



The non-negligibility of greenhouse gas emission from a combined pre-composting and vermicomposting system with maize stover and cow dung

Lei Zhang¹ · Tingting Zhao¹ · Enhui Shi¹ · Zunhao Zhang² · Yan Zhang³ · Yuxiang Chen¹

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Abstract

The acceptance of combined pre-composting and vermicomposting systems is increasing because of the advantage in rapidly stabilizing organic wastes and reducing emission of greenhouse gasses (GHG). However, GHG emission during the pre-composting phase is often neglected when evaluating the system. This study aimed to quantify GHG emission from a combined pre-composting and vermicomposting system and to investigate the effects of earthworms on GHG emission. A combined system using *Eisenia fetida* was employed to stabilize maize stover and cow dung (mixing ratio 60:40). The inoculating densities were 60 (T₁), 120 (T₂), and 180 (T₃) earthworms per kilogram of substrate. A traditional composting system without earthworms was set as a control (T₀). The results indicated that earthworms increased CO₂ while decreased CH₄ and N₂O emissions compared to the control. Higher emission of CO₂ suggested that the earthworms promoted the degradation of the substrates. Lower emission of CH₄ and N₂O showed the advantage of the combined system because CH₄ and N₂O possess extremely higher global warming potential than that of CO₂. T₂ is recommended for stabilizing maize stover and cow dung when making a tradeoff between stabilization rate and reduction of GHG. The percentages of GHG emission during pre-composting relative to total GHG emission in T₁, T₂, and T₃ were 34%, 35%, and 30%, respectively. GHG emission is non-negligible when using a combined system, especially the emission of GHG during the pre-composting phase cannot be ignored.

Keywords Greenhouse gasses · *Eisenia fetida* · Biodegradable wastes · Carbon · Nitrogen · Vermicomposting

Introduction

Composting is an efficient approach for stabilizing agricultural organic wastes. The obtained compost can be used as a natural fertilizer for improving soil structure (Zhu-Barker et al. 2017). Thermophilic composting and vermicomposting are widely used for bioconversion of agricultural wastes into natural fertilizers. Thermophilic composting or traditional composting is a high-

temperature process (> 45 °C) (Nigussie et al. 2017), and complex microbes get involved in it. The emission of greenhouse gasses (GHG) such as carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) during composting is a major concern when it comes to the utilization of this biological method to stabilize organic wastes (Christensen et al. 2009). The production of CO₂ during the composting process is due to the degradation of organic carbon materials, and it is a major component of GHG. CH₄ is generated from anoxic or anaerobic microsites (Chan et al. 2011; Zhu-Barker et al. 2017). Incomplete ammonia oxidation and incomplete denitrification are responsible for the emission of N₂O (Zhu-Barker et al. 2017). At the same time, large amounts of nitrogen are lost during traditional composting because high temperature increases ammonia volatilization (Pagans et al. 2006). Compared to traditional composting, vermicomposting is a mesophilic process (< 30 °C). The decomposition and stabilization of the organic wastes are the combined action of earthworms and associated microorganisms in the process of vermicomposting (Nigussie et al. 2017; Mupambwa and

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✉ Yuxiang Chen
chen@jlu.edu.cn

- ¹ College of Biological and Agricultural Engineering, Jilin University, Changchun 130022, China
- ² The Electron Microscopy Center, Jilin University, Changchun 130000, China
- ³ Costal Research and Extension Center, Mississippi State University, Starkville, MS 39567, USA

Mnkeni 2018). In comparison with traditional composting, vermicomposting is more acceptable for the reduced greenhouse gas emissions and faster stabilization rates. In particular, vermicomposting emits less CH_4 under aerobic conditions created by the burrowing action of the earthworms (Giroto and Cossu 2019). However, the mesophilic condition during vermicomposting cannot kill all pathogens. Weeds inside the wastes cannot be eradicated totally. Thereby, a combined system of thermophilic composting and vermicomposting has been proposed to produce high agronomic value and pathogen-free end products (Nigussie et al. 2017). In fact, nearly all the current vermicomposting systems are carried out as combined systems. In such combined systems, the thermophilic composting, also called pre-composting, plays a role of pre-treatment and is generally introduced prior to the mesophilic process (Lim et al. 2016). This pre-composting of organic wastes involving thermal stabilization usually lasts for 2–3 weeks (Lim et al. 2016) and fulfills the following functions: firstly, efficient pre-composting avoids exposure of the earthworms to high temperature (Chattopadhyay 2012); secondly, the pathogens borne by the organic waste and weed seeds in the substrates can be eradicated after this pre-treatment phase; thirdly, volatile gasses which are toxic to earthworms can be released by the pre-treatment; lastly, pre-treatment enhances initial microbial degradation and softening of the wastes (Lim et al. 2016; Nigussie et al. 2017). In most researches pertinent to vermicomposting processes, pre-composting is a common practice to render the organic waste suitable for the following vermicomposting step (Lim et al. 2016). Currently, a short duration of thermophilic composting is frequently used to pre-treat the waste before vermicomposting at the later stage. Although pre-composting is a common practice in combined systems, the emission of GHG during the pre-composting phase is often neglected when evaluating the advantage of a combined pre-composting and vermicomposting system in reducing GHG.

There is a large body of literature concerning the emission of GHG during vermicomposting, and the reports are contradictory. Some of the studies showed that vermicomposting systems increased the emission of GHG (Nigussie et al. 2017), whereas others indicated that the emission of GHG was decreased by the vermicomposting systems (Chan et al. 2011; Wang et al. 2014; Nigussie et al. 2016). Moreover, most of previous studies about the emission of GHG from vermicomposting systems tended to focus on the emission from the mesophilic stage, and the emission from the thermophilic stage (pre-treatment stage) is often neglected (Nigussie et al. 2017). However, Hobson et al. (2005) reported that pre-treatment removed considerable amount of carbon and nitrogen prior to vermicomposting, which caused significantly reduced emission of CH_4 and N_2O during the second phase (vermicomposting phase) compared to the emission from other studies. According to Hobson et al. (2005), considerable amount of GHG was emitted during pre-treatment, but limited

information is available about the amount of GHG emission in the thermophilic pre-composting phase when using combined pre-composting and vermicomposting systems to stabilize organic wastes. The emission of GHG during the pre-composting phase should be included when evaluating emission of GHG from a combined system. The aims of this study were (1) to quantify the emission of GHG during pre-composting and vermicomposting phases in a combined system and (2) to investigate the effect of earthworm *Eisenia fetida* on the emission of GHG during stabilizing maize stover and cow dung.

Materials and methods

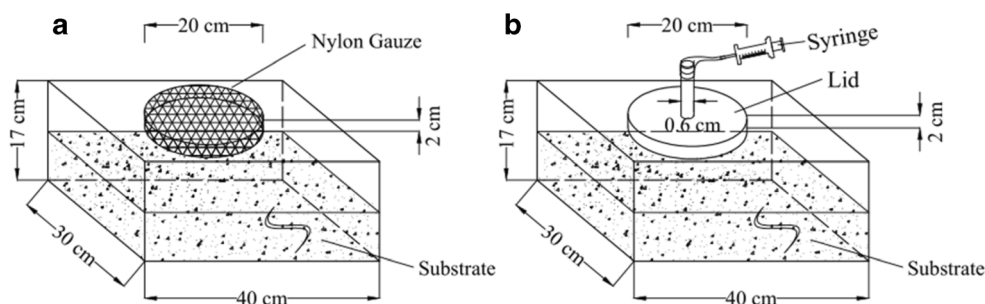
Materials

Maize stover and cow dung were obtained from a farm near Changchun, Jilin Province, China. The variety of the maize is Nianjiu, which is widely cultivated in Northeast China. Total organic carbon contents of the maize stover and cow dung were 479.32 and 432.35 g kg^{-1} dry matter (DM), respectively. Total nitrogen contents of the maize stover and cow dung were 5.75 and 8.06 g kg^{-1} DM, respectively. Maize stover and cow dung were naturally air-dried. Maize stover was cut into the length less than 5 mm, and dry cow dung was broken into small lumps for the experiment. The earthworm species is *E. fetida*, which was purchased from a local market. Adult earthworms were used in this vermicomposting system. The average weight of the earthworm is around 0.30 g. The earthworms were cultured in a mixture of maize stover and cow dung for 20 days in the laboratory. The container used in the experiment was made of polypropylene box with 40-cm length \times 30-cm width \times 17-cm height (Fig. 1a). These containers were drilled on the bottom and the walls. The holes were sealed and the lids of the containers were screwed tightly when gas sample collection was carried out (Fig. 1b).

Experimental design

The mixing ratio of maize stover to cow dung was 60:40 based on dry weight, and this mixing ratio was obtained from the preliminary study. One kilogram of the well-mixed substrates was placed into each container. The mixed substrates were pre-composted for 15 days (Lim et al. 2016), and this phase was designated as pre-composting. After the pre-composting phase, adult earthworms, *E. fetida*, were inoculated. The earthworm density was set as 0 (which served as the control), 60, 120, and 180 earthworms per kilogram substrate. Corresponding groups were designated as T_0 , T_1 , T_2 , and T_3 , respectively. This phase, designated as phase II, lasted for 45 days. During phase II, T_0 continued the traditional composting while other groups entered the vermicomposting

Fig. 1 The container used in the experiment. Composting and vermicomposting (a). Gas sampling (b)



phase. The moisture content of the substrates was maintained around 55–65% during pre-composting and phase II by periodic sprinkling of adequate quantity of sterilized water. Each treatment was done in triplicate. The substrates in all containers were turned thoroughly every 3 days during the pre-composting phase, and then turning was not performed during phase II. Sampling was taken at 15-day intervals. The temperature inside the substrate was monitored daily during pre-composting and then was recorded at each sampling date.

Earthworm biomass

All earthworms were carefully taken out from the substrate at each sampling date. The earthworms were cleaned with distilled water to remove the substrate. Filter paper was used to absorb the water attached on their body. Total biomass of earthworms in each treatment was recorded respectively.

Gas sampling

The lids of the containers were screwed tightly and the holes were sealed during gas sampling (Fig. 1b). Gas samples were taken using an air-tight syringe at four time points: 0, 10, 20, and 30 min, respectively. The samples were injected into pre-evacuated aluminum foil gas-collecting bags. The gas samples were analyzed using gas chromatography (Shimazu, GC-2010PLUS). The concentration of CO₂ was determined using a thermal conductivity detector. N₂O and CH₄ concentrations were measured using an electron capture detector and a flame ionization detector, respectively. Gas fluxes (G_f) in g kg⁻¹ DM day⁻¹ for CO₂ and mg kg⁻¹ DM day⁻¹ for CH₄ and N₂O were calculated according to Czepiel et al. (1996) and Nigussie et al. (2016):

$$G_f = \left(\frac{\Delta C}{\Delta t} \right) \times \left(\frac{V}{A} \right) \times \left(\frac{M}{V_s} \right) \times \left(\frac{P}{P_0} \right) \times \left(\frac{273}{T} \right) \times 24 \times \left(\frac{A}{W} \right) \quad (1)$$

where ΔC is the change in the concentration of gas (ppm) at time interval Δt (hour), V is the headspace volume (L), A is the surface area of the container (m²), M is the molecular weight of the gas, V_s is the volume occupied by 1 mol of gas at standard temperature and pressure, P is the barometric pressure (bar), P_0 is the standard pressure, T is the temperature inside the container (K), and W is the initial dry weight of the substrate (kg).

The cumulative emissions ($CE_{t(ab)}$) were calculated according to Nigussie et al. (2016):

$$CE_{t(ab)} = \frac{(t_b - t_a) \times (G_{ta} + G_{tb})}{2} \quad (2)$$

where ($CE_{t(ab)}$) is the cumulative emission between the measurement day a (t_a) and day b (t_b), and G_{ta} and G_{tb} are gas fluxes at the two measurement dates.

Total cumulative emission was calculated as the sum of cumulative emissions on each day according to Nigussie et al. (2016):

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

The emissions of CH₄ and N₂O were converted into CO₂ equivalents (CO₂-eq) using global warming potentials of 25 and 298 for CH₄ and N₂O, respectively (Solomon et al. 2007). The sum of the three gasses was the total emission of GHG.

Chemical parameter analysis

pH values of the substrate were determined using a digital pH meter. The collected samples taken at each date were suspended in deionized water at a ratio of 1:10 (w/v). The solution was agitated mechanically for 20 min and filtered. The filtrate was used to determine pH values.

Total carbon was measured using the potassium dichromate and concentrated sulfuric acid oxidation method (Nelson and Sommers 1996). Total nitrogen was measured according to the semi-micro Kjeldahl method (Jackson

1973). Lost carbon and nitrogen were calculated according to Nigussie et al. (2016).

$$\text{Carbon/Nitrogen Loss (\%)} = \frac{(W_i C_i - W_f C_f)}{W_i C_i} \times 100\% \quad (4)$$

where W_i and W_f are total dry weight at the beginning and end of the experiment and C_i and C_f are carbon or nitrogen concentration at the beginning and the end of the experiment.

Statistical analyses

The statistical analyses were carried out using SPSS 16.0. The homogeneity of variances was tested using Levene’s test. One-way ANOVA was used to test the effect of sampling date on the total biomass of earthworms and the effect of earthworm density on the carbon and nitrogen changes in the substrate and on the cumulative emission of gasses. A two-way ANOVA was used to determine the effect of sampling date and earthworm density on the temperature, pH, and fluxes of gasses. Post hoc comparisons of the means were performed by using a Student–Newman–Keuls (SNK) test at $\alpha = 0.05$. All values were given as mean \pm SD.

Results and discussion

Earthworm biomass

Total biomass of the earthworms fluctuated in each treatment followed by a decrease at the end of the experiment (Fig. 2), which was consistent with reports by Marhan et al. (2007). The shortage of available nutrients in the substrate in the later stage may be one of the reasons for the decrease of earthworm biomass (Castillo et al. 2013). Moreover, the ambient condition during vermicomposting was not so favorable for earthworm growth due to the formation of toxic intermediate metabolites during the degradation of substrates (Marhan et al.

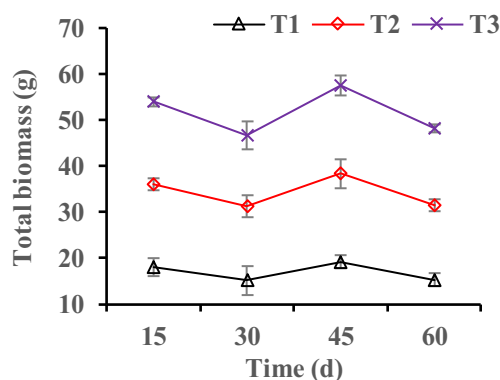


Fig. 2 Total biomass of earthworms. The earthworm density was 60, 120, and 180 adult earthworms per kilogram substrate in T₁, T₂, and T₃

2007; Suthar et al. 2017), which may also be one reason for the decline of total biomass of the earthworms. In T₂ and T₃, total biomass of earthworms showed significant difference between different sampling dates ($p < 0.05$), while in T₁, no significant difference was observed between different sampling dates. This was due to relatively lower earthworm density and less intense competition for food in T₁.

Changes in temperature and pH of the substrate

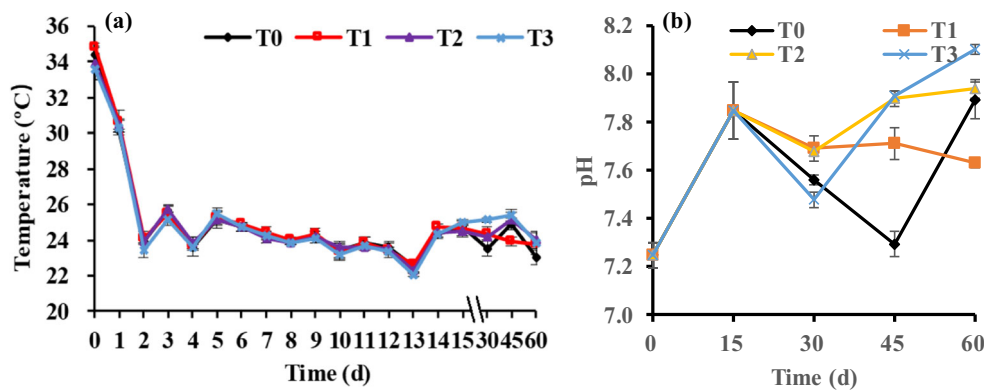
Temperature profiles are shown in Fig. 3a. In the first 2 days, the temperature decreased drastically from 35 to around 24 °C and fluctuated within a narrow range until the end of the experiment. The highest temperature was lower than 45 °C in the present study, which was not consistent with that in traditional composting systems. Generally, the temperature in traditional composting systems reaches at least 45 °C (Nigussie et al. 2017). However, it has been reported that low temperature is typical in small-scale composting such as home composting (Lim et al. 2016). The substrate in the present system was not conducive to the heat buildup, and the heat produced in the substrate quickly dissipated to the surrounding. As a result, the temperature was lower than that in traditional composting systems.

The changes in pH are shown in Fig. 3b. It was obvious that the pH change was greatly influenced by the number of earthworms applied. For all treatments, pH values increased from 7.25 to 7.85 during the first 15 days (pre-composting phase), and then decreased rapidly until day 30. From day 30 to day 45, pH of T₀ continued to drop to almost the initial value (pH 7.25), while pH for other three treatments exhibited an increase. From day 45 to day 60, except T₁, all other three treatments showed an increase in pH. The pH value for T₃, in particular, increased to around 8.10. The increase of pH during the pre-composting phase may be due to the decomposition of nitrogen organic matter and the formation of ammonium ions. The decline of pH may be due to the mineralization of organic matter, the production and fixation of CO₂, and the formation of carboxylic, phenolic, and humic acids. Carboxylic, phenolic, and humic acids and ammonium ions exert opposite effect on the pH of the substrate. Carboxylic, phenolic, and humic acids reduce the pH value; however, ammonium ions increase the pH value of the substrate (Sharma and Garg 2019). The increase or decrease of pH in the substrate during the composting process is decided by the joint effect of the above basic and acidic compounds.

Changes in carbon and nitrogen mass

Retained carbon contents at the end of the experiment in T₂, T₃, and T₄ were 42.54%, 39.96%, and 40.35% of the initial carbon, while it was 63.04% in T₀ (Table 1). The percentage of lost carbon was equivalent to the result

Fig. 3 Changes in temperature (a) and pH (b) during the whole process. The earthworm density was 0 (which served as the control), 60, 120, and 180 adult earthworms per kilogram substrate in T₀, T₁, T₂, and T₃



from Shah et al. (2012), who reported that 67% of the initial carbon of the original substrate was lost during composting. More carbon was lost in the combined system than that in the traditional composting system (control group), which indicated that more organic carbon was degraded in the combined system. This was in line with the results from Nigussie et al. (2016), in which vermicomposting increased total carbon loss. In the present study, most of the carbon was lost as CO₂. In T₁, T₂, and T₃, 34.09, 37.26, and 43.61 g carbon kg⁻¹ substrate were lost as CO₂, and 32.29 g carbon kg⁻¹ substrate was lost as CO₂ in T₀. However, 2.97, 1.73, and 1.41 mg carbon kg⁻¹ substrate were lost as CH₄ in T₁, T₂, and T₃, and it was 4.09 mg carbon kg⁻¹ substrate in T₀. The carbon lost as CH₄ was much lower than that lost as CO₂. This was consistent with the finding by Nigussie et al. (2016), who reported that most carbon was lost as CO₂ during composting and vermicomposting.

Total nitrogen loss was higher in the traditional composting system (T₀) (Table 2). Retained nitrogen in T₁, T₂, and T₃ was 89.84%, 91.65%, and 87.42% of initial nitrogen, respectively. However, it was only 79.68% in T₀. More nitrogen was

retained compared to the findings by Shah et al. (2012), who reported that 46% of the initial nitrogen of the original substrate was lost during composting. This may be due to the differences in the substrates, composting systems, and composting periods. Eklind and Kirchmann (2000) reported that nitrogen loss was 43–62% after 590 days of composting of organic household waste. Results about nitrogen loss from the present study were comparable with those results from Yang et al. (2019), who reported that nitrogen losses were 13–16% in the composting of dairy manure and rice straw. In the present study, most of nitrogen was lost as NH₃. In T₁, T₂, and T₃, 20.33, 27.89, and 35.40 mg NH₃-N kg⁻¹ substrate were lost, respectively. While in T₀, the loss amounted to 13.01 mg NH₃ kg⁻¹ substrate. Different from the situation of NH₃, nitrogen loss as N₂O was significantly higher ($p < 0.05$) in the traditional composting system than in the combined systems of T₁, T₂, and T₃ (Table 2). The decomposition of nitrogen organic matter produces NH₄⁺ in composting or vermicomposting systems. NH₃ volatilization is common during the composting or vermicomposting process. Under aerobic condition, NH₃ is oxidized to nitrous acid by ammonia-oxidizing bacteria and further converted into nitric acid by

Table 1 Changes in carbon mass after pre-composting and the whole process

Treatments	Retained carbon after pre-composting (% of initial carbon)	Retained carbon after the whole process (% of initial carbon)	Lost carbon	
			CO ₂ -C (g kg ⁻¹)	CH ₄ -C (mg kg ⁻¹)
T ₀	88.01 ± 0.33 ^a	63.04 ± 1.47 ^a	32.29 ± 1.33 ^{cd}	4.09 ± 0.17 ^a
T ₁	86.19 ± 0.56 ^a	42.54 ± 4.15 ^b	34.09 ± 2.48 ^{bc}	2.97 ± 0.50 ^b
T ₂	87.10 ± 0.58 ^a	39.96 ± 3.31 ^b	37.26 ± 2.99 ^b	1.73 ± 0.15 ^c
T ₃	86.49 ± 0.32 ^a	40.35 ± 0.86 ^b	43.61 ± 0.63 ^a	1.41 ± 0.33 ^c

The earthworm density was 0 (which served as the control), 60, 120, and 180 adult earthworms per kilogram substrate in T₀, T₁, T₂, and T₃. Values (mean ± standard deviation) bearing different superscript letters within the same column are significantly different ($p < 0.05$)

Table 2 Changes in nitrogen mass after pre-composting and the whole process

Treatment	Retained nitrogen after pre-composting (% of initial nitrogen)	Retained nitrogen after the whole process (% of initial nitrogen)	Lost nitrogen	
			NH ₃ -N (mg kg ⁻¹)	N ₂ O-N (mg kg ⁻¹)
T ₀	96.39 ± 2.05 ^a	79.68 ± 1.75 ^b	13.01 ± 0.003 ^d	13.19 ± 1.92 ^a
T ₁	93.91 ± 1.33 ^a	89.84 ± 1.93 ^a	20.33 ± 0.002 ^c	5.64 ± 0.18 ^b
T ₂	94.90 ± 1.34 ^a	91.65 ± 0.37 ^a	27.89 ± 0.003 ^b	0.91 ± 0.11 ^c
T ₃	94.41 ± 2.04 ^a	87.42 ± 2.60 ^a	35.40 ± 0.002 ^a	0.60 ± 0.00 ^c

The earthworm density was 0 (which served as the control), 60, 120, and 180 adult earthworms per kilogram substrate in T₀, T₁, T₂, and T₃. Values (mean ± standard deviation) bearing different superscript letters within the same column are significantly different ($p < 0.05$)

nitrite-oxidizing bacteria. Nitrous acid and nitric acid can be transformed into N₂ by denitrifying bacteria under anaerobic condition. N₂O is an intermediate product in transformation of nitrous acid/nitric acid into N₂. The ammonia-oxidizing process is a rate-limiting step of the nitrification process (Lv et al. 2019). This may be one of the reasons for the lower N₂O than NH₃ in the present study.

CO₂ emission from the degradation process

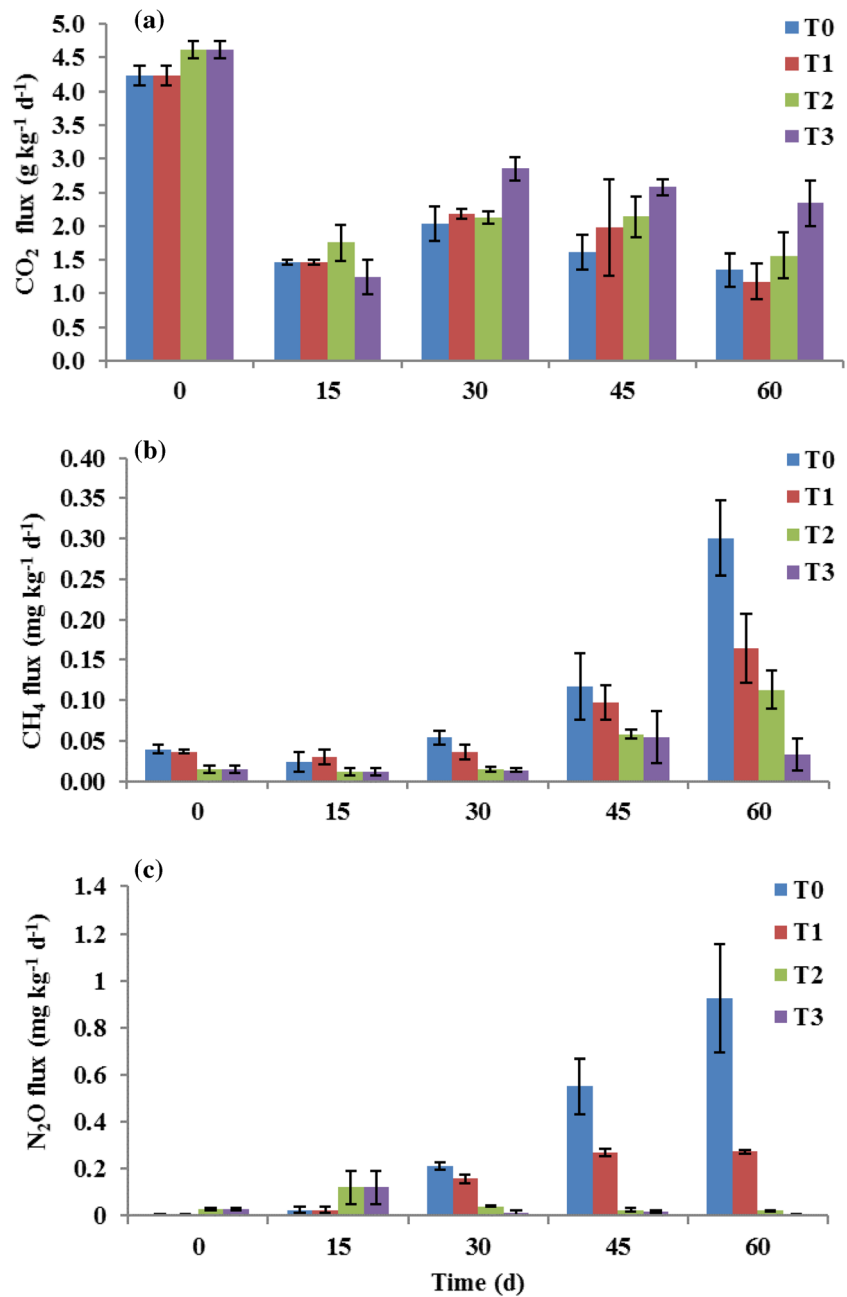
As shown in Fig. 4a, the emission of CO₂ was the highest during the initial stage of the experiment. On day 0, the flux of CO₂ emission was around 5.00 g kg⁻¹ DM day⁻¹. By the end of the experiment, it decreased to 1.35 to 2.34 g kg⁻¹ DM day⁻¹. The sampling time and earthworm density significantly affected CO₂ emission ($p < 0.01$). The lower CO₂ emission during phase II suggested that the substrates tended to be stable as composting proceeded. This was corroborated by the temperature profiles. The rapid evolution of CO₂ in the initial stage can be attributed to the decomposition of the easily biodegradable substances (Fernández-Gómez et al. 2010). The cumulative emission of CO₂ in T₁, T₂, and T₃ was higher than that in the control (Fig. 5a). This is supported by the retained amount of carbon in the corresponding groups. During the pre-composting phase, the cumulative emissions of CO₂ was around 43.00 g CO₂ kg⁻¹ substrate. The cumulative emissions of CO₂ during the mesophilic vermicomposting phase in T₁, T₂, and T₃ were 82.27, 88.86, and 112.15 g CO₂ kg⁻¹ substrate, respectively. In T₀, the cumulative emission of CO₂ was 75.68 g CO₂ kg⁻¹ substrate from day 15 to day 60. There were significant differences in cumulative emission of CO₂ in four earthworm density treatments ($p < 0.01$). The presence of the earthworms promoted the emission of CO₂ in this study. This was in line with some other studies. Lubbers et al. (2013) reported that earthworms increased emission of CO₂ during a relatively short experimental period, and the earthworm-induced CO₂ emission decreased with experimental duration. The movement of earthworms inside the substrate increased the oxygen penetration,

which promoted the aerobic degradation of organic matter (Ba et al. 2020). This is one of the reasons for the increased emission of CO₂ in treatments with earthworms compared to the control. The substrate composition affects the emission of CO₂. Wang et al. (2014) reported that CO₂ emission was not altered during vermicomposting of duck manure, while the addition of reed straw increased CO₂ emission by 20%. The percentages of CO₂ emission during the pre-composting phase relative to the total CO₂ emission in T₀, T₁, T₂, and T₃ were 36%, 34%, 35%, and 30%, respectively (Fig. 6a). That is to say, at least one-third of total CO₂ was emitted during the pre-composting phase using a combined pre-composting and vermicomposting system. The emission of CO₂ during pre-composting should be included when the emission of CO₂ from a combined system is estimated.

CH₄ emission from the degradation process

In most cases, the emission of CH₄ was higher in T₀ compared to T₁, T₂, and T₃ ($p < 0.05$) (Fig. 4b). T₀ was not inoculated with earthworms. The highest flux in T₀ appeared on day 60 with the value of 0.30 mg kg⁻¹ DM day⁻¹. It was twice the CH₄ flux from T₁, three times the flux from T₂, and nine times the flux from T₃. It was reported that CH₄ production can be inhibited to some extent under vermicomposting. The lower emission of CH₄ in the initial stage was in agreement with other studies (Andersen et al. 2010; Zhu-Barker et al. 2017). CH₄ was formed under anoxic conditions, and NO₃⁻ has an inhibitory effect on methanogenesis (Zhu-Barker et al. 2017). CH₄ emission started when oxygen and NO₃⁻ inside the substrates were depleted. The cumulative emissions of CH₄ during the pre-composting phase were less than 0.50 mg kg⁻¹ substrate in T₀, T₁, T₂, and T₃ (Fig. 5b). From day 15 to day 60, the cumulative emissions of CH₄ in T₀, T₁, T₂, and T₃ were 5.00, 3.46, 2.04, and 1.70 mg kg⁻¹ substrate, respectively. This indicated that the emission of CH₄ was significantly decreased by the presence of earthworms ($p < 0.01$). The emission of CH₄ is

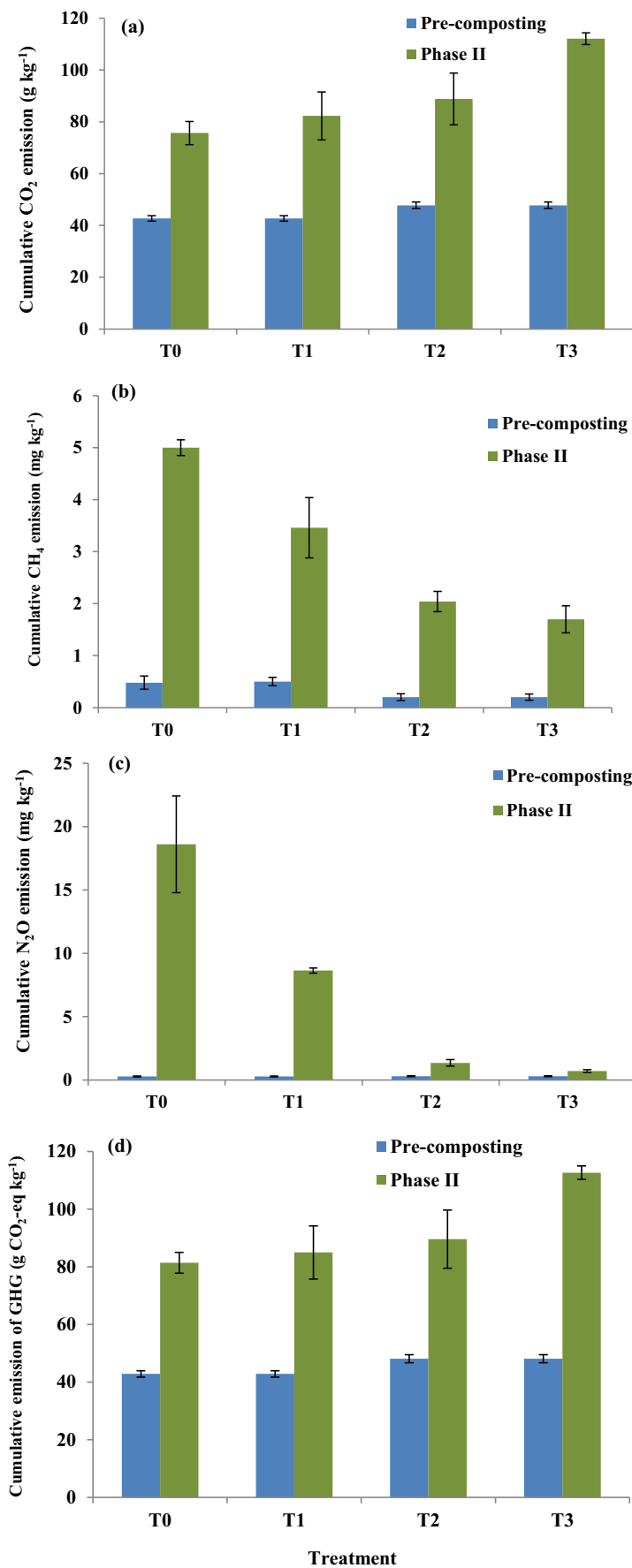
Fig. 4 Fluxes of CO₂ (a), CH₄ (b), and N₂O (c) during the stabilization of maize stover and cow dung. The earthworm density was 0 (which served as the control), 60, 120, and 180 adult earthworms per kilogram substrate in T₀, T₁, T₂, and T₃



dependent on oxygen level. Methanogens are strictly anaerobic organisms and are found in anoxic environments (Welte and Deppenmeier 2014). High aeration condition can decrease CH₄ emission. Thus, forced aerated composting is often adopted because of the advantage of high processing rate and good control of process variables (Tsutsui et al. 2013). Earthworms improve air circulation inside the compost pile through continuous turning of the substrates like mechanical aeration, thereby maintaining aerobic conditions (Nigussie et al. 2016; Lee et al. 2018). The percentages of cumulative emission of CH₄

during pre-composting relative to the total emission of CH₄ was 9%, 13%, 9%, and 11% in T₀, T₁, T₂, and T₃, respectively (Fig. 6b). The emission of CH₄ was a little higher in the later stage, though the absolute amount was quite low. The emission on day 60 was 0.30, 0.16, 0.11, and 0.03 mg kg⁻¹day⁻¹ DM in T₀, T₁, T₂, and T₃, respec-

Fig. 5 Cumulative emission of CO₂ (a), CH₄ (b), N₂O (c), and total GHG (d) during the stabilization of maize stover and cow dung. The earthworm density was 0 (which served as the control), 60, 120, and 180 adult earthworms per kilogram substrate in T₀, T₁, T₂, and T₃



tively. The values were below $0.04 \text{ mg kg}^{-1} \text{ day}^{-1}$ DM on day 0 in all treatments. The amount of CH_4 emitted was much lower compared to that of CO_2 , which was analogous to the results obtained by Nigussie et al. (2016), who reported that most carbon was lost as CO_2 during composting and vermicomposting processes. The production of methane accounted for only 0.5 – 1% of total carbon loss in the study conducted by Nigussie et al. (2016). In the present study, the production of methane accounted for 3 – 8% in treatments with earthworms and 11% in the control group.

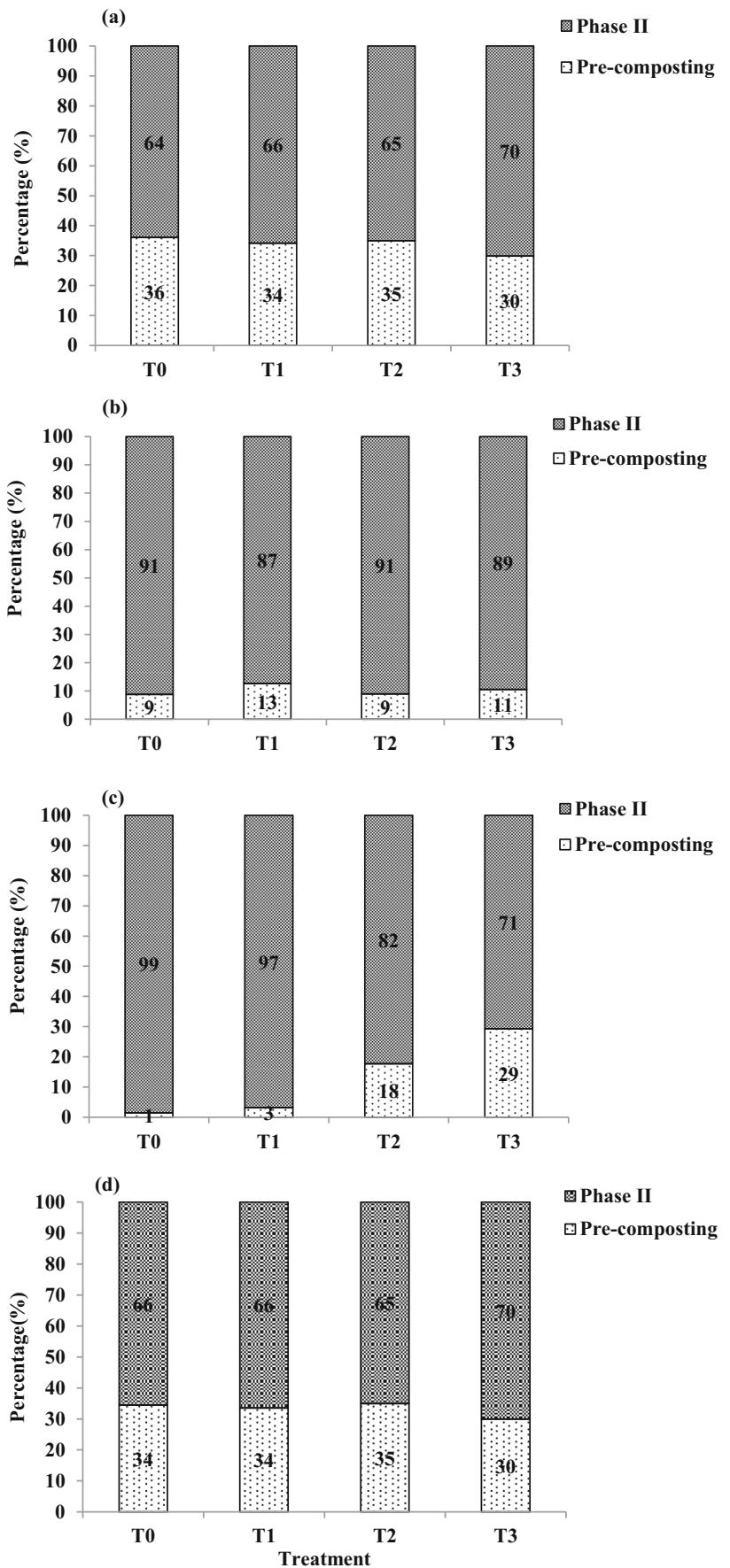
N_2O emission from the degradation process

N_2O flux from each group is shown in Fig. 4c. In T_1 , the flux of N_2O became stable starting from day 45, and the value was $0.27 \text{ mg kg}^{-1} \text{ DM day}^{-1}$. In T_2 and T_3 , the peak values of N_2O flux appeared on day 15. In the control group (T_0), the flux of N_2O showed an increasing trend. It was $0.92 \text{ mg kg}^{-1} \text{ DM day}^{-1}$ by the end of the experiment in T_0 . This demonstrated that the flux of N_2O was significantly affected by the earthworm ($p < 0.05$). From day 30 to day 60, the application of earthworms caused decrease of N_2O emission. This was consistent with the results by Wang et al. (2014), who reported that N_2O flux was decreased by the earthworms during vermicomposting of duck manure. Cumulative emission of N_2O in T_0 , T_1 , T_2 , and T_3 during the pre-composting phase was around 0.30 mg kg^{-1} substrate (Fig. 5c). Cumulative emissions of N_2O from day 15 to day 60 in T_0 , T_1 , T_2 , and T_3 were 18.61, 8.64, 1.35, and 0.70 mg kg^{-1} substrate, respectively. This indicated that earthworms decreased the emission of N_2O . The decreasing extent was related to the earthworm density ($p < 0.01$, $r = -0.923$). This was different from some reports. Hu et al. (2013) and Tsutsui et al. (2013) found that high N_2O emission was triggered immediately when the anoxic condition was switched to oxic condition due to the inactivation of N_2O reductase by oxygen. The emission of N_2O from the ingested substrates was increased compared to the group without earthworms (Chapuis-Lardy et al. 2010), which was due to the positive influence on the relative abundance of denitrifiers by earthworms through soil ingestion and subsequent egestion (Chapuis-Lardy et al. 2010). Nigussie et al. (2016) and Wu et al. (2015) reported that organic nitrogen transformations such as mineralization, volatilization, nitrification, and denitrification during vermicomposting were affected by earthworms and their interaction with associated microbes, although earthworms hardly produced any N_2O themselves. In addition to that, the gut of earthworms was an optimal environment for N_2O -producing microflora, and the microflora was stimulated by the passage of earthworm gut (Wu et al. 2015). It was reported that the low redox potentials, organic carbon richness, and nearly neutral pH of the

earthworm gut could lead to the selective stimulation of ingested microorganisms capable of anaerobiosis (Depkat-Jakob et al. 2013). Under this circumstance, the ingested fermentative, methanogenic, and nitrate-reducing bacteria can be activated during gut passage (Depkat-Jakob et al. 2013). The result about N_2O emission was partially consistent with the report from Chen et al. (2014), who reported more N_2O emission in earthworm-worked dry soil than that without earthworms. Under this circumstance, N_2O was thought to be a byproduct of the nitrification process. This means that it was released during hydroxylamine oxidation to nitrite by ammonia-oxidizing microorganisms. However, in wet soil, earthworms reduced N_2O emission. The presence of earthworms could favor the reduction of N_2O to N_2 . Also, earthworms altered soil microenvironment to slow N_2O diffusion, and therefore, N_2O was reduced to N_2 before it was diffused into the air (Chen et al. 2014). Although there is no soil used in the present study, the substrates of maize stover and cow dung were inhabited by a diversity of microorganisms. The attached microorganisms acted like the microbiota in the soil and interacted with earthworms to affect the emission of N_2O . The mechanism of N_2O emission is quite complex. Though many studies found that denitrification or nitrifier denitrification was a main pathway for N_2O production during the composting process, N_2O production through nitrification also played an important role (Tsutsui et al. 2013). It needs further study to identify the origin of N_2O in a combined pre-composting and vermicomposting system. The dominant microorganism species should be taken into account as an important factor in the future for clarifying the mechanism of the emission of N_2O in addition to the physiochemical conditions.

The percentages of cumulative emissions of N_2O during pre-composting relative to the total emission of N_2O were 1%, 3%, 18%, and 29% in T_0 , T_1 , T_2 , and T_3 , respectively (Fig. 6c). The lower percentage in the control group was because of its higher total emission of N_2O . Nearly 30% of N_2O was emitted during pre-composting in T_3 . In the present study, small-size earthworm *E. fetida* decreased the emission of N_2O . This was different from the report by Depkat-Jakob et al. (2013), who found that small-size earthworm was capable of emitting N_2O . *E. fetida* is a typical small-size earthworm, and it is often used in vermicomposting systems. The effect of earthworms on the emission of N_2O in the present study was similar to that obtained by Wang et al. (2014). Luth et al. (2011) attributed the discrepancy to the available organic nitrogen in the system. They suggested that there was probably a threshold of available organic nitrogen that determined whether earthworms would decrease or increase the emission of N_2O . This needs to be further investigated in the combined pre-composting and vermicomposting system with maize stover and cow dung.

Fig. 6 The percentage of CO₂ (a), CH₄ (b), N₂O (c), and total GHG (d) emission during the pre-composting phase and phase II. The earthworm density was 0 (which served as the control), 60, 120, and 180 adult earthworms per kilogram substrate in T₀, T₁, T₂, and T₃



Total GHG emission from the degradation process

CH₄ and N₂O were converted into CO₂ equivalents using conversion coefficient suggested by Solomon et al. (2007), and total emission of GHG was obtained (Fig. 5d). During the pre-composting phase, GHG emissions were around 43.00 g CO₂-eq kg⁻¹ substrate. During phase II, GHG emissions in T₀, T₁, T₂, and T₃ were 81.40, 84.95, 89.33, and 112.41 g CO₂-eq kg⁻¹ substrate, respectively. The percentages of GHG emission during pre-composting relative to total GHG emission in T₀, T₁, T₂, and T₃ were 34%, 34%, 35%, and 30%, respectively (Fig. 6d). This indicated that during the pre-composting phase, around one-third of GHG was emitted when using a combined pre-composting and vermicomposting system. Taking up a significant portion of total GHG emission, the GHG emitted during the pre-composting phase should be considered one of the noteworthy factors when evaluating a combined pre-composting and vermicomposting system. The total emissions of GHG in T₀, T₁, T₂, and T₃ were 124.22, 127.77, 137.65, and 160.73 g CO₂-eq kg⁻¹ substrate, respectively. It indicated that the presence of earthworms increased the emission of GHG and the emission amount increased with earthworm density. The emission of GHG in T₃ was significantly higher than that in other three groups ($p < 0.01$). The combined system had an advantage though its total GHG emission was higher than that in the traditional composting system. This was due to the faster decomposition and stabilization in the combined system which was indicated by the higher emission of CO₂. More importantly, the emission of N₂O ($p < 0.01$) and CH₄ ($p < 0.01$) was significantly decreased by the combined system, and the global warming potential of N₂O and CH₄ is extremely higher than that of CO₂. T₂ is recommended for stabilizing maize stover and cow dung when the tradeoff between the effectiveness of stabilization and the reduction of GHG is considered.

Conclusions

GHG mainly emitted as CO₂ from the combined pre-composting and vermicomposting system used in the present study. The GHG emission during the pre-composting phase accounted for around 30–35% of total emission from the combined system. Earthworms had an impact on the emission amount of GHG during the whole process. The emission of CO₂ was increased by the presence of earthworms, whereas the emissions of CH₄ and N₂O were decreased by the earthworms. On the whole, the emission of GHG was increased by earthworms in the present study. T₂ is recommended for stabilizing maize stover and cow dung at the present study context. Both of the emission amounts of GHG during pre-

composting and vermicomposting phases cannot be neglected when evaluating a combined system.

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Data availability The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Competing interests The authors declare that they have no conflict of interest.

Ethics approval The present study was carried out at the College of Biological and Agricultural Engineering, Jilin University, Changchun, China. The experimental protocol used in the study was approved by the Institutional Animal Care and Use Committee of Jilin University, Changchun, China.

Consent to participate All authors agree to participate in this study.

Consent to publish All authors allow the publication of this paper.

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