



Vermicomposting as a technology for reducing nitrogen losses and greenhouse gas emissions from small-scale composting



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ABSTRACT

Thermophilic composting produces a significant amount of greenhouse gases. The objectives of this study were (i) to evaluate the effectiveness of vermicomposting to reduce nitrogen losses and greenhouse gases emissions compared to thermophilic composting, and (ii) to determine the effect of different variables (*i.e.* carbon:nitrogen ratio, earthworm density, moisture content and carbon quality) on greenhouse gases emissions and earthworm growth during vermicomposting. The results showed that vermicomposting significantly reduced nitrogen loss by 10–20% compared to thermophilic composting. Vermicomposting decreased nitrous oxide emissions by 25–36% and methane emissions by 22–26%. A higher earthworm density increased carbon dioxide emissions by 3–14%, but decreased methane emissions by 10–35%. Earthworm density had a marginal effect on nitrous oxide emissions. Vermicomposting decreased nitrous oxide emissions by 40% with higher moisture and by 23% with lower moisture. Vermicomposting also decreased methane emissions by 32% and 16% with higher and lower moistures respectively. This study showed that the addition of labile carbon sources increased carbon dioxide and methane emissions and earthworm growth, but did not affect nitrous oxide emissions. In conclusion, vermicomposting is effective at reducing nitrogen losses and greenhouse gas emissions from composting. Therefore, vermicomposting could represent an option for reducing greenhouse gas emissions from composting, particularly in developing countries where the existing technical solutions are expensive and difficult to implement.

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1. Introduction

Municipal solid waste management has become a matter of global concern due to rapid urban population growth and the high costs associated with waste management (Marshall and Farahbakhsh, 2013). Studies have shown that biodegradable materials constitute over half of municipal solid waste (Taeporamaysamai and Ratanatamskul, 2016), with their results indicating that bioconversion of municipal solid waste into soil amendments (compost) is a viable option for sustainable waste management. High compost demand by urban farmers (Nigussie et al., 2015) also emphasises the need for municipal waste composting.

Thermophilic composting and vermicomposting are effective

techniques commonly used to convert biodegradable waste into soil amendments. Thermophilic composting is a composting process at high temperatures (>45 °C), but vermicomposting is a mesophilic (<30 °C) process that involves earthworms and associated microorganisms in decomposing and stabilising organic materials (Lim et al., 2016). Major similarities and differences between thermophilic composting and vermicomposting are summarised by Lim et al. (2016).

Compost, particularly in many (sub-) tropical countries, contains low amounts of plant nutrients (especially nitrogen) and hence is unable to meet crop nutrient requirements. Nitrogen (N) is lost during composting through ammonia (NH₃), nitrogen oxides (NO_x), nitrous oxide (N₂O) or dinitrogen (N₂) (Awasthi et al., 2016; Chan et al., 2016). The total N loss may account for 40–70% of initial N (Vu et al., 2015). These N losses reduce the fertilising value of compost and contribute to environmental problems. Furthermore, significant greenhouse gas (GHG) emissions (methane and nitrous oxide) from thermophilic composting are widely reported (Lim et al., 2016). Anoxic hotspots in the compost piles produce

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methane (CH₄), while nitrification in aerobic microsites and denitrification in anaerobic microsites are responsible for N₂O production (Chan et al., 2011).

Moisture, temperature, pH, ammonium concentration and substrate C:N ratio are the key factors that control N losses and GHG emissions from thermophilic composting (Chowdhury et al., 2014; Santos et al., 2016). Temperatures above 45 °C increase NH₃ volatilisation and a high pH (about 9; pKa = 9.25) shifts the NH₄⁺ ↔ NH₃ equilibrium to ammonia (Chowdhury et al., 2014). High temperatures also inhibit the nitrification process and thereby increase ammonia volatilisation. About 36–70% of total N losses have been observed during the active stage of thermophilic composting (Chowdhury et al., 2014). High oxygen consumption during the active stage leads to anoxic microsites, which are centres of GHG production. Similarly, high activities of methanogens (Ermolaev et al., 2014) and subsequent CH₄ emissions (Vu et al., 2015) have been reported during the thermophilic stage. Controlling pH through the use of additives (Awasthi et al., 2016), lowering mineralisable C and/or increasing the substrate C:N ratio by the addition of a bulking agent (Santos et al., 2016; Wang et al., 2014) reduce N losses and GHG emissions. However, these practices are difficult to scale up to large-scale systems, particularly in non-mechanised agricultural systems. Furthermore, it is a challenge to implement these practices under field conditions where there is shortage of labour. Vermicomposting, a mesophilic (<30 °C) composting technique, might therefore be a good alternative for reducing N losses and GHG emissions.

Earthworms improve air circulation in the compost pile through continuous turning of substrate, thereby maintaining aerobic conditions. They also affect N transformations such as mineralisation, volatilisation, nitrification and denitrification through their interaction with associated microbes (Velasco-Velasco et al., 2011; Wang et al., 2014). Hence, earthworms could influence N losses and GHG emissions during composting. Little is known about N losses and GHG emissions during vermicomposting (Lim et al., 2016), and the literature is contradictory. For instance, Wang et al. (2014) found that earthworms reduce GHG emissions during vermicomposting. Other studies have shown that earthworms induce N₂O emissions (Hobson et al., 2005) because their gut contains denitrifying bacteria. Hence, the effects of earthworms on N loss and GHG emissions require clarification. Moreover, previous studies have not considered variables such as mineral N concentration, C quality, moisture content and earthworm density, which could influence N loss and GHG emissions from vermicomposting. The mineral nitrogen and available C contents influence the denitrification processes in the earthworm gut (Lubbers et al., 2013). It is therefore essential to understand the effect of earthworms on N₂O emissions from materials with different substrate quality (i.e. C:N ratio, labile C sources such as glucose). Moisture is another factor that determines the magnitude of GHG emissions (Jiang et al., 2011), since a higher moisture content increases anaerobic patches in the compost pile that result in greater GHG emissions. However, there are no data on the effect of earthworms on GHG emissions with varying moisture contents. Studies have shown that a higher earthworm density increases N transformation (Ndegwa et al., 2000). Earthworm density could also influence the mixing of substrates – aeration – and subsequently influence GHG emissions. Hence, the objectives of the present study were: (i) to evaluate the effectiveness of vermicomposting in reducing N losses and GHG emissions compared with thermophilic composting, and (ii) to determine the effect of substrate C:N ratio, earthworm density, carbon quality and moisture on GHG emissions from vermicomposting. It was hypothesised that (i) vermicomposting decreases N losses and N₂O and CH₄ emissions compared to thermophilic composting, (ii) higher earthworm density reduces

CH₄ and N₂O emissions from vermicomposting, and (iii) the addition of labile carbon (glucose) increases CH₄ and N₂O emissions from vermicomposting, with the effect of glucose being greater at higher moisture content of the compost.

2. Materials and methods

2.1. Experimental set-up

Three experiments were each carried out for 45 days each. The first experiment (Experiment I) was undertaken to quantify total N loss and GHG emissions from two different composting methods as affected by the substrate C:N ratio. The composting experiment was carried out in small-scale reactors at the University of Copenhagen, Denmark using vegetable waste. Details of the reactors are described in Section 2.2 and illustrated in Fig. 1. Two composting methods, namely thermophilic composting and vermicomposting, and two C:N ratios (24 and 30) were used (Table 1). The experimental design was a complete random design arranged in a 2 × 2 factorial arrangement, and the treatments were replicated three times. The reactors were rotated every week to reduce the effect of external variables on the composting processes. The two C:N ratios were chosen after a pilot study showed that C:N ratios between 24 and 30 were optimal for both composting methods with the substrates used. Only a small number of earthworms survived (<43%) when the C:N ratio was below 24, possibly due to the high moisture content in vegetable waste (87–91%) creating anaerobic conditions in the reactors. Thermophilic conditions (>45 °C) could not be achieved when the substrate C:N ratio was above 30.

The second experiment (Experiment II) was undertaken to determine the effect of earthworm abundance on GHG emissions from vermicomposting. Four different substrates and two earthworm densities (i.e. 1 kg earthworm m⁻² and 3 kg earthworm m⁻²) were used. The results were analysed using a complete random design in a 4 × 2 factorial arrangement.

The third experiment (Experiment III) was conducted to test whether easily degradable C substrate (glucose) reduces N₂O emissions from vermicomposting by immobilisation of mineral nitrogen and/or increases CH₄ emissions, since labile C pools could be used by methanogenic microorganisms. Consequently, two levels of available C (i.e. with and without the addition of glucose) and two moisture levels (i.e. 75% and 85%) in the presence or absence of earthworms were applied. The experimental design was a complete random design arranged as a 2 × 2 × 2 three-way factorial experiment with three replicates. The details of these experiments are presented in Sections 2.2, 2.3 and 2.4.

2.2. Experiment I

2.2.1. Composting materials

Vegetable waste, a representative household waste in many countries, was collected from a food processing plant in Denmark. The vegetable waste consisted of a mixture of carrot, cabbage, lettuce and red beet. It contained 87–91% moisture, total carbon of 400–410 g kg⁻¹ dry matter, total nitrogen of 15–35 g kg⁻¹ dry matter, 1.4–2.6 g NH₄⁺ kg⁻¹ dry matter and 0.2–0.4 g NO₃⁻ kg⁻¹ dry matter. The variation in nitrogen content between the different components was used to vary the C:N ratio, with minimum changes in other properties such as moisture content. Dry barley straw was chopped to <2 cm pieces and mixed thoroughly with the vegetable waste in two ratios, namely 5:1 and 10:1 (vegetable:straw), to produce the intended C:N ratios. The barley straw had 5% moisture content, total carbon content of 441 g kg⁻¹ dry matter and total nitrogen content of 9 g kg⁻¹ dry matter. The chemical properties of substrates used for the experiments are presented in Table 1.

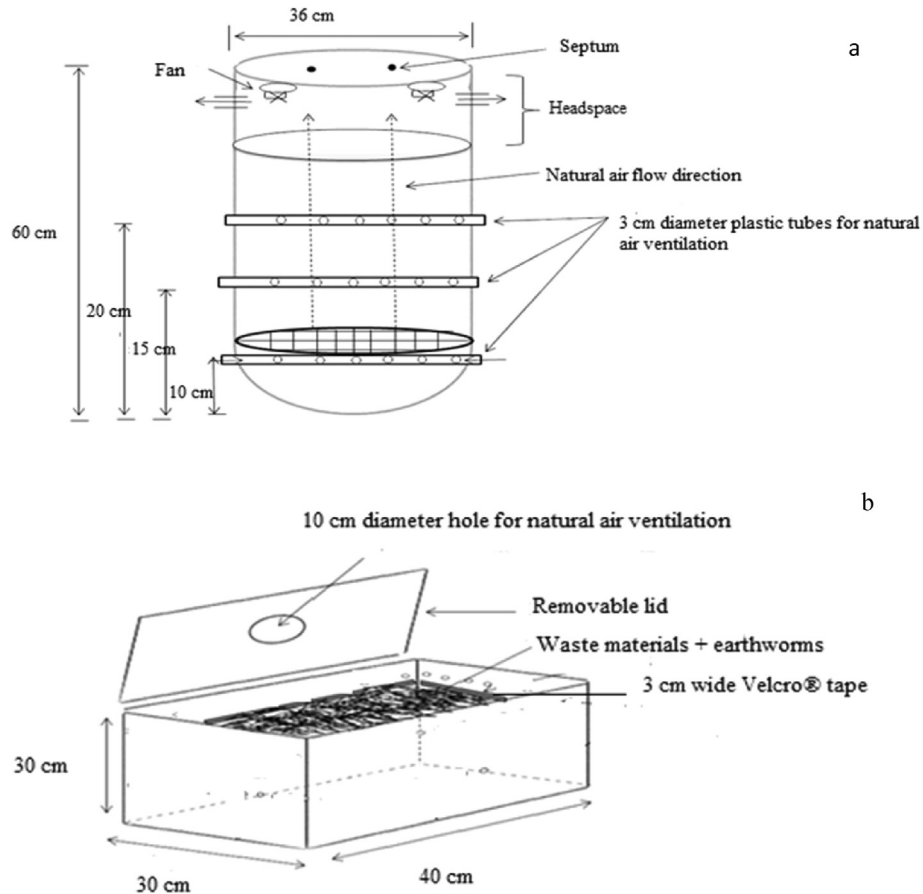


Fig. 1. The reactor design: (a) thermophilic composting, (b) vermicomposting. The reactor for thermophilic composting was adopted from Vu et al. (2014).

Table 1

Selected chemical properties of the starting materials used for the composting experiment.

Treatment code	Mixing ratio ^a (vegetable: straw)	Total C	Total N	C:N	NH ₄ ⁺	NO ₃ ⁻	pH
Experiment I							
		g kg ⁻¹ DM			mg kg ⁻¹ DM		
Mix A	5:1	443.8	14.5	30.6	6220.5	91.5	8.4
Mix B	10:1	425.9	17.9	23.8	8160.5	119.7	8.2
Experiment II (vegetable: cattle manure: straw)							
Mix A	5:0:1	443.8	14.5	30.6	6220.5	91.5	8.4
Mix B	10:0:1	425.9	17.9	23.8	8160.5	119.7	8.2
Mix C	4:1:0.25	391.4	19.5	20.1	1038.8	269.0	7.2
Mix D	3:1:0	382.1	26.6	14.2	1440.3	478.0	6.6

^a Wet basis; C = carbon; N = nitrogen; DM = dry matter.

2.2.2. Thermophilic composting

The thermophilic composts were prepared in 60-L polyethylene compost reactors. The reactors were insulated with a 5-cm foam layer in order to minimise heat loss. Perforated plastic tubes 3 cm in diameter were positioned at 10, 15 and 20 cm height to ensure natural air ventilation in the reactor. Two plastic tubes (3 cm in diameter) were also connected to the reactor headspace to circulate air in the reactor (Fig. 1a). Five kg (wet basis) substrate was then applied to the reactors and the moisture content was adjusted to 50–60% by spraying water. The mixtures were turned every two days in the first week and once a week until the end of the experiment.

2.2.3. Vermicomposting

The same substrates were used for vermicomposting, but in 36-L polyethylene containers (30 cm width x 40 cm length x 30 cm height) (Fig. 1b). Different-sized containers were used because the containers for the thermophilic composting also included the headspace (approximately 24 L). The containers were otherwise filled to the same degree in both composting methods. A 10 cm diameter hole was also made in the lids of the containers to ensure air circulation in the vermicompost bin. A 3-cm-wide Velcro[®] tape was glued to the top of the container to prevent the escape of earthworms.

Prior to earthworm addition, one kg (wet basis) straw pellets

(<1 cm) were added for bedding material. The straw pellets used had a C:N ratio of 62.0 (445 g C and 7.2 g N kg⁻¹ dry matter) and a pH value of 7.20. Pre-decomposed cattle manure (273 g C and 21.2 g N kg⁻¹ dry matter) was also mixed with the straw pellets to create habitats for the earthworms. The same amount of straw pellets and pre-decomposed cattle manure was added to the thermophilic composting reactors in order to provide the same substrate.

Adult *Eisenia fetida* were used for the experiment. The earthworms were kept on moist paper at 15 °C for 24 h to empty their guts prior to being weighed and put in the vermicomposting bins. The earthworms were then added to each vermicomposting reactor at the stocking rate of 3 kg earthworm m⁻² (i.e. equivalent to 360 g earthworms per container). A continuous-flow vermicomposting system was used because it is the most commonly used vermicomposting method in small-scale systems (Abbasi et al., 2015). This means that the bedding materials were put in first, then inoculated with earthworms, and finally covered continuously with 10–15 cm layers of waste. The first half (i.e. 2.5 kg of substrate) was added at the start of the experiment, and the other half added after two weeks. The moisture content in the vermicompost reactors was kept at 80–85% by occasional spraying of water. The temperatures in the thermophilic and vermicompost reactors were recorded every 2 h using a temperature data logger (Tinytag View 2-TV-4020, United Kingdom).

2.3. Experiment II

Four different substrates were prepared from vegetable waste, pre-decomposed cattle manure (273 g C and 21.2 g N kg⁻¹ dry matter) and barley straw (Table 1). The vermicomposting was prepared as described in Experiment I. In this study, however, adult *Eisenia fetida* were added at two stocking densities, namely 1 kg m⁻² and 3 kg m⁻², equivalent to 120 g and 360 g earthworms per container respectively.

2.4. Experiment III

A mixture of vegetable waste and pre-decomposed cattle manure was selected for this experiment. Glucose (i.e. labile C source) was added at the rate of 5% (dry weight base). Two moisture contents (75% and 85%) were used to determine the relationship between moisture and glucose on GHG emissions during vermicomposting. The earthworms were added at a stocking rate of 3 kg m⁻². Treatments without the addition of glucose and earthworms were used as controls. The treatments without earthworms were mesophilic (i.e. the temperature was <45 °C) because the mixtures were regularly turned manually. Hence, the treatments without earthworms represented small-scale household aerobic composting (Chan et al., 2011).

2.5. Gas sampling

The gas samples were collected every two days for the first week after the addition of substrates and then twice a week until the end of the experiments. All ventilation tubes on the thermophilic reactors (Fig. 1a) were air-tightened with rubber plugs at the time of gas sampling. The vermicompost reactors were placed in an airtight polyethylene container (40 cm width x 50 cm length x 40 cm height) during gas sampling. Two mini fans (12 V) were installed in all the reactors to ensure homogenous distribution of air in the headspace during sampling. Gas samples were taken using a 60 ml air-tight syringe at five time points (at 0, 20, 40, 60 and 80 min) and injected into pre-evacuated 3 ml screw-capped Exetainer[®] vials. The linearity assumption was checked once a month by collecting gas

samples at 0, 20, 40, 60, 80 and 100 min. The gas samples were analysed using gas chromatography (Bruker 450-GC, 2011; United Kingdom). The CO₂ concentration was measured using a thermal conductivity detector (TCD), whereas N₂O and CH₄ were measured using an electron capture detector (ECD) and flame ionisation detector (FID) respectively. The emission rate in mg kg⁻¹ initial dry matter day⁻¹ was calculated using Equation (1) (Czepiel et al., 1996):

$$\text{Emission rate} = \left(\frac{\Delta C}{\Delta t}\right) \times \left(\frac{V}{A}\right) \times \left(\frac{M}{Vs}\right) \times \left(\frac{P}{Po}\right) \times \left(\frac{273}{T}\right) \times 24 \text{ hr} \times \left(\frac{A}{W}\right) \quad (1)$$

where ΔC is the change in concentration of gas (ppm) at time interval Δt (hour), V and A are the headspace volume (litre) and reactor surface area (m²) respectively, M is the molecular mass of the gas of interest (44, 16 and 44 g for CO₂, CH₄ and N₂O respectively), V_s is the volume occupied by 1 mol of a gas at standard temperature and pressure (22.4 L), P is the barometric pressure (bar), P_o is the standard pressure (i.e. 1.013 bar), T is the temperature inside the chamber during the deployment time in Kelvin, and W is the initial dry mass of the composting material (kg).

The cumulative emissions were calculated using the trapezoid formula (Equation (2)) (Ly et al., 2013):

$$A_{t(ab)} = \frac{(t_b - t_a) \cdot (F_{ta} + F_{tb})}{2} \quad (2)$$

where $A_{t(ab)}$ is the cumulative emission between the measurement days (between t_a and t_b), t_a and t_b are the dates of the two measurements, and F_{ta} and F_{tb} are the gas fluxes at the two measurement dates.

Therefore, the total cumulative emission was calculated as the sum of cumulative emissions on each day using Equation (3):

$$\text{Total cumulative emission} = \sum A_{t(ab)} \quad (3)$$

The global warming potential (CO₂-equivalents) of each treatment was then calculated by multiplying total cumulative CO₂, CH₄ and N₂O emissions by 1, 34 and 298 respectively (IPCC, 2013). Global warming potential was calculated for all three greenhouse gases and for the combination of CH₄ and N₂O.

2.6. Chemical analyses

Compost samples were collected for the analyses of pH, total carbon, total nitrogen, NO₃⁻ and NH₄⁺. Half of the samples were oven dried at 40 °C and milled to determine pH, total carbon and total nitrogen, while the other half was stored in a freezer at -18 °C for NH₄⁺ and NO₃⁻ measurements. The pH was measured in water (1:10 ratio, w/v). Total carbon and total nitrogen were determined using isotope ratio mass spectroscopy (IR-MS). Compost samples were mixed with 1 M KCl in a 1:100 compost:solution ratio (w/v) and shaken for 1 h. The extracts were then analysed for NH₄⁺ and NO₃⁻ using a flow injection analyser (FIA star[™] 5000 analyser, Denmark).

The final and initial total C and total N contents were used to calculate the C and N mass balances as:

$$\text{Relative Mass Balance} = \frac{(Q_i C_i - Q_f C_f)}{Q_i C_i} \times 100 \quad (4)$$

where Q_i and Q_f are total dry weight at the beginning and end of the experiments respectively, and C_i and C_f are carbon or nitrogen concentrations at the beginning and end of the experiments respectively.

2.7. Statistical analyses

The statistical analyses were carried out using the SAS version 9.3 statistical package. Analysis of variance (ANOVA) was used to test the significant sources of variation, and subsequent Tukey test was used to compare the treatment means if the factors' effect was significant at $P < 0.05$. Treatments in Experiments I and II were arranged in a complete random design and two-way ANOVA was performed accordingly. Three-way ANOVA was used in Experiment III. The assumptions of ANOVA were checked before data analysis.

3. Results

3.1. Comparison of thermophilic compost and vermicompost (Experiment I)

3.1.1. GHG emissions

Total cumulative CO_2 varied by composting method ($P < 0.01$), C:N ratio ($P < 0.001$) and their interaction ($P < 0.001$) (Fig. 2). Vermicomposting increased total cumulative CO_2 emissions compared with thermophilic composting. The composting method and C:N ratio affected total cumulative CH_4 emissions ($P < 0.05$ and

$P = 0.04$ respectively). Vermicomposting decreased CH_4 emissions by 22% from high C:N and 26% from low C:N compared with thermophilic composting. The higher C:N ratio (addition of straw) decreased CH_4 emissions by 13–18% ($P = 0.04$). As with CH_4 emissions, the composting method and C:N substrate affected cumulative N_2O emissions ($P = 0.05$ and $P = 0.001$ respectively). Vermicomposting decreased N_2O emissions by 36% from low C:N substrate and by 25% from high C:N material.

The total GHG budget for both composting methods and C:N ratio is presented in Fig. 2. Total GHG emissions varied between composting method ($P < 0.001$) and substrate C:N ($P < 0.05$) if CO_2 emissions were excluded from the total GHG budget. Accordingly, vermicomposting decreased total GHG emissions by 20–30% relative to thermophilic composting (Fig. 2d). Vermicomposting had a higher total GHG budget than thermophilic composting ($P < 0.001$) when CO_2 was accounted for in the total GHG budget (Fig. 2e). The addition of straw reduced total GHG emissions ($P < 0.05$) in both scenarios (including and/or excluding CO_2 from the GHG budget).

3.1.2. Nitrogen and carbon balance

Total N loss varied between composting methods ($P = 0.02$) and C:N ratios ($P = 0.01$). Vermicomposting reduced total N loss by 10%

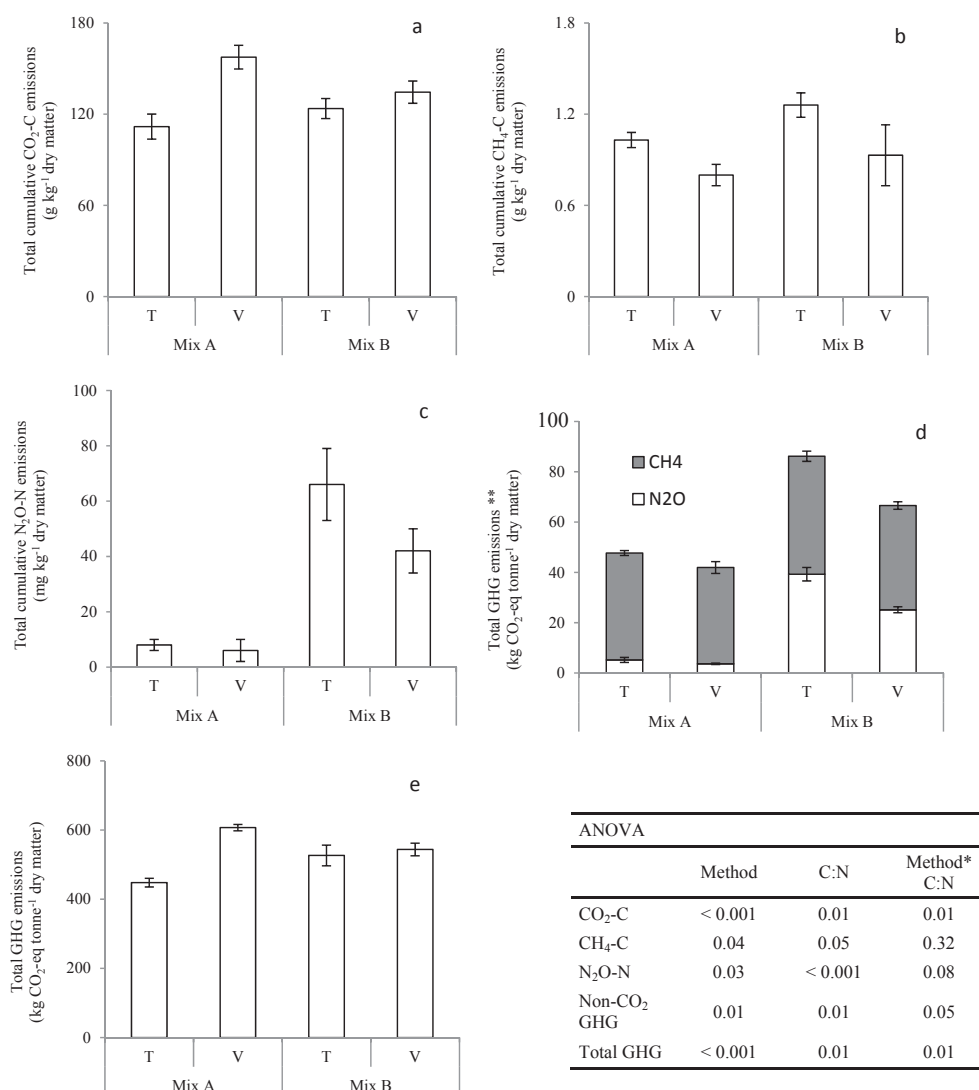


Fig. 2. Total cumulative GHG emissions after 45 days of composting: (a) CO_2 -C, (b) CH_4 -C, (c) N_2O -N, (d) total non- CO_2 GHG emissions, (e) total GHG emission including CO_2 . T = Thermophilic composting; V = vermicomposting; ** indicates that CO_2 emission is not included; bars indicate the standard error of the means ($n = 3$).

from the high C:N substrate and by 20% from the low C:N substrate (Table 2). Total C varied between composting methods ($P = 0.001$) and substrate C:N ($P = 0.001$) (Table 2). Vermicomposting increased total C loss irrespective of the substrate C:N (Table 2). C loss varied between 44% and 46% of initial C content after vermicomposting and 40–43% of initial C content after thermophilic composting.

3.2. Effect of earthworm density on GHG emissions (Experiment II)

Analysis of variance showed that higher earthworm density increased CO_2 emissions in all substrates ($P = 0.04$). Higher earthworm density decreased CH_4 emissions by 12–36% ($P = 0.011$) and had a marginal effect on N_2O emissions ($P = 0.05$). The addition of more straw reduced the earthworm density effect on N_2O emissions (Fig. 3). Both CH_4 and N_2O emissions varied significantly between substrates ($P < 0.001$). Vermicomposting of vegetable waste (i.e. mixtures A and B) produced the highest amounts of CO_2 and CH_4 compared with the substrates also containing pre-decomposed cattle manure (i.e. mixtures C and D) (Fig. 3). The highest N_2O emissions were found in substrates containing pre-decomposed manure compared with vegetable wastes. Higher earthworm density did not affect the total GHG budget if CO_2 was excluded from total GHG emissions (Fig. 3d). However, higher earthworm density marginally influenced total GHG emissions ($P = 0.08$) when CO_2 was included (Fig. 3e).

3.3. Effect of C quality and moisture on GHG emissions (Experiment III)

The addition of glucose increased the total cumulative CO_2 and CH_4 emissions from vermicompost ($P < 0.001$ and $P < 0.001$ respectively) (Fig. 4). The glucose effect on N_2O emissions was non-significant ($P = 0.13$). The higher moisture content increased total cumulative CH_4 emissions ($P < 0.001$) and N_2O emissions ($P = 0.01$). The effect of moisture on CH_4 and N_2O , however, was only pronounced in the treatments without earthworms (Fig. 4). Earthworm treatments decreased CH_4 emissions by 9–53% ($P < 0.001$) and N_2O emissions by 16–59% ($P < 0.001$) compared with non-earthworm treatments. Earthworms decreased N_2O emissions by 40% with higher moisture and by 23% with lower moisture. Earthworms also decreased CH_4 emissions by 32% and 16% with higher and lower moisture respectively.

3.4. Change in chemical properties

The composting method and C:N ratio affected C and N concentration in the end product ($P < 0.01$ and $P < 0.001$ respectively).

The C concentration was higher in thermophilic composting than in vermicomposting (Table 3). Vermicomposting resulted in a higher total nitrogen concentration than thermophilic composting for all substrates. Total N concentration increased by 26–33% after vermicomposting, but only by 18–20% after thermophilic composting. The composting method did not affect NH_4^+ concentration ($P = 0.23$), however NH_4^+ concentration varied significantly between substrates ($P < 0.001$) (Table 3). NO_3^- concentration varied between the composting methods ($P = 0.03$) and C:N ratio ($P < 0.001$). Vermicomposting increased NO_3^- concentration by 14% and 33% with the high and low C:N ratio respectively compared with thermophilic composting (Table 3). During Experiment III, vermicomposting also increased NO_3^- concentration by 164–401% ($P < 0.0001$) compared with non-earthworm treatments (Supplementary Fig. 1). Earthworm density, however, did not affect NH_4^+ and NO_3^- concentrations.

3.5. Earthworm biomass

The earthworm biomass increased in all vermicomposting experiments (Fig. 5). The relative change in earthworm biomass was significantly affected by substrate C:N ratio ($P < 0.001$), but not by the initial earthworm density ($P = 0.14$). Mixtures containing decomposed cattle manure (i.e. mixtures C and D) increased the earthworm biomass by more than 80%, while the mixtures of vegetable waste and straw (i.e. mixtures A and B) increased the earthworm biomass by just 40%. Higher moisture content increased earthworm biomass by 50–57% ($P < 0.001$) compared with the lower moisture content. The addition of glucose marginally increased the earthworm biomass (Fig. 5).

4. Discussion

4.1. Earthworms and GHG emissions from vermicomposting

4.1.1. Methane and nitrous oxide

The hypothesis that vermicomposting decreases CH_4 and N_2O emissions irrespective of the substrate quality (C:N ratio and presence of a labile C pool) (Figs. 2 and 4) was confirmed. In agreement with these findings, earthworms have been found to decrease CH_4 emissions by > 40% during vermicomposting of manure (Wang et al., 2014) and N_2O emission by > 80% during vermifiltration of pig slurry (Luth et al., 2011). Vermifiltration is a wastewater treatment using epigeic earthworms, which is a different system from that used in the present study. Wang et al. (2014) also observed a 6–27% decrease in N_2O emission during vermicomposting of manure. The present study, however, is the first to evaluate the effectiveness of vermicomposting to reduce

Table 2
Carbon and nitrogen mass balances after 45 days of thermophilic composting and vermicomposting (Mean \pm SEM; $n = 3$).

Treatments	C balance				N balance		
	Total C retained % of initial carbon	C loss ($\text{CO}_2\text{-C}$)	C-loss ($\text{CH}_4\text{-C}$)	Carbon unaccounted	Total N retained % of initial nitrogen	N loss ($\text{N}_2\text{O-N}$)	N losses as ($\text{NH}_3\text{-N}$, N_2 , NO_x)
T_mix A	60.4 \pm 0.8	25.0 \pm 1.2	0.19 \pm 0.02	14.4 \pm 0.7	77.0 \pm 1.9	0.09	22.8 \pm 2.2
T_mix B	57.0 \pm 1.2	30.3 \pm 1.7	0.22 \pm 0.01	12.5 \pm 2.0	69.6 \pm 0.7	0.41	29.8 \pm 1.3
V_mix A	56.5 \pm 0.6	33.6 \pm 0.5	0.14 \pm 0.02	9.7 \pm 1.4	79.4 \pm 1.1	0.04	21.3 \pm 0.9
V_mix B	54.5 \pm 1.0	31.3 \pm 0.5	0.20 \pm 0.01	14.0 \pm 0.6	76.3 \pm 1.9	0.30	22.2 \pm 1.4
ANOVA							
Method	0.01	<0.001	0.03	0.24	0.02	0.03	0.02
Mix	0.01	0.01	0.02	0.81	0.01	<0.001	0.06
Method \times mix	0.30	0.01	0.28	0.10	0.14	0.10	0.24

T = thermophilic composting; V = vermicomposting; mix A = 5:1 (waste:straw ratio); mix B = 10:1 (waste:straw ratio); N = nitrogen; C = carbon.

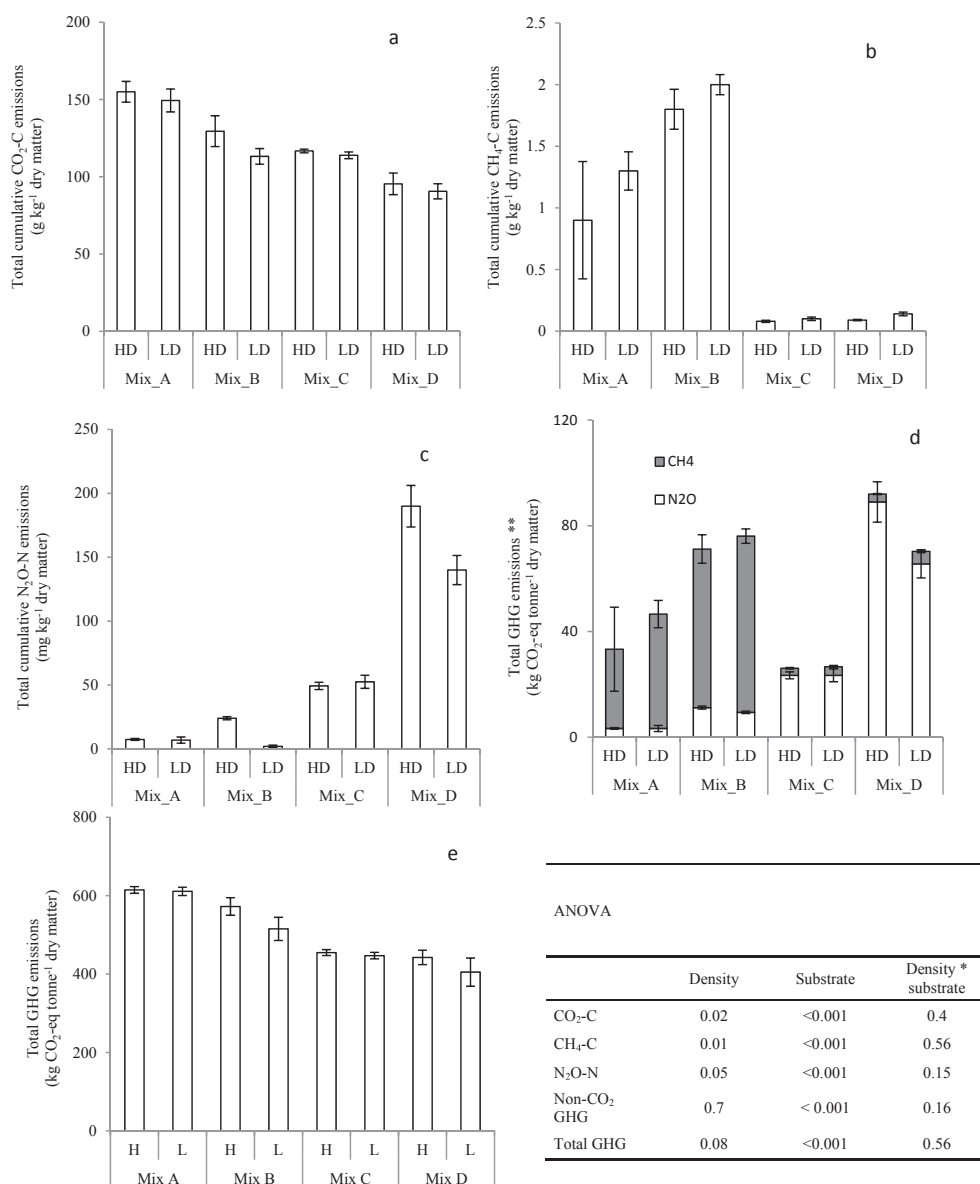


Fig. 3. Total cumulative GHG emissions from different earthworm densities and substrates: (a) CO₂-C emissions, (b) CH₄-C emissions, (c) N₂O-N emissions, (d) total non-CO₂ GHG emissions, (e) total GHG emissions including CO₂. HD = earthworm density at 3 kg m⁻²; LD = earthworm density at 1 kg m⁻²; Mix A = 5:1 (waste:straw ratio); Mix B = 10:1 (waste:straw ratio); DM = dry matter; Mix C = 4:1:1/4 (waste:manure:straw ratio); Mix D = 3:1:0 (waste:manure:straw mixture); ** indicates that CO₂ is not included; bars indicate the standard error of the means (n = 3).

GHG emissions using a wide range of variables (C:N ratio, carbon quality, moisture and earthworm density). Continuous turning of the substrates by earthworms and subsequent higher air circulation explained the decrease in CH₄ and N₂O emissions after vermicomposting, which is also supported by the lower CH₄ emissions with the higher earthworm density, as hypothesised. The higher temperature (45–56 °C) in the thermophilic phase compared with vermicomposting (≤27 °C) (Supplementary Fig. 2) may also contribute to the differences in CH₄ emissions between the composting methods. High microbial activities during the active phase of composting increase temperature and oxygen consumption, and subsequently increase CH₄ production (Chowdhury et al., 2014; Vu et al., 2015). Chowdhury et al. (2014) found a positive correlation between composting temperature and CH₄ emissions. N₂O emission occurs during both nitrification and denitrification processes, implying that excess aeration increases nitrification and

consequently results in high N₂O production (Jiang et al., 2011). Surplus air circulation and denitrification from the earthworms gut could therefore explain the slight increase in N₂O emission at higher earthworm density. The effect of earthworm density on N₂O was less pronounced in straw-amended substrates, suggesting that initial nitrogen content, aeration and other composting parameters might influence the effect of earthworm abundance on N₂O emissions.

Increasing the C:N ratio (addition of straw) reduced CH₄ and N₂O emissions from both composting methods, as hypothesised (Figs. 2 and 3). The addition of straw or bulking agents improves porosity and regulates moisture in a compost pile and thereby reduces CH₄ and N₂O emissions (Santos et al., 2016; Vu et al., 2015). The addition of glucose also increased CH₄ emissions as hypothesised. Higher CH₄ emissions from the addition of glucose to substrates (Fig. 4) implies that labile C pools could easily be used by

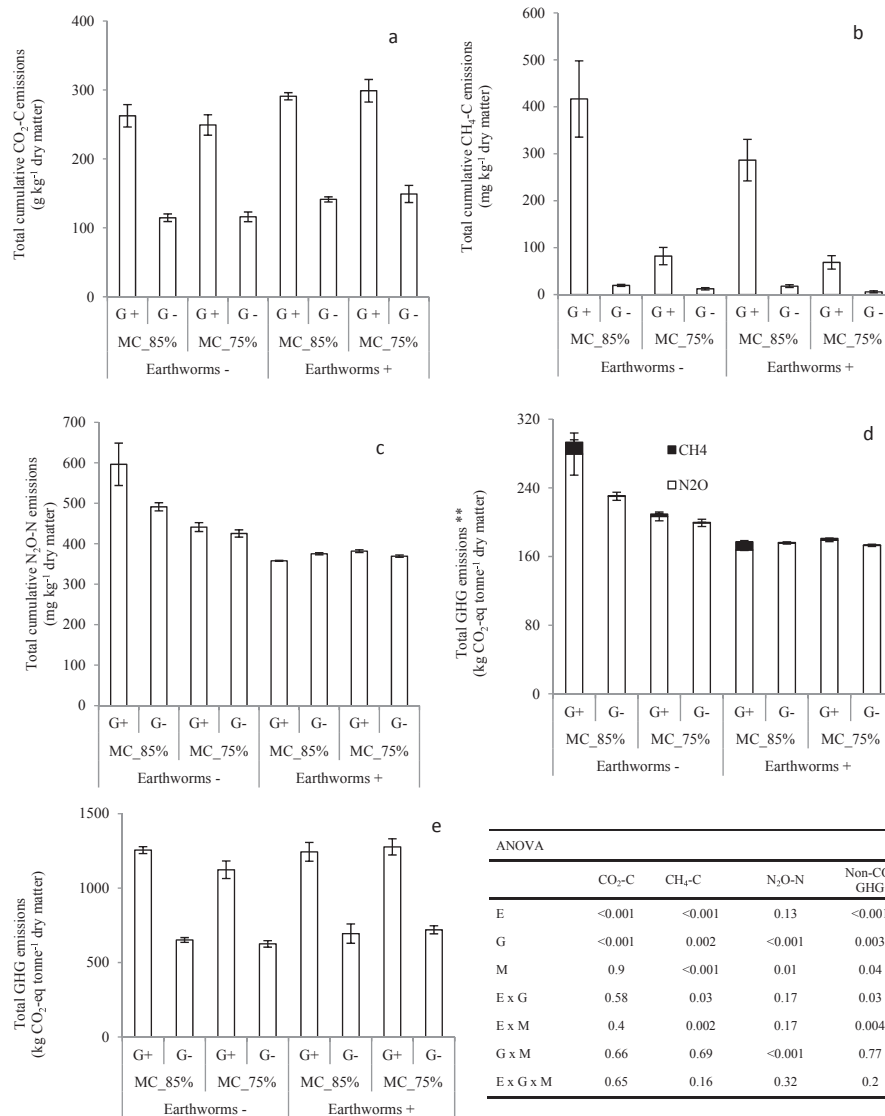


Fig. 4. Total cumulative GHG emissions from different qualities of substrate: (a) CO₂-C emissions, (b) CH₄-C emissions, (c) N₂O-N emissions, (d) total non-CO₂ GHG emissions, (e) total GHG emissions including CO₂. G+ = glucose added; G- = glucose not added; MC_85% = moisture content of 85%; MC_75% = moisture content of 75%; Earthworms - = without earthworms; Earthworms + = earthworms added; ** indicates that CO₂ is not included; bars indicate the standard error of the means (n = 3).

Table 3

Chemical properties of the end products after 45 days of thermophilic composting and vermicomposting.

	Total C	Total N	C:N	NH ₄ ⁺	NO ₃ ⁻	pH
	g kg ⁻¹ DM			mg kg ⁻¹ DM		
T _{mix} A	412.3	17.3	23.8	558.7	327	8.0
T _{Mix} B	383.8	21.1	18.2	1398.7	440	7.7
V _{mix} A	399.2	18.3	21.9	884.5	374	7.6
V _{mix} B	376.7	23.1	16.3	1450.9	583	6.8
ANOVA						
Method	0.01	<0.001	<0.001	0.03	0.23	<0.001
Mixture	<0.001	<0.001	<0.001	0.002	0.001	0.004
Method × mixture	0.39	0.07	0.90	0.22	0.38	0.06

T = thermophilic composting; V = vermicomposting; mix A = 5:1 (waste:straw ratio); mix B = 10:1 (waste:straw ratio); N = nitrogen; C = carbon; DM = dry matter.

methanogenic microorganisms to produce CH₄ (García-Marco et al., 2014). The addition of labile C sources (glucose) also increased microbial oxygen demand and thereby caused anaerobic microsites

for CH₄ and N₂O production. Higher CH₄ emissions from vegetable waste than from pre-decomposed manure (Fig. 3) confirmed the hypothesis that a high proportion of easily decomposable materials increases CH₄ production. The effect of glucose addition on N₂O emissions, however, was inconsistent with this study's hypothesis. N₂O emissions were mainly affected by NO₃ concentration. Higher N₂O emissions from pre-decomposed manure than from vegetable mixtures (Fig. 3) could be explained by the higher concentration of NO₃ (Table 1). A high moisture content increases anaerobic patches in compost piles and thereby increases CH₄ and N₂O emissions. However, this study showed that earthworms reduced the moisture effect on CH₄ and N₂O, as hypothesised (Fig. 4), because the earthworms mixed the substrates continuously and thereby counterbalanced the moisture effect. The results are consistent with the 34% decrease in N₂O due to earthworms in the soil with higher moisture (i.e. 97% water-filled pore space) (Chen et al., 2014).

4.1.2. Carbon dioxide

Total cumulative CO₂ emissions were higher from

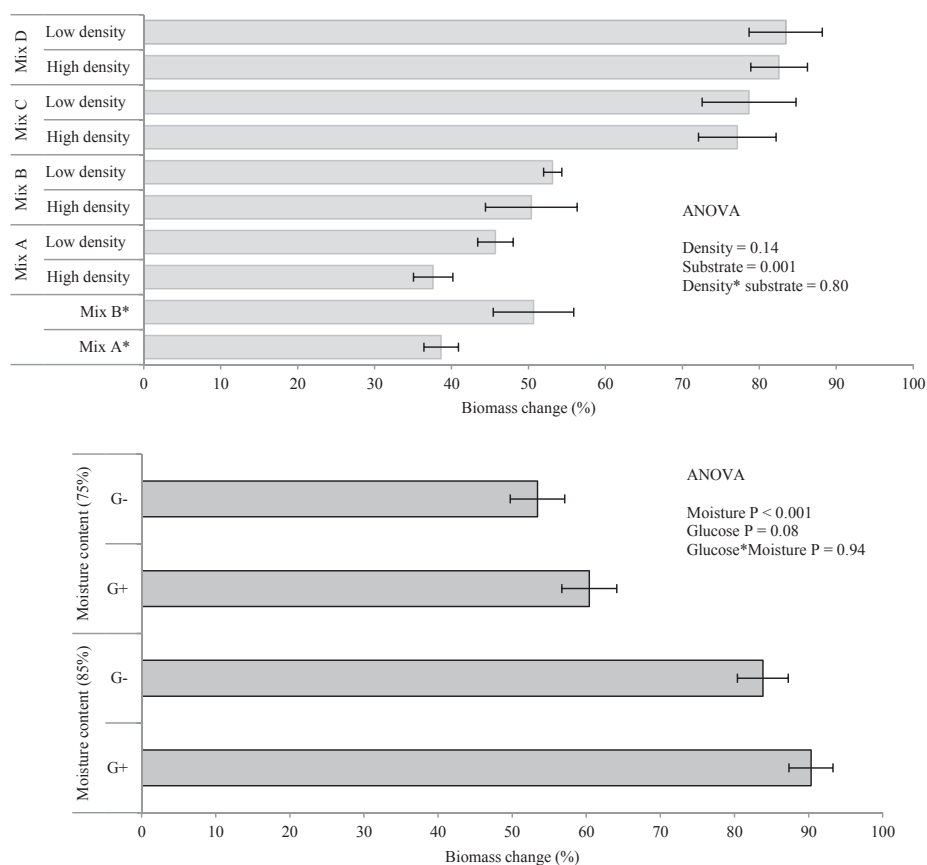


Fig. 5. Change in earthworm biomass after 45 days of vermicomposting: high density = 3 kg m^{-2} ; low density = at 1 kg m^{-2} ; Mix A = 5:1 (waste:straw ratio); Mix B = 10:1 (waste:straw ratio); Mix C = 4:1:1/4 (waste:manure:straw ratio); mix D = 3:1:0 (waste:manure:straw mixture); G+ = glucose added; G- = glucose not added; * indicates biomass change during experiment I; bars indicate the standard error of the means ($n = 3$).

vermicomposting than from thermophilic composting, as hypothesised. Experiment III also confirmed this hypothesis since vermicomposting increased total cumulative CO_2 emissions by 11–26% relative to non-earthworm treatments. Higher total cumulative CO_2 emissions indicate a higher biodegradation rate and stabilisation (Chan et al., 2011). The results from the present study therefore suggest that vermicomposting enhances decomposition of waste materials compared with thermophilic compost. Similarly, the CO_2 flux after 45 days was between 0.15 and $0.5 \text{ g CO}_2\text{-C kg}^{-1}$ dry matter day^{-1} in vermicompost and 0.98 – $1.3 \text{ CO}_2\text{-C kg}^{-1}$ dry matter day^{-1} in thermophilic compost, implying that vermicompost was at a more advanced stage of decomposition than conventional compost. It is possible to argue that earthworm respiration could contribute higher CO_2 emissions from vermicomposting. However, this argument cannot explain the higher CO_2 emissions in the earthworm treatments because the difference observed was greater than the earthworm respiration. The effect of earthworms on CO_2 emissions was less clear in the low C:N and substrates with glucose (Figs. 2 and 4) because a higher proportion of easily decomposable materials (*i.e.* low C:N and glucose-added substrates) resulted in much higher decomposition (Chowdhury et al., 2014), which exceeded the earthworms' effect. The higher CO_2 emissions at higher earthworm density implied that decomposition of vermicompost could be accelerated by increasing the earthworm population from the optimal earthworm density ($1.2 \text{ kg earthworms m}^{-2}$) recommended by Ndegwa et al. (2000).

4.1.3. Total GHG emissions

This study used two different scenarios to assess the GHG

budget from the two composting methods. The first scenario excluded CO_2 emissions from the total GHG budget, as discussed in Section 4.1.1. Under this scenario, vermicomposting had a lower total GHG budget than thermophilic composting, as hypothesised. The lower GHG budget from vermicomposting was explained by the lower N_2O and CH_4 production (Figs. 2 and 4). The second scenario included CO_2 emissions in the GHG budget, and under this scenario total GHG emissions were higher from vermicomposting than from thermophilic composting. This variation was expected because CO_2 emissions were higher during vermicomposting, and CO_2 contributed over 75% of the total GHG budget in all treatments. Similarly, Andersen et al. (2010) and Chan et al. (2011) reported an 80% contribution of CO_2 to total GHG emissions during household waste composting. Higher CO_2 emissions indicate a greater stability of the remaining material (*i.e.* a higher decomposition). It is therefore important to exclude CO_2 from the total GHG budget of composting experiments (Schott et al., 2016; Vu et al., 2015), including as biogenic CO_2 from short-term pools, as plant litter is usually excluded from greenhouse gas balances (IPCC, 2013). Substrate quality determined the relative contribution of N_2O and CH_4 to the total GHG budget (Figs. 2–4). The contribution of CH_4 was higher than N_2O in vegetable waste composting due to the high proportion of easily degradable C, which favours CH_4 production. The N_2O contribution was higher from cattle manure, which was explained by the high mineral N concentration in cattle manure.

4.2. N and C balance

Total N loss was lower after vermicomposting than thermophilic

composting, as hypothesised. The N loss as N_2O was small (less than 1% of the total N loss) (Table 3), therefore other gaseous losses such as NH_3 , NO_x and N_2 explain the variation in N loss between the two composting methods. No N loss via leaching was observed during the experimental period. NH_3 is the major N loss from composting (Jiang et al., 2011), and temperatures above 45 °C increase NH_3 volatilisation (Chowdhury et al., 2014). Hence, higher temperatures (45–56 °C) during the active phase (Supplementary Fig. 1) explain the higher N loss during thermophilic composting. The active phase was short (*i.e.* three days), typical of small-scale composting systems (Bustamante et al., 2013; Lleó et al., 2013), however this temperature is sufficient to produce a significant NH_3 loss (Chowdhury et al., 2014; Vu et al., 2015). Total N loss was lower compared with previous studies on manure composting (Chowdhury et al., 2014; Vu et al., 2015) and this difference can be explained by the lower N concentration in the substrates (Table 1) relative to manure (25–38.6 g kg^{-1} dry matter) (Chowdhury et al., 2014; Vu et al., 2015). The NO_3^- concentration increased during composting and was higher after vermicomposting, implying higher N mineralisation, nitrification and lower NH_3 loss in the vermicompost than in conventional compost. Moreover, the more rapid decrease in NH_4^+ concentration after thermophilic composting suggested high NH_3 loss.

Vermicomposting increased total C loss compared with thermophilic composting, irrespective of substrate quality (Table 3). CO_2 loss accounted for over 65% of C loss (Table 3), therefore higher C loss after vermicomposting was explained by (i) earthworms decomposing C (Lubbers et al., 2013) (ii) earthworms mixing the substrate, thereby increasing the accessibility of the materials for decomposers, and (iii) earthworm casts increasing decomposition (Sierra et al., 2013). The unaccounted C was between 9 and 14%, which is comparable with several studies (Chowdhury et al., 2014; Vu et al., 2015). The unaccounted C indicates C that was not measured between sampling dates (Vu et al., 2015) and/or C loss via volatile compounds (Chowdhury et al., 2014). The sampling frequency might be inadequate for capturing all CO_2 emissions, particularly from vermicomposting where intensive decomposition occurs. The increase in earthworm biomass (Fig. 5) also explains some of the unaccounted carbon during vermicomposting.

4.3. Implications for small-scale farmers

Gaseous N losses and GHG emissions have been reported from thermophilic composting (Chowdhury et al., 2014), and these emissions reduce the agronomic value of compost and contribute to climate change and nuisance odour. The addition of bulking agents such as crop residues (Santos et al., 2016; Vu et al., 2015), the control of aeration rate (Chowdhury et al., 2014) and the control of pH (Awasthi et al., 2016) have been identified as means of reducing N losses and GHG emissions from composting. However, it is difficult to apply these practices in many developing countries, for instance because of the competing uses of crop residues, mainly for animal feed and fuel in smallholder farming systems (Nigussie et al., 2015), and the need for labour to turn compost piles, determining factors in the adoption of composting in developing countries (Nigussie et al., 2015). Technologies such as forced aeration are expensive and difficult to scale up to field conditions. It is therefore suggested that vermicomposting is a more practical and less expensive method than the existing solutions to reduce GHG emissions, enhance the fertilising value of compost and close the nutrient loop in smallholder farming systems. Temperatures above 45 °C are essential in order to remove pathogens from compost (Velasco-Velasco et al., 2011). However, literature shows the effectiveness of vermicomposting at eradicating pathogens

(Edwards, 2011; Rodríguez-Canché et al., 2010). Pre-composting prior to vermicomposting could be the solution to removing pathogens and weed seeds from vermicomposting (Wang et al., 2014), however N loss and GHG emissions during pre-composting need further investigation because higher N losses and GHG emissions might occur during the pre-composting stage (Velasco-Velasco et al., 2011).

The experiments in this study were designed to correspond to the activity of small-scale farmers in developing countries, therefore care should be taken when extrapolating the results to large-scale enterprises. For example, temperatures may reach values above 60 °C (Tortosa et al., 2012) during large-scale thermophilic composting. Hence, N losses and GHG emissions could be greater in large-scale composting systems. The earthworm effect on GHG budget might be smaller in large-scale vermicomposting because a high input of fresh material might offset the effect of earthworms on aeration (Luth et al., 2011).

Studies have shown earthworm-induced N_2O emissions from soils (Lubbers et al., 2013) because denitrifying bacteria in the earthworm gut contribute to measurable N_2O fluxes. However, caution should be exercised when interpreting this fact because substrate quality, earthworm species and earthworm abundance used for vermicomposting are different from soil experiments. Vermicomposting uses nitrogen-rich substrates, higher earthworm density ($>1 kg m^{-2}$) and particular epigeic earthworms (compost worms), consequently the earthworms' effect on aeration exceeds the earthworm gut effect (denitrification). In contrast, soil usually has $<3%$ organic carbon and/or nitrogen and low earthworm density ($<2.1 g m^{-2}$), consequently the earthworm effect on denitrification is greater.

5. Conclusions

This study showed that vermicomposting reduces total N loss, CH_4 and N_2O emissions compared with thermophilic composting methods, irrespective of the substrate quality (*i.e.* C:N ratio, moisture content and presence of a labile C pool). Higher earthworm abundance reduces CH_4 emissions and accelerates the decomposition process. The addition of labile C sources increases CO_2 and CH_4 emissions during composting. Vermicomposting increases CO_2 emissions, implying that vermicompost is at a more advanced stage of decomposition than thermophilic compost. Numerous solutions have been identified for reducing N losses and non- CO_2 GHG emissions from composting, however the existing technologies are harder to apply in developing countries because they are expensive and difficult to upscale to larger systems. Vermicomposting, on the other hand, is low cost method, making it feasible for many resource-poor farmers to produce high fertilising value compost. Further studies, however, are needed to understand the effect of earthworms on N losses and GHG emissions from composting using different parameters such as substrate quality, earthworm species and feeding frequency.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jclepro.2016.08.058>.

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