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RESEARCH ARTICLE

The influence on carbon, nitrogen recycling, and greenhouse gas emissions under different C/N ratios by black soldier fly

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Abstract

Currently, sustainable utilization, including recycling and valorization, is becoming increasingly popular in waste management. Black soldier fly larvae (BSFL) can convert the carbon (C) and nitrogen (N) from organic waste into biomass and improve properties of the substrate to reduce greenhouse gas and NH_3 emissions. In this study, the recycling of C and N and the emissions of greenhouse gas and NH_3 during BSFL bio-treatment of mixtures of pig manure and corncob were investigated under different C/N ratios. The results indicated that initial C/N ratios of feedstock are a crucial parameter affecting the biomass generation of larvae. The BSFL recycled approximately 4.17–6.61% of C and 17.45–23.73% of N from raw materials under different C/N ratios. Cumulative CO_2, CH_4, NH_3, and N_2O emissions at the different C/N ratios ranging from 15 to 35 were 107.92–151.68, 0.08–0.76, 0.14–1.17, and 0.91–1.18 mg kg^{-1}, respectively. Compared with conventional composting, BSFL treatment could reduce the total greenhouse gas emissions by over 90%. The study showed that bio-treatment of mixtures of pig manure and corncob with a proper C/N ratio by BSFL could become an avenue to achieve higher nutrient recycling, which is an eco-friendly process.

Keywords Bio-waste · *Hermetia illucens* · Bioconversion feedstock · Recycle · Piggery manure · Corncob

Introduction

Currently, the intensification of crop and livestock production has generated substantial levels of agricultural waste (Chang et al. 2019). Mismanagement of these bio-wastes might bring about many environmental challenges, such as odor nuisance, spreading of diseases, and air and groundwater pollution (Rehman et al. 2017a). In environmental management, composting is one of the most proper technologies for controlling livestock manure and crop residue, and it can diminish the volume and mass of bio-waste, destroy the weed seeds, as well as produce soil-amendment materials (Jiang et al. 2011; Peng et al. 2019). In China, it is reported that pig manure production had increased to about 490 million tons, and the total yield of corncobs is estimated to have reached over 20 million tons (Wang et al. 2018; Li et al. 2015). Nevertheless, recently, only 26.0% of the total amount of crop residues and 32.9% of manure were treated by composting (Chang et al. 2019). Moreover, the biodegradation of organic matter can result in substantial loss of valuable carbon (C) and nitrogen (N) during the long composting process, which both reduces the end-product quality and has a negative impact on the ecosphere. Many studies also confirmed that the loss of N caused by NH_3 and N_2O emission during composting could reach 9.6–46 and 0.2–6%, respectively (Luo et al. 2014). About 14–59% and 0.8–14% of initial total organic carbon (TOC) in organic wastes are lost in the form of CO_2 and CH_4, respectively (Chang et al. 2019; Luo et al. 2014). Under this circumstance, the development of ecologically green technique as an alternative for low-quality composting is extremely important.

Wancheng Pang and Dejia Hou contributed equally to this work and should be considered co-first authors.

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Sustainable use of waste, including recycling and valorization, is the current trend in waste management (Sánchez et al. 2015). Waste valorization is usually conducted by biological processes, such as bioconversion of these wastes with larvae of insects. Black soldier fly (*Hermetia illucens* L.) larvae (BSFL), a bio-waste treatment, is an ecologically green technology as it could convert various wastes (e.g., swine manure, food waste, and sludge) and provide high-quality products compared with other waste treatment technologies, such as composting or anaerobic digestion (Cai et al. 2018; Pang et al. 2020; Lalander et al. 2019). In particular, BSFL farming can be used for converting agricultural waste as an ideal protein source for livestock to replace increasingly expensive commercial feed (Wang et al. 2019). Numerous researches highlighted the economic benefits of BSFL used as animal feed or biodiesel production and the residues produced as bio-fertilizer (Choi et al. 2009; Xiao et al. 2018; Quilliam et al. 2020). On the contrary, there are fewer studies generally focused on the environmental benefits of BSFL in terms of sustainability. Mertenat et al. (2019) and Ermolaev et al. (2019) found that BSFL treatment food waste has potential to decrease greenhouse gas (GHG) emissions compared with conventional composting. However, these researchers also expressed the need for further exploration based on various raw materials and process parameters which would undoubtedly be indispensable to studies on the direct GHG emissions and C and N conversion during BSFL conversion bio-waste process for evaluating its environmental impact.

To enhance the sustainability of BSFL conversion system, the search for more effective process parameter to shift toward a cleaner production process while maintaining the economic benefits, reducing the loss of gaseous C and N and increasing C and N recovery by BSFL, would be desirable. With this in view, the present study envisages adjusting the C/N ratio of the feeding substrates by mixing pig manure and corncob with complementary properties and optimize the BSFL conversion system, in that a suitable C/N ratio could reduce gaseous C and N losses, as well as enhance the efficiency of the BSFL biological process. Specifically, the flow direction of C and N in the following three parts would be monitored: direct GHG and NH_3 emission, BSFL biomass, and nutrients of residues. This study aims to present data for life cycle assessment evaluation of BSFL treatment as a greener alternative and optimize C/N ratio of the mixtures of pig manure and corncob in BSFL treatment process.

Materials and methods

Raw materials

Wuhan strain of the BSFL (*Hermetia illucens* L.) was obtained from the State Key Laboratory of Agricultural Microbiology of Huazhong Agricultural University (HZAU), Wuhan, China. The fresh pig manure was collected from the National Pig Breeding Experimental Center at HZAU. The chopped corncob was from the experimental field of HZAU. The main physicochemical properties are shown in Table 1.

Experimental design and operation

Pig manure and corncob were mixed at different mass ratios to initial C/N ratios of 15, 20, 25, 30, and 35, respectively, and hereafter labeled as E1–E5. The moisture content of the substrates was adjusted at 70 ± 2%. A total of 1.0 kg (wet weight) substrate was added in each 3 L cylindrical glass bottles with an enclosed lid. Based on preliminary trials, about 1600 3-day-old BSFL were inoculated onto the substrates. The experiment was terminated when the first prepupae appeared in the container of each replicate; the remaining larvae were separated from the residues and total weight of larvae and survival rate were recorded per bottle**.** All the treatments were incubated at 30 .0 ± 1.0 °C. All the experiments were replicated three times.

Measurements of gas emissions

The CO_2, CH_4, and N_2O samples were detected daily. According to a method of Wu et al. (2018), on each sample day, the reactor was enclosed with the lid, and the headspace gas (30 mL) was collected with a 50-mL gastight syringe at 0 and 300 s. The bottles were kept open after gas is taken and gas sample was injected into pre-evacuated 20 ml screw-capped Exetainer® vials. The gas samples were analyzed using gas chromatograph (GC-7890A; Agilent Technologies, Santa Clara, CA, USA). The CO_2 and CH_4 concentrations were measured using a flame ionization detector, whereas N_2O was measured using an electron capture detector. NH_3 was trapped in a 0.01 mol/L H_2SO_4 solution and measured via titration with 0.02 mol/L NaOH (Komilis and Ham 2006).

Solid sample collection and analysis

Representative substrate samples were collected after gas sampling for the determination of some physiochemical properties. Total nitrogen (TN) and TOC were tested in the air-dried samples. TN was measured using the Kjeldahl method (Song et al. 2013). TOC in the mixtures was measured by $K_2Cr_2O_7$ and H_2SO_4 (Chang et al. 2019). Solid sample inorganic N (ammonium-N and nitrate-N) was measured (sample: 2 mol L^{-1} KCl, 1:5 w/v ratio and shaken for 1 h) using the indophenol blue spectrophotometric method and ultraviolet spectrophotometry at 220 and 275 nm, respectively (UV-1800, SHIMADZU, Japan) (Wu et al. 2015). Dissolved

Table 1 Some physical and chemical characteristics of pig manure and corncob

	TOC (g/kg)	TKN (g/kg)	Moisture content (%)	C/N ratio	pH
Pig manure	370.24 ± 7.28	24.31 ± 0.41	71.86 ± 1.62	15.23	6.68 ± 0.25
Corncob	513.87 ± 9.23	5.36 ± 0.35	7.74 ± 0.38	95.87	5.37 ± 0.11

TOC, total organic carbon; TKN, total Kjeldahl nitrogen

organic carbon (DOC) in the substrate samples were measured using $K_2Cr_2O_7$ oxidation (sample:water, 1:10 w/w ratio and shaken for 1 h) as described by Zhu et al. (2015). The moisture content was measured by drying the samples in an oven at 105 °C for 24 h.

Statistical analysis

The experiments and sample analyses for all important parameters in this study were performed in triplicates. Data were analyzed by one-way analysis of variance (ANOVA). Multiple comparisons between treatments were compared using the least significant difference test. SPSS 22 was used for all statistical analyses.

Results and discussions

Larval survival rate and yield

Table 2 displays the larval survival rates and their fresh weights. There were no significant differences between all treatments (F = 1.337, $P = 0.322$, df = 14) in larval survival rate and rather a high survival rate was found in E1–E5 (94.76–97.23%), which were similar to most published values. Oonincx et al. (2015) reported survival rates for chicken, pig, and cow manure in the range of 82.2–97.0%. Gold et al. (2020) found that the mean survival rates were 90–99% when using mill by-products, human feces, poultry slaughterhouse waste, and canteen waste as substrates. These results suggest that BSFL can develop on bio-wastes with a wide range of C/N ratio. Table 2 also shows that there was a significant difference (F = 11.775, $P = 0.001$, df = 14) in fresh larval weight among the five treatments of different C/N ratios. The highest larval yield was obtained in E3 treatment (79.47 ± 3.19 g), followed by E4 (73.65 ± 2.78 g), E2 (71.72 ± 3.05 g), E1 (65.64 ± 2.42 g), and E5 (61.71 ± 2.91 g). Lalander et al. (2019) indicated that volatile solids in substrate are likely the key element controlling the larval development. In the present study, the higher percentage of pig manure in the substrate contains less volatile solids and thereby slower larval growth. In contrast, corncob is rich in cellulose, hemicelluloses, and lignin and was difficult to degrade by BSFL, which tend to limit the treatment performance of the larvae. Therefore, adjusting the C/N ratio to balance the substrate nutrition will enhance BSFL ability to utilize the required nutrients to a higher degree. This is in agreement with the results obtained by Rehman et al. (2017a), who demonstrated that an appropriate C/N ratio enhances the BSFL production owing to a better nutrient balance, which is essential to establish positive synergism for biological growth. Overall, C/N ratio of 25 (E3) proved to be the best for BSFL biomass generation among the mixtures of pig manure and corncob.

Table 2 Impact of using mixtures of pig manure and corncob in different C/N ratio on BSFL survival rate and its fresh weight

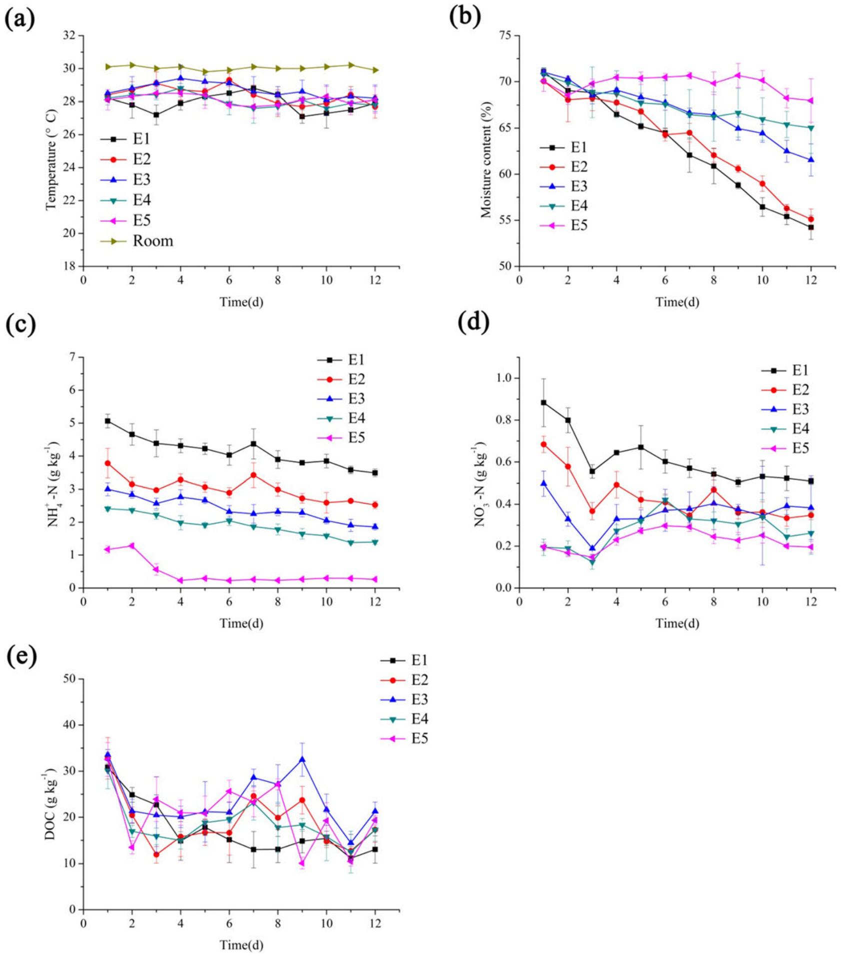
Treatment	Initial C/N ratio	Survival rate (%)	Fresh larval weight (g)
E1	15	95.66 ± 1.14a	65.64 ± 2.42b
E2	20	97.22 ± 0.88a	71.72 ± 3.05a
E3	25	97.23 ± 1.39a	79.47 ± 3.19a
E4	30	96.84 ± 2.01a	73.65 ± 2.78a
E5	35	94.76 ± 1.09a	61.71 ± 2.91c

Values followed by different letters within a column differ significantly at the 0.05 probability level

Changes in the physico-chemical properties of substrate during BSFL bioconversion process

Figure 1a shows the variation in temperature during the BSFL treatment process. BSFL is a temperate tropical and warm-season species, and the suitable temperature (27–30 °C) allows the BSFL to quickly transform organic wastes and shorten the growth cycle (Chen et al. 2019; Tomberlin et al. 2009). The temperature of all treatments was relatively steady at around 28 °C throughout the experiment almost approaching ambient temperature. This might be attributed to the feeding behavior of *H. illucens*, whereby they burrow and thus aerate the feeding medium (Ewusie et al. 2018), thereby maintaining temperature at the same level, indicating that BSFL waste treatment process is a mesophilic process. The moisture content showed variation and gradually decreased in the treatments studied (Fig. 1b). The moisture content of E1 and E2 decreased at a faster rate as compared with other treatments. The moisture content of E1–E5 was reduced from 71.06 to 54.23, 70.05 to 55.11, 71.03 to 61.54, 70.76 to 65.03, and 70.06 to 67.96%, respectively. During bioconversion, BSFL improves air circulation through continuous activity and digests the substrate, thus leading to evaporation and consumption of water. The differences in final moisture contents were

Fig. 1 Changes of temperature (**a**), moisture content (**b**), NH+ 4-N (**c**), NO− 3-N (**d**), and DOC (**e**) during the bioconversion process



possibly due to the addition of corncob which increased porosity and reduced the bulk density, improving water-holding capacity in raw materials.

The changes in ammonium-N (NH+ 4-N) and nitrate-N (NO− 3-N) were as shown in Fig. 1c and d, respectively. The initial NH+ 4 concentration of the treatments ranged between 1.17 and 5.06 g/kg owing to the differences in the composition of the different treatments. Organic N was firstly converted into NH+ 4via ammonification and subsequently oxidized into NO− 3 by nitrifying bacteria in the presence of O$_2$ (Sánchez et al. 2015). In this study, a slowly decreasing tendency of NH+ 4-N concentration was found. However, NO − 3-N concentration of five treatments showed an initial decrease and then increase until relatively stable during the study period, whereas the content of NH+ 4-N was significantly higher than that of NO− 3-N content. These findings are inconsistent with the reporting of Wang et al. (2018) and Lv et al. (2018), who showed that with time, NH+ 4-N concentration decreased significantly while accumulation of NO− 3-N increased during composting. This phenomenon could be explained as the transformation of NH+ 4-N by nitrifying bacteria to NO− 3-N (Lv et al. 2018). In this present study, transformation of NH+ 4 to NH$_3$ may be the main reason for the reduction in NH+ 4-N content during BSFL bio-waste treatment rather than nitrification. According to Yu et al. (2019), nitrification index (NH+ 4/NO− 3 ratio) reflects compost maturity and a value below 3 indicates mature compost. In our study, the nitrification index of final substrate from each treatment was above 3 except for E5, indicating that most of the resulting residues still required further degradation or curing. Besides, the DOC of the residues showed a trend of fluctuation without an apparent relationship to the C/N ratio (Fig. 1e). BSFL grow quickly and thus continuously utilize the most easily degradable C parts of the substrate which may result in a decrease in DOC concentration. After DOC cannot supply enough nutrients for BSFL, the formation of soluble organic matter by the degradation of solid polymeric material in the substrate may lead to increasing the DOC concentration (Lazcano et al. 2008). Therefore, a constantly fluctuating trend was observed during the composting.

CO$_2$ and CH$_4$ emissions during BSFL bioconversion process

CO$_2$ emissions from the biodegradation process of organic matter are usually not considered in the evaluation of global warming potential due to the biogenic origin of CO_2 (Sánchez et al. 2015). However, it is crucial for quantifying CO_2 emissions to better understand C cycling in BSFL bio-waste treatment. Figure 2a shows the daily CO_2 emissions during the bioconversion process. The CO_2 evolution rate of E2 (16.56 g kg^{-1} day^{-1}), E3 (16.98 g kg^{-1} day^{-1}), and E4 (14.83 g kg^{-1} day^{-1}) peaked by first day, while E5 (12.08 g kg^{-1} day^{-1}) and E1 (12.08 g kg^{-1} day^{-1}) peaked on 4th and 8th days, respectively. Thereafter, the CO_2 evolution rate gradually dropped until the end of the experiment. Several studies indicated that large emission amounts of CO_2 occurred at the initial stages of organic waste biodegradation (Lv et al. 2018; Santos et al. 2017; Barthod et al. 2018). The CO_2 emission rate relates to BSFL and microbial activities and the stability of substrates during the conversion process (Lv et al. 2018). In the present study, CO_2 emissions showed a trend of gradual reduction, suggesting stabilization of substrate by BSFL treatment.

The cumulative CO_2 emission for the five treatments during the study is presented in Fig. 2b. The total CO_2 emitted were 107.95, 126.91, 151.68, 148.84, and 124.26 g kg^{-1} for the E1–E5, respectively. Total CO_2 emission in E1, E3, and E5 was significantly different ($F = 66.794$, $P = 0.002$, $df = 8$), whereas it did not differ significantly between E3 and E4 ($F = 0.452$, $P = 0.693$, $df = 5$) or between E2 and E5 ($F = 0.193$, $P = 0.683$, $df = 5$). The maximum cumulative CO_2 emission was observed in E3 treatment, followed by E4, E3, E5, and E1. It is noteworthy that with the increase in larval output, cumulative CO_2 emission increased, indicating BSFL respiration may be the main contribution to difference in total CO_2 emissions from each treatment. A similar result was reported by Chen et al. (2019), who investigated the influences of different moisture contents on BSFL development, and they found that the tendency of total CO_2 production was consistent with that of the yield of harvested BSFL. Lalander et al. (2019) and Rehman et al. (2017a) opined that a balanced nutrition (C/N ratio) enhances the BSFL process performance and helps with the biodegradation of substrate which cannot be well digested by BSFL. Consequently, a potential explanation might be that the suitable C/N ratio can accelerate the decomposition and bioconversion of organic materials by BSFL and thereby increasing CO_2 loss through BSFL respiration from the treatment process. Overall, higher total cumulative CO_2 emissions also show higher biodegradation and greater stability of the residue, which is of benefit to composting process (Chan et al. 2011).

Similar patterns in CH_4 emission rate were observed in all treatments (Fig. 2c), which peaked in the first day, and decreased sharply, then approached zero after the 7th day. It is noteworthy that BSFL treatment of bio-waste had a lower emission rate and a shorter emission time compared with conventional compost (generally more than 15 days) (Luo et al. 2013; Yang et al. 2019; Jiang et al. 2011). CH_4 is mainly generated through methanogenic bacteria breakdown of

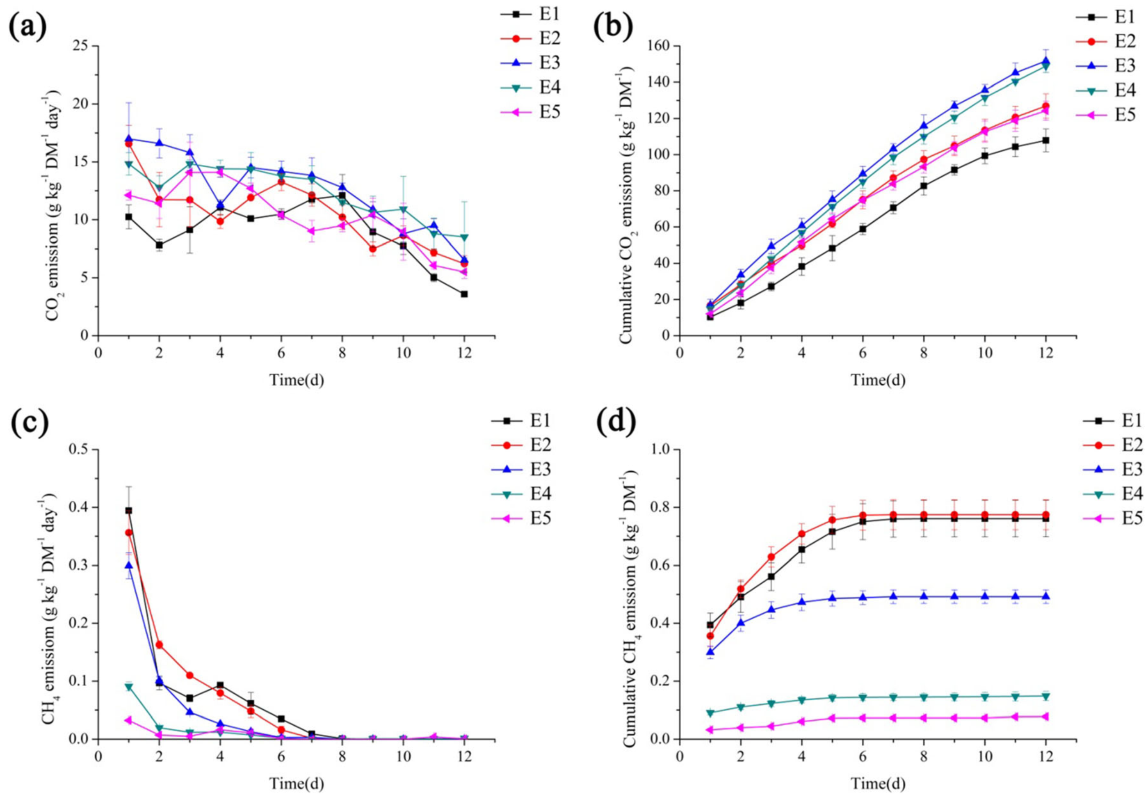


Fig. 2 Changes of CO_2 (**a**), cumulative CO_2 (**b**), CH_4 (**c**), and cumulative CH_4 (**d**) emissions during BSFL bioconversion process

CO_2/H_2 and acetic acid in anaerobic microsites (Takai 1970). In the initial stages, the raw materials contain larger particles and BSFL activity range is insufficient to cover the entire substrate, thus part of feeding substrate internal area will be in an anaerobic state resulting in a considerable fraction of CH_4 production. As the bioconversion process continued, BSFL improves air circulation and breakdown of larger particles through continuous turning and degrading of the substrate, thereby removing anaerobic conditions (Ermolaev et al. 2019).

The cumulative CH_4 emitted were 0.76, 0.77, 0.49, 0.15, and 0.08 g/kg for treatments E1–E5, respectively, during the study (Fig. 2d). A significant difference ($F = 167.226$, $P = 0.000$, $df = 11$) in cumulative CH_4 emission was observed among E1, E3, E4, and E5, whereas the difference between E1 and E2 was not significant ($F = 0.057$, $P = 0.823$, $df = 5$). According to Jiang et al. (2011), a higher C/N ratio (lower proportion of pig manure) caused lower CH_4 emission by increasing the void space and reducing the easily degradable C sources. Nevertheless, several reports indicated that the cumulative CH_4 emission is very low during BSFL bioconversion of food waste. The discrepancy could be attributed in part to the properties of the raw materials (Mertenat et al. 2019; Ermolaev et al. 2019). In the initial phase, the pH of food waste, being acidic, inhibits methanogen activity (Barthod et al. 2018), after which larval activity destroys the anaerobic activities. Thus, CH_4 emissions remained at a low level during the study. In spite of this, compared with the results from previous studies (Table 3), the CH_4 emissions of BSFL treatment were quite lower than those from conventional composting.

NH_3 and N_2O emissions during BSFL bioconversion process

NH_3 is included in various environmental studies because it becomes imperative for the agricultural value of the end-product and protection of ecosystems (Sánchez et al. 2015). The NH_3 volatilization is relatively stable throughout the BSFL conversion process except for E1 (Fig. 3a). This emission pattern is different from the thermophilic composting process, where an initial emission peak is generally found early in the biodegradation process, when easily available N source is transformed into inorganic nitrogen, such as NH+ 4-N by the microorganisms (Luo et al. 2014; Ermolaev et al. 2015; Wu et al. 2019). Previous research indicated that the significant factor of NH_3 volatilization was high temperature in the biodegradation process, and temperatures above 45 °C speed up NH_3 emissions (Chang et al. 2019; Nigussie et al. 2016). Higher temperatures (45–56 °C) during the active phase increase NH_3 emissions resulting in higher N loss during composting (Nigussie et al. 2016). It was observed in this study that BSFL bioconversion of bio-waste was a mesophilic process, and the relatively constant temperature of 28 °C cannot stimulate further volatilization of NH_3. Consequently, there is no significant fluctuation in the NH_3 emission rate in the entire conversion process.

As shown in Fig. 3b, the cumulative NH_3 emission curves present a good correlation between C/N ratio and NH_3 emission. With the increase of C/N ratio, the cumulative emissions of NH_3 were 1.17, 0.79, 0.57, 0.34, and 0.14 g kg^{-1} for treatments E1–E5, respectively. Obviously, the higher C/N ratio led to lower NH_3 volatilization. Similar results were found by Jiang et al. (2011), who conducted composting of pig manure with corn stalk under different C/N ratios. Also, studies by Jiang et al. (2011) and Meng et al. (2016) suggested that the high C/N ratio of substrate improves microbial assimilation and thus reduces NH_3 emissions. On the other hand, a higher proportion of corncob as part of the BSFL feedstock improves the water-holding capacity, therefore partly reducing NH_3 volatilization. Figure 1b thus supports the above-mentioned conclusion. Furthermore, the initial NH+ 4concentrations of the substrates (Fig. 1c) may have contributed to the differences in NH_3 emissions. Thus, the C/N ratio dominates the total NH_3 emission in this system. It is noteworthy that BSFL bio-waste treatment showed more potential in mitigating NH_3 emissions compared with composting in other research (Table 3). As previously commented, it appears that the relatively constant temperature of the system does not cause further loss of NH_3. Besides, part of the available N in the raw materials is converted into larval biomass rather than being biodegraded by microorganisms and then volatilized in the form of NH_3.

The N_2O emission patterns of the BSFL composting were significantly different from conventional composting and vermicomposting that immediately had a relatively high N_2O release in the initial stage of composting. During the entire BSFL conversion process, the daily N_2O discharge did not follow any specific pattern regardless of C/N ratio (Fig. 3c), with almost undetectable N_2O emissions in all the treatments. Cumulative N_2O emission was not significantly different ($F = 2.711$, $P = 0.092$, $df = 14$) among E1–E5, and the total emissions were all less than 1.5 mg kg^{-1} (Fig. 3d). This should be a matter of great joy since N_2O is a potent GHG, with a global warming potential of 298 times higher than that of CO_2, and causes destruction of the ozone layer (Tsutsui et al. 2013; IPCC 2013). These results corresponded with research by Ermolaev et al. (2019), who reported that N_2O concentrations in BSFL treatment of food waste do not differ significantly with the ambient air. The N_2O production during biodegradation process is a complicated system since the formation of N_2O is involved in different generated pathways (nitrification, nitrifier denitrification, and denitrification among others), which is regulated by the NH+ 4 and NO− 3 concentration, available C sources, O_2 content, pH, and temperature in the substrate (Sánchez et al. 2015; Cayuela et al. 2012; Wang et al. 2018). BSFL activity could create a

Table 3 Total GHG emissions from BSFL treatment and composting

Treatment	CO_2 (g/kg)	CH_4 (g/kg)	N_2O (mg/kg)	NH_3 (g/kg)	GHG emissions (kg CO_2-eq t^{-1} DM)	References
E1	107.92 ± 2.31	0.76 ± 0.06	1.03 ± 0.27	1.17 ± 0.21	26.15 ± 0.17	Current study
E2	126.91 ± 6.70	0.77 ± 0. 05	1.14 ± 0.05	0.79 ± 0.19	26.52 ± 0.44	Current study
E3	151.68 ± 3.29	0.49 ± 0.02	0.91 ± 0.02	0.57 ± 0.11	16.93 ± 0.11	Current study
E4	148.84 ± 3.52	0.15 ± 0.02	1.18 ± 0.08	0.34 ± 0.09	5.75 ± 0.14	Current study
E5	124.26 ± 5.26	0.08 ± 0.01	1.36 ± 0.02	0.14 ± 0.03	3.12 ± 0.03	Current study
Composting	–	~ 4.5[a]	~ 640[a]	~ 1.90[a]	~ 343.72[a]	Jiang et al. (2011)
Composting	–	~ 3.2[a]	~ 628[a]	~ 3.88[a]	~ 296.11[a]	Luo et al. (2013)

GHG, greenhouse gas; DM, dry matter

−, not reported; ~, approximately

[a] Cumulative emissions during the first 12 days of composting, dry weight basis

conducive aerobic environment that leads to inhibition of denitrification and thus limits the N_2O production. Several composting research also indicated that poor aeration gives rise to higher N_2O emissions (Hao et al. 2001; Awasthi et al. 2016; Tsutsui et al. 2013). Nevertheless, Lv et al. (2018) observed that the C/N ratio significantly affect N_2O emission in vermicomposting of sewage sludge, probably due to denitrifying bacteria in the earthworm gut which produce measurable N_2O fluxes. Larvae of *Hermetia illucens* have been shown to change the bacterial community of their feeding substrate (Jeon et al. 2011; Ermolaev et al. 2019). Moreover, Jiang et al.'s (2019) research found that BSFL gut microbiome could invade and interact with the substrate microorganisms, thus altering the microbial community structure, and the formation of a new intermediate habitat between the gut and medium. This intermediate habitat might be able to promote reproduction of bacteria beneficial to metabolism, thereby improving biodegradation (Jiang et al. 2019). The authors inferred that the influences of N_2O production might be related to the establishment of symbiotic bacterial communities in BSFL gut and medium microorganisms. But the BSFL-microbe-waste system is a complicated system that still requires lots of experiments and data to support this hypothesis. In addition, this study shows that using BSFL to treat

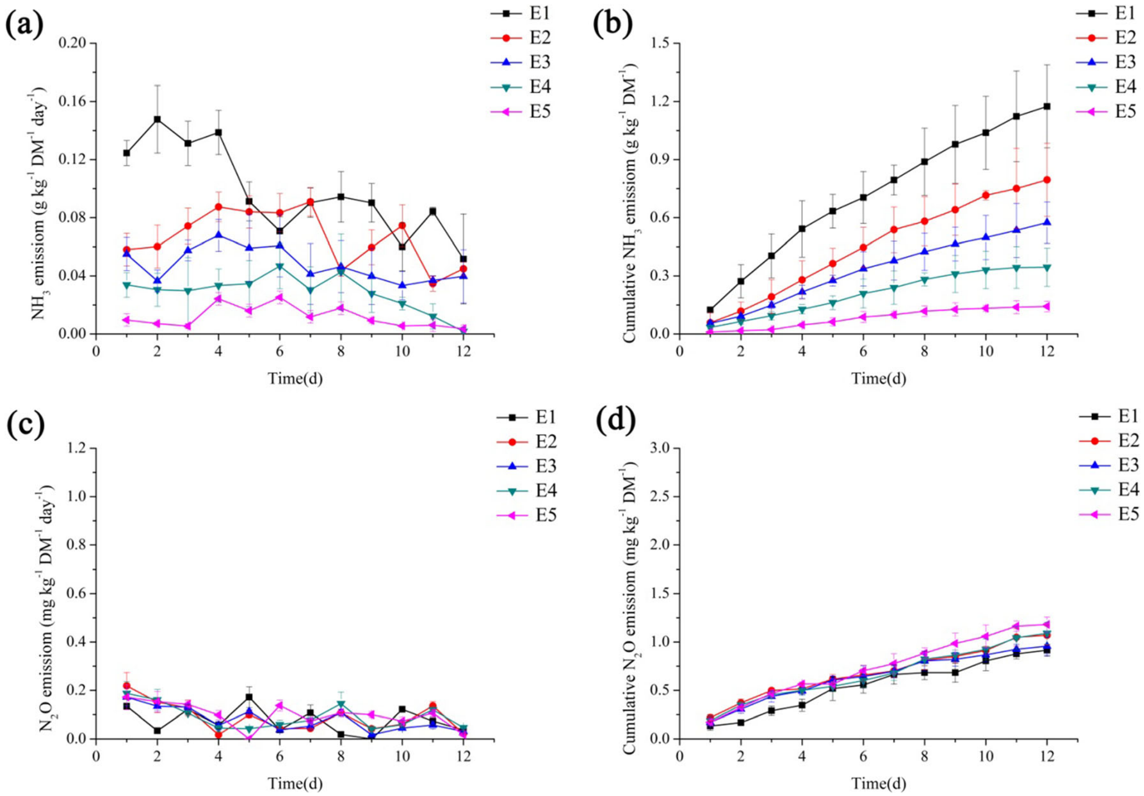


Fig. 3 Changes of NH_3 (**a**), cumulative NH_3 (**b**), N_2O (**c**), and cumulative N_2O (**d**) emissions during BSFL bioconversion process

Table 4 Carbon and nitrogen balance in BSFL bio-treatments

Treatments	Carbon balance[a] (%)						Nitrogen balance[b] (%)					
	CO_2-C	CH_4-C	Other C loss	Total C loss	Larva-C	Residue-C	NH_3-N	N_2O-N	Other N loss	Total N loss	Larva-N	Residue-N
E1	9.89 ± 1.31	0.19 ± 0.06	5.29 ± 0.58	15.37 ± 2.37	5.92 ± 0.26	78.71 ± 3.27	5.32 ± 1.06	< 0.01	11.47 ± 2.20	16.79 ± 3.22	17.45 ± 0.58	65.76 ± 4.47
E2	11.01 ± 2.13	0.19 ± 0.04	11.03 ± 1.64	22.23 ± 3.78	6.61 ± 0.82	71.16 ± 4.08	4.01 ± 0.67	< 0.01	14.67 ± 2.86	18.68 ± 3.74	19.83 ± 0.77	61.49 ± 5.01
E3	12.31 ± 2.76	0.11 ± 0.02	10.98 ± 1.79	23.40 ± 3.33	6.58 ± 0.76	70.02 ± 3.16	3.21 ± 0.76	< 0.01	12.46 ± 2.48	15.67 ± 4.04	22.57 ± 1.21	61.76 ± 3.27
E4	11.47 ± 2.26	0.03 ± 0.01	8.06 ± 1.03	19.56 ± 2.84	4.96 ± 0.37	75.48 ± 4.18	2.09 ± 0.74	< 0.01	6.84 ± 0.93	8.93 ± 1.16	23.73 ± 0.26	67.34 ± 3.76
E5	9.19 ± 1.58	0.01 ± 0.00	17.38 ± 2.76	26.58 ± 4.04	4.17 ± 0.26	69.25 ± 4.19	0.97 ± 0.08	< 0.01	9.34 ± 1.25	10.31 ± 2.34	22.56 ± 1.09	67.13 ± 3.29

[a] Percentages of initial total organic carbon of raw materials on dry weight basis

[b] Percentages of initial total nitrogen of raw materials on dry weight basis

organic waste has a significant advantage over earthworms in reducing N_2O emissions.

GHG emissions and C and N balance

Total GHG emissions are shown in Table 3. Although CO_2 is one of the GHGs and originates from the biodegradation process, its global warming potential is the least (IPCC 2013; Sánchez et al. 2015). Hence, only CH_4 and N_2O were taken into account in calculating the total GHG emissions in this study. Taking the global warming potential of CO_2 as 1, that of CH_4 and N_2O was 34 and 298, respectively (IPCC 2013). In this study, CH_4 is a major contributor to GHG released from the system, which provided over 85% of total GHG emissions in all treatments. The results suggest that with increasing C/N ratio of the substrate, the total GHG emissions in BSFL biotransformation was decreased. The total GHG ranged from 3.12 to 26.52 g CO_2-eq/kg, which does not exceed 10% of those from conventional composting. However, total GHG emission in this study was higher than those in previous BSFL treatment food waste studies (Mertenat et al. 2019; Ermolaev et al. 2019). The inconsistency between these studies may be ascribed to differences in the nature of raw materials. It is thus advised that measures are taken in the early stage of BSFL conversion process to mitigate CH_4 generation.

The balance of C and N is presented in Table 4. The study is perhaps the first to evaluate the flow direction of C and N in BSFL bio-waste conversion process using C/N ratio as a variable. CO_2 emissions were the major contributor to TOC losses (9.19–12.31%), resulting from BSFL and microbial metabolism in a natural process. The CH_4 emission C loss was much lower than CO_2 emissions C loss, which was only accounting for 0.01–0.19% of initial TOC. The TOC losses of all treatments ranged from 15.37 to 26.58% of initial TOC of raw materials. Perednia et al. (2017) observed a higher level (28.54%) of CO_2-C and CH_4-C loss when BSFL were used to treat rabbit manure. The main routes for the loss of total gaseous N were in the form of NH_3 (0.97–5.32%), whereas initial TN of raw material lost is negligible due to N_2O emission ($< 0.01\%$). Although NH_3 loss was enhanced at lower substrate of C/N ratio, the degree of enhancement was relatively lower compared with the TN in the raw materials. For all the treatments, the loss of TN ranged from 8.93 to 18.68% of the initial TN. The results of total C and N loss showed that high C/N ratio could reduce N loss but simultaneously increase C loss.

A diagram of the BSFL treatment system and the potential application of BSFL and residues in diverse fields are as shown in Fig. 4. In general, the quality of BSFL and residues relies on the nutritive contents of feedstock. In this study, the

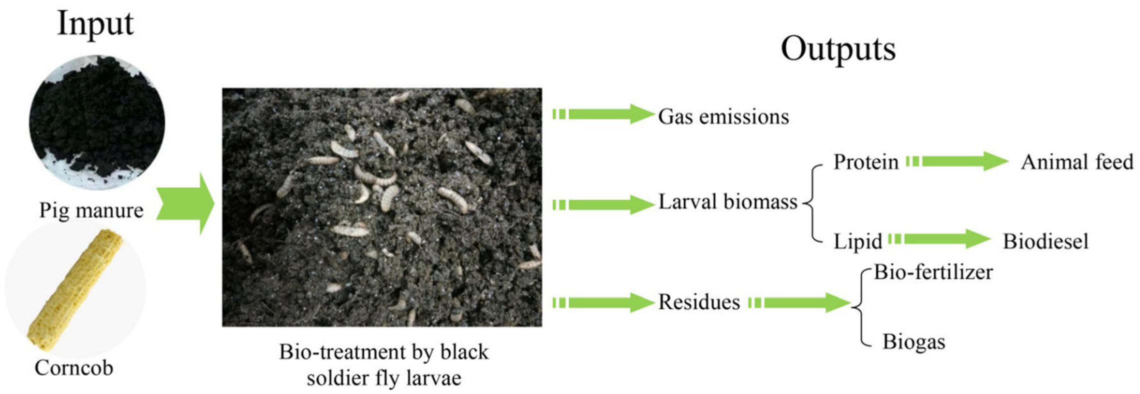


Fig. 4 Diagram of the BSFL treatment system and application of BSFL and residues in diverse fields

C and N in the substrate would be emitted into the atmosphere by microbial decomposition to cause environmental pollution without any treatment, while BSFL can recycle C and N in the substrate into stable and readily harvestable biomass, such as fat and protein. BSFL recycled 4.17–6.61% of C from organic waste into biomass. Although there was a significant difference (F = 7.677, $P = 0.04$, df = 14) in C recovery in BSFL among treatments E1–E5, probably due to the fact that the initial C in the feedstock was much higher than recovered by the larvae that resulted in the observed difference, which is not obvious. It is reported that TN over 90% in the form of organic N is first transformed into NH+ 4-N and subsequently easily volatilized as NH_3 in the composting process (Chang et al. 2019). In this study, between 17.45 and 23.73% of initial TN could be recycled from organic waste to BSFL biomass of high value-added insect protein instead of being mineralized it into inorganic N by microbes and then emitted into the atmosphere. The results revealed high N recovery in larvae and residues, which agrees with the findings of Ermolaev et al. (2019). Currently, the commercial value of animal feeds primarily depends on its protein content (Lim et al. 2019). Hence, it is expected that enhancing yield of BSFL protein which correlates with the N content can lead to a lower price and thus being more competitive than fishmeal. Furthermore, the residues still contain 69.25–78.71% of C and 61.49–67.34% of N. These valuable nutrients potentially can further be converted to bioenergy (Win et al. 2018) or used as high-quality bio-fertilizer (Rehman et al. 2017b).

In terms of total GHG emissions, the addition of corncob to increase C/N ratio is a viable way to enable BSFL cope with mixtures of pig manure and corncob. From a practical application point of view, however, it is difficult to upscale to larger systems using a mixture with a high proportion of corncob, because the yield and environmental impact of pig manure are much higher than that of corncob, especially in China. Thus, based on the conversion of C and N from waste into BSFL biomass, mitigation of GHG emissions, and larval output, a C/N ratio of 25 is the most favorable condition for BSFL bioconversion of mixtures of pig manure and corncob.

Conclusions

The appropriate C/N ratio of BSFL feedstock comprising of a mixture of pig manure and corncobs can provide balanced nutrition and suitable properties of substrate and thus can lead to increased larval biomass, keep the environmental footprint low, and improve N recovery from waste. BSFL bioconversion of organic waste could reduce CH_4, N_2O, and NH_3 emissions compared with conventional composting methods. Various analyses (C and N recovery, GHG emissions, larval biomass output, and practical application potential) indicated that C/N ratio of 25 is recommended for the bioconversion of mixtures of pig manure and corncob by BSFL. Overall, the findings of this study may contribute to improving the environmental and economic benefits of BSFL conversion system. Further studies on a large-scale production of biodiesel and animal feeds using BSFL should consider feedstock components that will enhance simultaneous C and N recovery.

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Compliance with ethical standards

Conflict of interest All the authors declare that they do not have any possible conflicts of interest.

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