S2). In line with this observation, the recorded time course in NH_3 emissions (Fig. 4C) showed higher emission levels for manure inoculated with BSFL and a high variability between trials. The variability was more pronounced in the treatment with BSFL (see larger standard error in Fig. 4C, and Fig. S3). The NH_3 emission time patterns differed between the treatments. While NH_3 emissions of manure incubated with BSFL peaked on day 6 and dropped in the following days, the NH_3 emissions of manure without BSFL remained constant over time. Overall nitrogen emissions were 57.5 ± 6.84 g per kg of DM larvae (mean ± standard error, Table 3)

Unlike CO_2, CH_4 and NH_3 which were measured continuously, N_2O was measured once a day. With this setup, the recorded N_2O emissions for both treatments were very low and not significantly different from zero (Table 2 and SI.4). The negative values near zero were caused by normal analytical errors (Fig. 4D and Fig. S3).

The GHG emissions in grams of CO_2 eq per kg of DM input manure did not differ significantly between treatments. As the calculation of CO_2 eq combines both CH_4 and N_2O, and N_2O emissions were negligible (Table 2), CO_2 eq were determined mainly by the

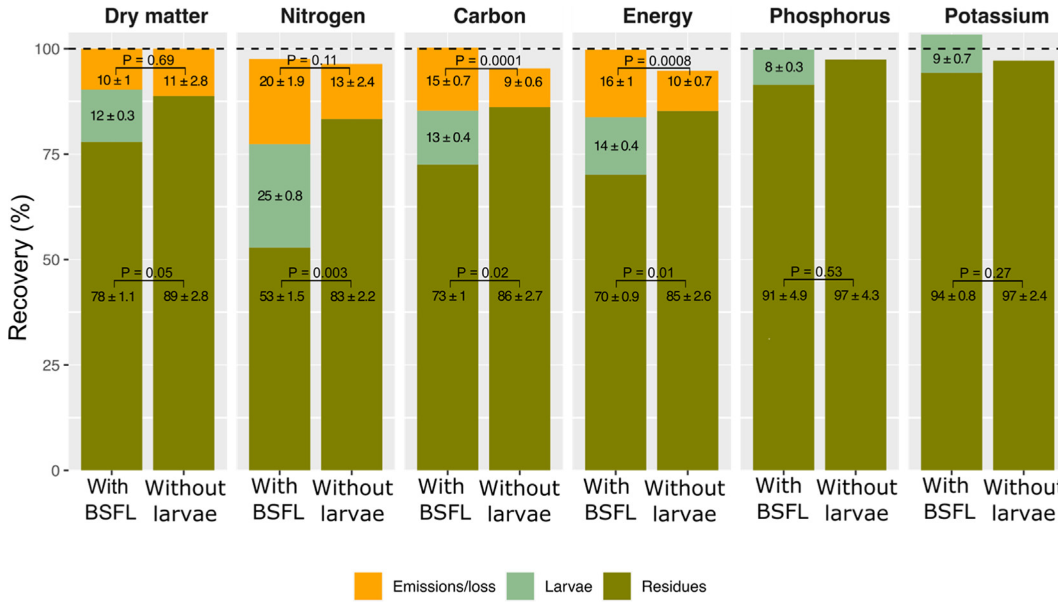

Fig. 1. Recovery of DM, nitrogen, carbon, energy, phosphorus and potassium of pig manure incubated with or without BSFL in residual material, larvae, and gaseous emissions and heat loss. DM recovery is by definition 100% because emissions were calculated by difference. See SI.4 for the detailed statistical parameters. See Fig. S2 for the raw nutrient balances per trial.

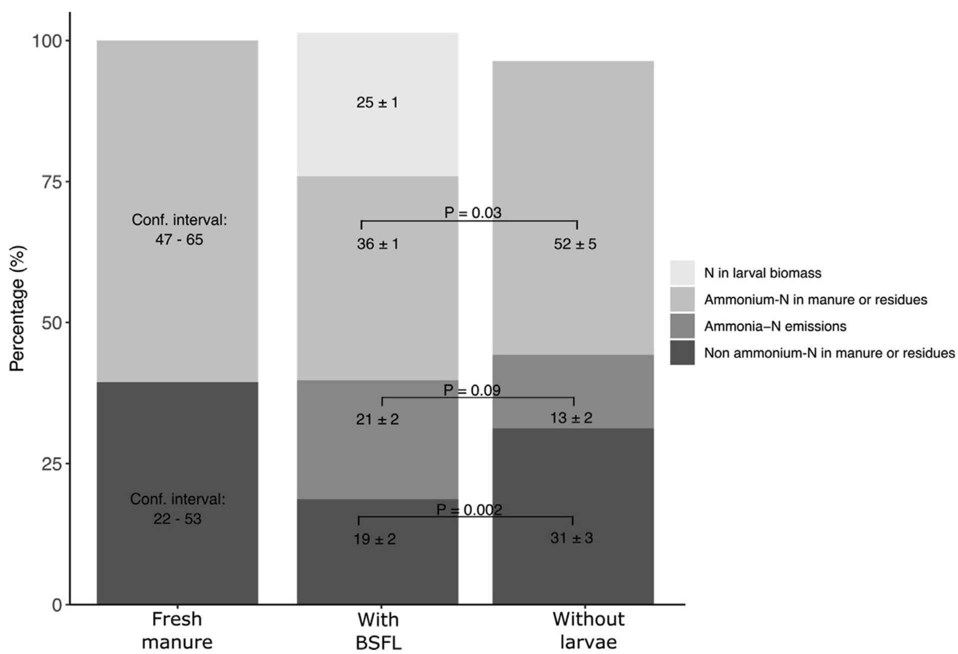

Fig. 2. Nitrogen balances partitioned among ammonium-nitrogen and non-ammonium nitrogen in fresh manure and residual material, ammonia-nitrogen in gaseous emissions and nitrogen accumulated in larval biomass. All bars show mean values (n = 4). For fresh pig manure, text labels within each bar show the 95% confidence interval, while for the two treatments, text labels show mean ± std. error. See SI.4. for detailed statistical parameters.

CH_4 emissions. Overall GHG emissions were 344 ± 43 g CO_2 eq per kg of DM larvae (mean ± standard error, Table 3).

4. Discussion

To evaluate the environmental sustainability of using BSFL as a manure management technology, broad-scale assessments, such as life cycle assessments and food system modelling, are needed. Our study, and specifically the results for manure incubated with BSFL, provides a reliable quantitative basis based on high analytical recoveries and complete nutrient balances for the elaboration of such broader scale studies. For instance, future life cycle assessments on manure processing with BSFL could use not only the emissions reported here, but also the chemical composition and bioconversion efficiencies at the larval density studied to estimate the avoided impacts associated with the use of BSFL ingredients for feed formulations and residual material as a fertilizer. In addition, our values could be used to quantify the potential of manure bioconversion with BSFL to increase the nitrogen use efficiency in livestock supply chains. These studies, combined with assessments of economic feasibility and technological adoption, should be used for

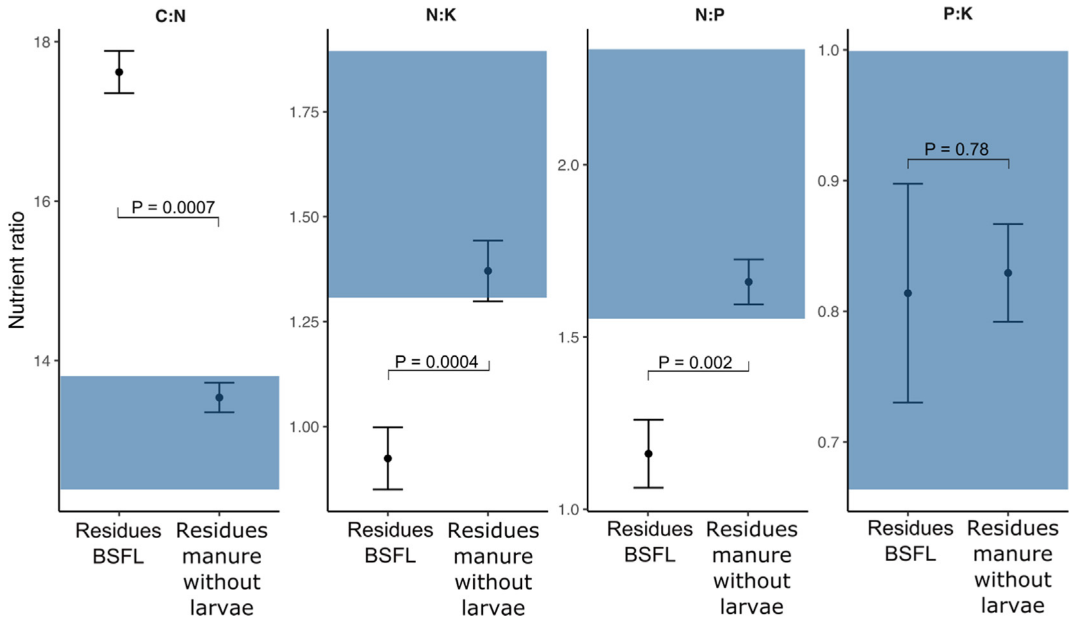

Fig. 3. Nutrient ratios (mean ± std. error) in the residual material of manure incubated with BSFL, and the residual material of manure incubated without BSFL. Shaded areas show the lower and upper confidence interval (95%) of each nutrient ratio found in fresh pig manure. See SI.4 for the detailed statistical parameters.

Table 2
Gaseous emissions, heat production and oxygen consumption per kg of DM initial manure. Means ± std. error followed by the same superscript in each column do not differ significantly ($P > 0.05$). N_2O emissions were not significantly different from zero for both treatments, but original mean and standard error are shown. See SI.4 for the detailed statistical parameters. See Table S2 for detailed emissions expressed in other metrics and per trial.

Treatment	CO_2 (g)	CH_4 (g)	N_2O (mg)	CO_2 eq (g)*	O_2 (g)	NH_3-N (g)	Energy (kJ)
Manure incubated with BSFL	247 ± 12^{a}	1.27 ± 0.17^{a}	0.73 ± 0.95^{a}	43 ± 5.4^{a}	213 ± 15^{a}	7.2 ± 0.70^{a}	3048 ± 201^{a}
Manure incubated without BSFL	148 ± 10^{b}	1.12 ± 0.02^{a}	0.56 ± 1.10^{a}	38 ± 1.1^{a}	124 ± 10^{b}	4.5 ± 0.83^{a}	1780 ± 132^{b}

* Global Warming Potential (GWP) was expressed as g CO_2 equivalents based on the GWP_{100} of CH_4 (34) and N_2O (298) with carbon feedback (IPCC, 2013).

decision making to evaluate whether manure bioconversion with BSFL can bring environmental, health and social benefits compared to other alternatives for treating surplus manure.

To accurately quantify the influence of the larvae on the nutrient flows and emissions when reared on manure we included the treatment consisting of manure without larvae. It is important to highlight that the treatment without larvae and its associated measurements (i.e., nutrients in residual material, gaseous emissions) should not be considered representative for current manure management practices (i.e., composting).

4.1. Bioconversion efficiencies

Attaining high nutrient bioconversion efficiencies in a manure bioconversion system with BSFL is desirable from an environmental perspective. As surplus manure leads to environmental pollution due to the excessive concentration of reactive nutrients susceptible to microbial decomposition, storing as much nutrient mass as possible in the larval biomass (i.e., high bioconversion efficiencies) reduces the amount of nutrients vulnerable to microbial decomposition in the residual material. In addition, high nutrient bioconversion efficiencies lead to higher total larval biomass fed with recycled nutrients that could decrease in a larger degree the need of producing raw materials (e.g., feed ingredients, biofuels) and therefore avoid the impacts associated with their production.

Bioconversion efficiencies of pig manure with BSFL have been reported mainly for DM and only a few studies covered carbon, nitrogen and potassium. While we found a DM bioconversion efficiency of 12.5%, previous studies with pig manure and BSFL reported DM bioconversion efficiencies of 1.8–2.1% (Miranda et al., 2019), 2% (Liu et al., 2018) and 5% (Oonincx et al., 2015). Similarly, our carbon (i.e., 12.8%) and phosphorus (i.e., 8.5%) bioconversion efficiencies, were higher than the 0.3–4.7% carbon bioconversion efficiency reported by Chen et al., (2019), and the 5% phosphorus bioconversion efficiency reported by Oonincx et al. (2015). Among the many factors that could explain such variation (Bosch et al., 2020), the different metrics used to calculate bioconversion efficiencies (e.g., fresh vs dry weight), the different experimental conditions (e.g., all cited studies were performed with 100 to 450 larvae/per container and different larval densities), the different nutrient composition of manure samples, and the different feeding regimes (i.e., single vs. multiple feeding) are likely the four most important.

4.2. Residual material

We determined the effect of incubating manure with BSFL on the reduction of the quantity of nutrients in the residual material (Fig. 1, Fig. 2). The lower nutrient levels of the residual material after BSFL treatment compared to manure incubated without larvae, are in line with Liu et al. (2019) who found 20% less organic matter, 13% less dissolved organic carbon and 25% less nitrogen in the residual material after manure bioconversion with BSFL compared to manure composted without BSFL. The lower nutrient levels and subsequent change in nutrient ratios in the residual material of manure incubated with BSFL can be explained by the

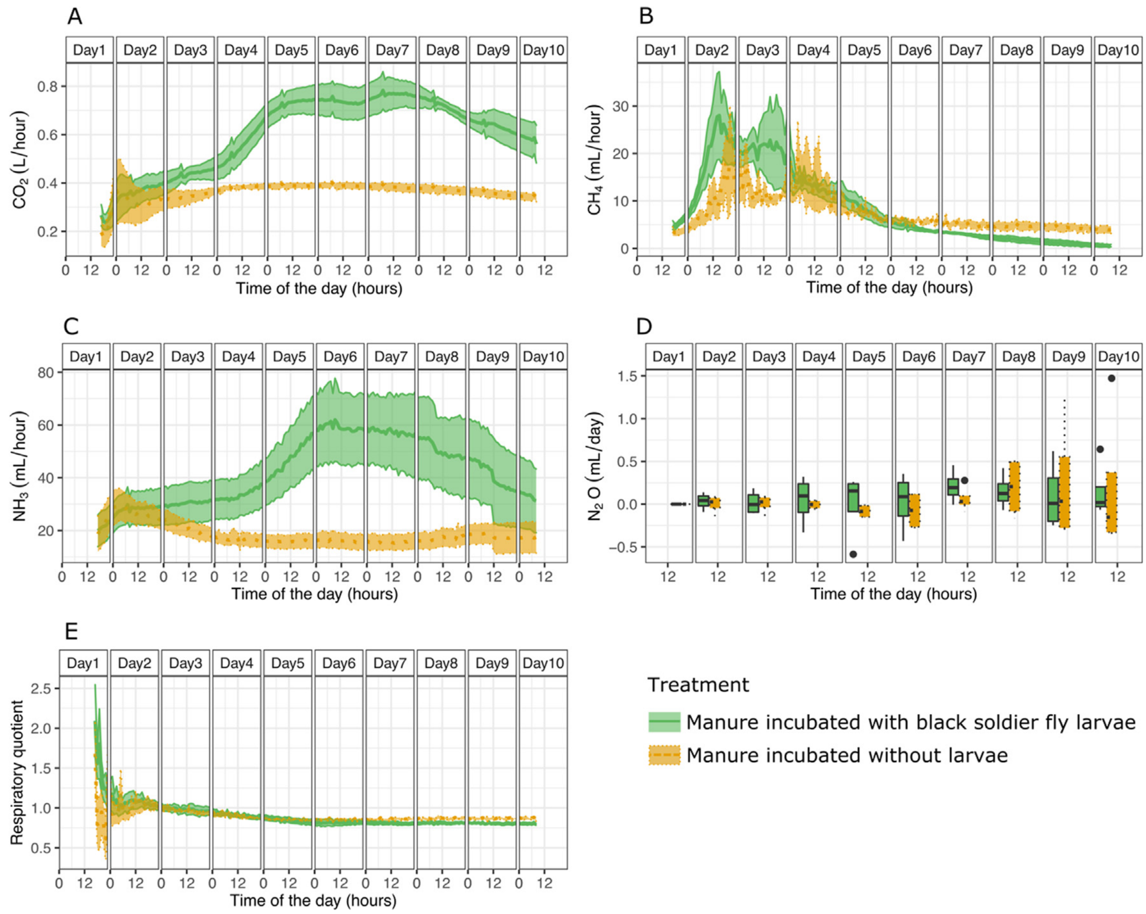

Fig. 4. Gaseous emissions (panels A-E) over time during the incubation of manure with black soldier fly larvae and without larvae. Lines in panels A, B, C and E show the mean of each treatment (n = 4) and the shaded areas the standard error of the mean. See Fig. S3 for emissions figures per trial.

Table 3
Direct gaseous emissions during BSFL rearing expressed per kg of dry matter larvae (mean ± standard deviation). These values correspond to the emissions during the growth of BSFL and do not account for feed, energy-use and processing-related emissions.

Study*	Diet	CO_2 (g)	CH_4 (mg)	N_2O (mg)	Nitrogen (g)	GWP - CO_2eq (g)
Ermolaev et al. (2019)	Restaurant food waste	1750 ± 170	49 ± 29	21 ± 13	–	8 ± 4.8
Mertenat et al. (2019)	Kitchen food waste	–	5.5	118	–	35
Pang et al. (2020)	Restaurant food waste with rice straw	1394 ± 343	14 ± 6	7 ± 1	–	2.5 ± 0.5
Parodi et al. (2020)	Food by-products	2750 ± 314	28 ± 29	53 ± 27	1.2 ± 0.7	17 ± 8.6
This study	Pig manure	1956 ± 105	10066 ± 2652	6 ± 14	58 ± 7	344 ± 43

* For Ermolaev et al. (2019) we used the values of treatment "L". For Pang et al. (2020) we used the values of treatments pH 5, pH 7 and pH 9.

sequestration of nutrients in the larval biomass and the slightly higher CO_2 and NH_3 emissions occurring during the bioconversion process (Fig. 1, Table 2).

Although the presence of BSFL increased NH_3 emissions during the rearing, if the residual material of manure bioconversion with BSFL is used as fertilizer, the lower levels of ammonium-nitrogen in the residual compared to fresh or stored manure, could lead to lower rates of ammonia volatilization when applied to the soil (Jiang et al., 2017; Sommer and Hutchings, 2001). Lower ammonia-nitrogen levels might not affect the fertilizer quality as NO_3^- nitrogen levels in the residual material are 15% higher than composted pig manure without BSFL (Liu et al., 2019)

4.3. Gaseous emissions

While Parodi et al. (2020) detected NH_3 emissions only after day 5 of the bioconversion process of food residues with BSFL, in this study NH_3 was emitted from day 1 (Fig. 4C). Considering that microbial enzymes are abundant in manure, the NH_3 emissions occurring early in the process are likely explained by the activity of microbial ureases breaking down manure urea into NH_3 and CO_2 (Aarnink and Elzing, 1998; Dai and Karring, 2014). Nitrogen emissions were not only earlier when BSFL was reared on manure but were also 48 times higher than those recorded when larvae grew on food residues (Table 3). This large difference between the two diets might be due to the fact that two thirds of the nitrogen contained in pig manure was present as ammonia-nitrogen, which can volatilize faster compared to the predominantly organic nitrogen likely contained in the food residues used by Parodi et al. (2020).

Even though most of the ammonia-nitrogen losses observed in both treatments were likely driven by the activity of microbial ureases, our results showed that after day 3, nitrogen losses were higher when BSFL were present (Fig. 4).

It is known that NH_3 volatilization in manure is a function of pH, temperature, moisture and air velocity over the surface of manure (Groot Koerkamp, 1994). Although we did not measure these parameters, we hypothesize that moisture and temperature were not important drivers for the higher NH_3 emissions observed for the treatment with larvae, and instead pH played a main role. Chen et al. (2019) showed that when BSFL is present in manure with 25% DM content, there were no substantial changes in substrate temperature and moisture levels in the first five days of bioconversion. As we started to observe differences in NH_3 production between treatments after the third day, it is likely that neither temperature nor moisture played a main role in the higher NH_3 emissions observed when BSFL were present. Instead, Liu et al. (2019) reported that the substrate pH is higher when black soldier fly is present in manure (pH 9) compared to when it is absent (8.5). Considering that slight changes in pH between pH 8 and 11 can lead to large changes in the NH_3-NH_4^+ equilibrium, and that nitrogen is only volatilized as NH_3 (Groot Koerkamp, 1994), a higher pH caused by the larval activity might have caused the larger NH_3 losses. Additional factors that might have influenced the larger NH_3 emissions are the higher exposure of the residual substrate to air currents due to the mechanical perturbation of the substrate caused by larval movement, and the larval excretion of uric acid which could be converted to NH_3 by microbes (Gold et al., 2018; Green and Popa, 2012).

A key topic that requires more attention is the quantification of the nitrogen contained in the larval body mass that originated from ammonium-nitrogen. The ammonium and non-ammonium nitrogen balances presented in Fig. 2 revealed the existence of a potential mechanism allowing BSFL or associated microbes to use ammonium-nitrogen. Quantifying this potential flow of nitrogen will allow the estimation of how much ammonium-nitrogen is involved in BSFL anabolism and will hopefully lead to the exploration of ways on how to utilize this mechanism to reduce NH_3 emissions.

Different processes might explain the temporal dynamics and occurrence of CO_2, CH_4 and N_2O emissions observed in this study. While microbial respiration was the main source of CO_2 emissions in the treatment without BSFL, the presence of BSFL increased the CO_2 emissions as both microbial and larval respiration occurred. Compared to the emissions reported for BSFL farmed in non-manure diets (Table 3), CO_2 emissions per kg of DM larvae were 40% and 11% higher than Pang et al. (2020) and Ermolaev et al. (2019) respectively, but 40% lower than Parodi et al. (2020). Besides the influence that factors such as type of diet, larval density, age of starter larvae at the start of the experiment could have on CO_2 emissions, a key factor that might explain the lower CO_2 emissions reported by Pang et al. (2020) and Ermolaev et al. (2019) is the lower gas sampling frequency (i.e., daily basis or once every 2 to 5 days). Such low sampling frequencies might have led to an underestimation of true emissions. On the other hand, the higher CO_2 emissions reported by Parodi et al. (2020) might be linked to differences in the quality of the diet. The respiratory quotient (RQ) (i.e., ratio between CO_2 produced and O_2 consumed that indirectly shows if protein, carbohydrates or fats are used as oxidation substrate) reported by Parodi et al. (2020) were above or equal to 1 in most of the growing periods indicating that larvae had access and were mainly using carbohydrates as oxidation substrate. Instead, the RQ for BSFL fed on manure was relatively constant around 0.8 (Fig. 4E), suggesting that protein and fats were the main macromolecules used to sustain larval metabolism.

Manure CH_4 emissions are mainly linked to the presence and abundance of Archaea (Petersen et al., 2014). CH_4 emissions, however, can decrease with aeration (Martinez et al., 2003). Even though CH_4 emissions were only slightly higher with the presence of BSFL (Table 2), the slight decline in CH_4 after day 4 in the BSFL treatment (Fig. 4B), might have been linked to the aeration caused by the larval movement through the crate when the larvae had grown to a larger size, and to the reduction in the quantity of substrate present. Compared to other studies, CH_4 emissions were much higher when larvae were reared on manure than when reared on food waste or food residues (Table 3). This highlights the importance of the substrate on the emissions during BSFL bioconversion, as in this case it is the manure Archaea and not the larvae that cause the high emissions. Therefore, judging whether manure-fed BSFL production systems can bring environmental benefits should be done comparing emissions with other manure uses at a life cycle level, and not comparing food waste fed BSFL with manure fed BSFL.

The N_2O emissions registered with our experimental setup were almost null in both treatments and did not have a defined time pattern. In addition, our N_2O measurements are in line with Chen et al. (2019) who found that N_2O emissions were close to zero after 9 days of manure bioconversion with BSFL. Compared to other diets N_2O emissions were equal or lower than those for food waste and food residues (Table 3).

5. Conclusion

In this study we provide a quantitative nutrient balance for pig manure bioconversion with BSFL. With recoveries ranging between 95 and 103%, our nutrient balances were virtually complete. BSFL incorporated 12, 25, 14, 8.5 and 9% of the carbon, nitrogen, energy, phosphorus and potassium initially contained in fresh manure. By storing nutrients in the larval body mass and releasing additional carbon dioxide, ammonia and heat, BSFL substantially reduced the carbon, nitrogen and energy levels originally found in fresh pig manure. CH_4 was the main GHG produced during manure bioconversion with larvae and was likely produced by manure's Archaea. Our study provides a reliable quantification of GHG and NH_3 emissions to quantify the environmental consequences of using BLFL in future life cycle assessments.

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CRediT authorship contribution statement

Alejandro Parodi: Conceptualization, Investigation, Formal analysis, Visualization, Writing - original draft. **Walter J.J. Gerrits:** Conceptualization, Writing - review & editing, Supervision. **Joop J. A. Van Loon:** Conceptualization, Writing - review & editing, Supervision. **Imke J.M. De Boer:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition. **André J.A. Aarnink:** Validation, Writing - review & editing. **Hannah H.E. Van Zanten:** Conceptualization, Writing - review & editing, Supervision.

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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2021.04.001. Raw data and custom R scripts developed for the analyses and visualizations can be found at https://doi.org/10.4121/14318780.

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