Waste Management 62 (2017) 43–51

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Earthworms change the quantity and composition of dissolved organic carbon and reduce greenhouse gas emissions during composting

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ARTICLE INFO

Article history:
Received 25 October 2016
Revised 3 February 2017
Accepted 6 February 2017
Available online 20 February 2017

Keywords:
Vermicomposting
Nitrous oxide
Feeding ratio
Eisenia fetida
Dendrobaena veneta

ABSTRACT

Dissolved organic carbon (DOC) has recently been proposed as an indicator of compost stability. We assessed the earthworms' effect on DOC content and composition during composting, and linked compost stability to greenhouse gas emissions and feeding ratio. Earthworms reduced total DOC content, indicating larger stability of vermicompost than of thermophilic compost. The concentrations of humic acid and fulvic acid were reduced by earthworms, whereas there was no significant effect on hydrophobic neutrals and hydrophilics. The humic acid fraction was depleted more quickly than the other compounds, indicating humic acid degradation during composting. The optimum feeding ratio decreased DOC content compared to the high feeding ratio. The lowest N_2O emissions were also observed at the optimum feeding ratio. Our study confirmed the use of DOC content and composition as an indicator of compost stability and suggested that feeding ratio should be considered when assessing the earthworms' effect on stabilisation and greenhouse gas emissions.

1. Introduction

Thermophilic composting and vermicomposting are two composting techniques commonly used to convert biodegradable waste into compost (Lazcano et al., 2008; Nigussie et al., 2016). Thermophilic composting is a microbially-mediated, high-temperature (>45 °C) process, while vermicomposting is a mesophilic (<30 °C) process that involves earthworms and associated microorganisms in the decomposition and stabilisation of organic materials (Munroe, 2007). The temperature during vermicomposting should remain within the range of 15–30 °C, as temperatures above 35 °C kill earthworms (Munroe, 2007).

Considerable decomposition while retaining higher nutrient concentrations was observed during vermicomposting compared with thermophilic composting (Nigussie et al., 2016; Lazcano et al., 2008). In contrast, high N losses occur during thermophilic composting because high temperatures (>45 °C) increase ammonia volatilisation (Pagans et al., 2006). Temperatures above 45 °C were considered essential for eradicating weeds and pathogens from compost (Ryckeboer et al., 2003), however, vermicomposting has also been shown to be effective at eradicating weeds and pathogens (Edwards, 2011), but the mechanisms of how earthworms kill weed seeds and pathogens is not known and the reports are contradictory. Hence the combination of thermophilic composting and vermicomposting has been proposed to produce compost of high agronomic value and pathogen-free (Lazcano et al., 2008). Generally, the combination also enables the organic fertilizer to be produced at a faster rate than either of the individual process (Lazcano et al., 2008). The first phase – thermophilic composting – occurs only for a short period of time, mainly to eradicate pathogens and eliminate toxic compounds, and the subsequent vermicomposting (*i.e.* the second phase) is carried out to accelerate the stabilisation process and improve the agronomic value of compost (Lazcano et al., 2008).

It is important that compost is sufficiently stable prior to soil application because unstable compost reduces plant growth. Unstable compost leads to oxygen depletion in the root zone, osmotic stress and contains phytotoxic compounds (Wichuk and McCartney, 2010). Compost is considered stable when the organic matter decomposition rate is reduced to a low level with no heat development. A number of indices are used to determine compost stability (Bernal et al., 2009; Khan et al., 2014). Evolution of CO_2 is the most commonly used indicator (Bernal et al., 2009; Nigussie et al., 2016), but this index is influenced by a number of factors such as substrate quality. Lack of heat development is another simple method for evaluating compost stability (Boulter-Bitzer et al.

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http://dx.doi.org/10.1016/j.wasman.2017.02.009
0956-053X/

2006), however it is also affected by aeration, pile size, moisture content, degree of insulation and other parameters. Therefore the use of one index to determine compost stability is potentially misleading. Indices such as a C/N ratio <12 and a NH_4^+-N: NO_3^--N ratio <0.16 are also recommended as a threshold level for indicating compost maturity (Khan et al., 2014). Recently, studies showed that the stability and maturity of the compost could be determined by spectroscopy, structural characterization and thermogravimetric analysis (Kumar et al., 2013). Numerous researchers have therefore suggested the combined use of multiple indices as indicator for compost stability (Bernal et al., 2009; Khan et al., 2014).

Dissolved organic carbon (DOC) has recently been proposed as an additional indicator of compost stability (Bernal et al., 2009; Santos et al., 2016). A maximum threshold DOC value of 4 g kg^{-1} dry matter is used as an indicator of stable compost (Khan et al., 2014). Not only the quantity but also the quality (*i.e.* chemical composition) of DOC can be used to assess compost stability. A batch fractionation procedure (Van Zomeren and Comans, 2007) is currently used to separate DOC into four fractions, *viz.*, humic acid (HA), fulvic acid (FA), hydrophobic neutral (HON), all considered as hydrophobic compounds, and hydrophilic (Hi) compounds (Straathof and Comans, 2015). A recent study has shown that the proportions of these four fractions vary between composts, independent of DOC concentration (Straathof and Comans, 2015). Hi compounds declined during composting likely because they were used as a substrate for microorganisms, and the hydrophobic compounds (HA, FA and HON) fractionally increased in stable compost (Straathof and Comans, 2015).

It is plausible that earthworms influence the DOC quantity and quality (composition) of compost because they ingest the substrates and thereby condition the microbial communities that influence the decomposition process. Previous studies have found higher stabilisation of compost as a result of vermicomposting compared to thermophilic composting using indices such as CO_2 evolution (Nigussie et al., 2016; Ngo et al., 2013) and biochemical analysis (Lazcano et al., 2008; Ravindran and Mnkeni, 2016). However, little is known about the effect of earthworms on the quantity and composition of DOC during vermicomposting.

Feeding ratio is defined as the ratio of substrate added over earthworm biomass (Ndegwa et al., 2000). A high feeding ratio decreases the conversion rate of fresh materials into vermicompost. Previous studies have shown that very high food supply reduces the biomass and reproduction of earthworms (Luth et al., 2011). Furthermore, Ndegwa et al. (2000) found that low feeding ratio increases the mineralisation of nutrients (particularly nitrogen) compared with high feeding ratio. High feeding ratio increases temperature and impedes air circulation in the pile (Luth et al., 2011), both of which affect GHG emissions. For instance, if food supply is too high (supra-optimal feeding ratio) per unit earthworm biomass, the temperature in the pile increases; high temperatures and anoxic patches not only result in increased earthworm mortality, but in greater GHG emissions as well. Feeding ratio is therefore an essential parameter that should be considered when assessing the effect of earthworms on stabilisation and greenhouse gas emissions. Recent reports have used substrate quality (Nigussie et al., 2016; Wang et al., 2014) and earthworm density (Nigussie et al., 2016) to evaluate the effect of earthworms on decomposition and GHG emissions. In addition, feed type affects the conversion rate of fresh materials into vermicompost (Edwards and Bohlen, 1996). However, the effect of feeding ratio on stabilisation processes and GHG emissions during vermicomposting is not known. The objectives of the present study were therefore (i) to evaluate the effect of earthworms on DOC quantity and composition of the compost, linking this effect to the initial substrate quality and feeding ratio, and (ii) to assess the effect of feeding ratio on GHG emissions from vermicomposting. We hypothesised that (i) earthworms reduce the DOC content of compost compared to non-earthworm composting, with the effect of earthworms being greater at the optimal feeding ratio; (ii) earthworms reduce the fractional contribution of Hi and hence increase the fractional contribution made by HA, FA and HON compared to non-earthworm composting, (iii) high feeding ratio increases GHG emissions from vermicomposting compared to the optimal feeding ratio, and (iv) high feeding ratio reduces compost stability, as assessed by CO_2 flux, compared to the optimal feeding ratio.

2. Materials and methods

2.1. Experimental setup

Pre-decomposed garden waste was obtained from Unifarm, part of Wageningen University and Research, and placed in plastic boxes (30 cm width × 40 cm length × 25 cm height). Three substrates with different composition that have undergone different degrees of decomposition were used as composting materials. The first substrate (substrate_1) was pre-composted for three months, and the second substrate (substrate_2) was pre-composted for nearly 1½ months. The third substrate (substrate_3) was prepared from substrate_1, substrate_2 and cattle manure at a ratio of 1:1:1 (weight basis). The cattle manure was obtained from Unifarm, and added to the third substrate to increase nitrogen availability in the mixture, whereas pre-decomposed materials were used in this experiment to avoid the development of high temperatures in the vermicompost bins. Hence there was no temperature effect in our experiment unlike previous composting experiments (Nigussie et al., 2016; Straathof and Comans, 2015).

Mixtures of adult individuals of two common composting earthworm species, namely *Eisenia fetida* and *Dendrobaena veneta* (approximate 2:1 ratio), were obtained from two earthworm breeding companies, 'De Polderworm' and 'Star Foods', the Netherlands. The earthworms were added at a stocking density of 3 kg earthworms m^{-2}. The substrates were added to the vermicomposting bin in two doses: (i) 1.5 kg substrate kg $earthworms^{-1}$ (recommended by Aira and Domínguez, 2008) – hereafter referred to as optimal ratio (OR) – and (ii) 3 kg substrate kg $earthworms^{-1}$ – hereafter referred to as the high ratio (HR). Treatments without earthworms were used as controls. The experiment had two factors arranged in a 3 × 3 (earthworm treatments (OR, HR and control) × substrate quality) complete randomised design with three replicates. The experiment was conducted for 60 days, and the moisture content in each container was adjusted approximately to 70–75% by spraying of water on top.

2.2. DOC fractionation

DOC was extracted using ultra-pure water, as described by Straathof and Comans (2015). Briefly, fresh compost was mixed with ultra-pure milli-Q water at a 1:10 ratio (w/v), shaken for one hour on a horizontal shaker and filtered through a 0.45 μm filter (Whatman™). Due to the heterogeneity of the compost samples, each sample was replicated four times and the replicates were finally pooled after the extracts had been filtered. A sub-sample (5 ml) was then taken and analysed for DOC concentration using San^{++} channel SFA (SKALAR, The Netherlands). The remaining samples were used for DOC fractionation.

The batch fractionation procedure (Van Zomeren and Comans, 2007) was used to separate the DOC fractions. Briefly, 40 ml of the DOC sample was added in a 50 ml centrifuge tube, acidified to pH 1.0 with 6 M HCl and allowed to stand overnight. This step allowed the humic acid (HA) fraction to precipitate and form pellets. The acidified solution was then centrifuged (20 min, 3500g) to separate the HA (*i.e.* the pellets) from the supernatant containing FA, HON and Hi (FaHiHON). About 25 ml of 0.1 M KOH (pH 12.0) was added to the pellets and shaken for 20 min to re-suspend the HA fraction. The supernatant was transferred to another 50 ml centrifuge tube. About 15 ml of the supernatant was added to a 3 gm DAX-8 resin (Sigma–Aldrich), shaken for one hour and filtered through 0.45 μm (Whatman ™). This step separated the hydrophilic compounds (Hi) from the supernatant. Finally, 15 ml 0.1 M KOH was added to the DAX-8 resin, shaken for one hour, and filtered through 0.45 μm (Whatman™) to separate the fulvic acid (FA) fractions. This step was repeated three or more times until the concentration in the samples was equal to the blank samples. The neutral (HoN) fractions were estimated from the DOC that was not dissolved under alkaline conditions (FaHiHON - (FA + Hi)). The concentrations of each fraction (*i.e.* HA, Hi, FA and HoN) were measured using San^{++} channel SFA (SKALAR, The Netherlands).

2.3. Gas sampling

The static chamber method was used to collect gas samples (Chan et al., 2010). The gas samples were collected every two days for the first week and then once a week until the end of the experiment, after 60 days. Gas samples were collected three times after closing the chamber (0, 20 and 40 min). Gas samples were also collected after 0, 20, 40, 60, 80 and 100 min every month in order to check the linearity assumption. The gas samples were measured using INNOVA 1412 photoacoustic field gas monitor (LumaSense Technologies, Ballerup, Denmark). The emissions rate in mg kg^{-1} initial dry matter day^{-1} was calculated as:

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

where ΔC is the change in concentration of gas (ppm) during time interval Δt in days, V is the headspace volume (litres), M is the molecular mass of the gas of interest (44, 16 and 44 g for CO_2, CH_4 and N_2O respectively), Vs is the volume occupied by 1 mol of a gas at standard temperature and pressure (22.4 L), P is the atmospheric pressure (bar), Po is the standard pressure (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 integration rule (Ly et al., 2013).

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

where At(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 Fta and Ftb 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 Eq. (3):

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

2.4. Chemical analyses

Compost samples were collected at the end of the experiment for the analyses of pH, NO_3^- and NH_4^+. The samples were stored at 4 °C prior to laboratory analysis. pH was measured from a compost:water ratio of 1:10 (w/v), whereas NO_3^- and NH_4^+ concentrations were determined using 1 M KCl. The compost samples were mixed with 1 M KCl at a ratio of 1:100 compost:solution (w/v) and shaken for one hour. The extracts were then analysed for NH_4^+ and NO_3^- concentrations using segmented flow analysis (SFA) (SKALAR analytical, the Netherlands).

2.5. Statistical analyses

A two-way analysis of variance (ANOVA) was performed to test for significant effects of earthworm treatments, substrate quality and their interactions. A separate ANOVA was performed - excluding the control treatment - to test for significant effects of two feeding ratios on the vermicomposting process. The data were checked for the assumptions of ANOVA prior to data analysis. Levene and Shapiro-Wilk's tests were used to test for homogeneity of variance and normality respectively. Data on the change in earthworm biomass did not fit with ANOVA assumptions and hence the data were log-transformed. Tukey test was used to compare the means if the factors' effect was significant at $P < 0.05$. Linear regression was performed between the CO_2 emissions in the last week as an independent variable and DOC quantity and DOC composition as a dependent variable. All statistical analyses were undertaken using SAS version 9.2.

3. Results

3.1. DOC quantity and composition

The effects of earthworms on DOC concentration are presented in Fig. 1. There was a significant effect of earthworm treatment ($P < 0.001$) and substrate quality ($P < 0.001$) on DOC concentration as well as a significant interaction between them ($P = 0.001$). The presence of earthworms reduced DOC concentration by 7–28%, depending on substrate quality and feeding ratio. The DOC concentration per total C mineralised was calculated, and earthworms decreased the total DOC concentration by 38–60% compared with the non-earthworm treatments. The effect of earthworms on DOC was observed more in substrate_3. DOC contents were lower than 4 g kg^{-1} dry matter with substrate_1, around 4 g kg^{-1} dry matter with substrate_2 and higher than the critical limit value of 4 g kg^{-1} dry matter for substrate_3, indicating decreasing stability of the final product. The optimal feeding ratio significantly ($P < 0.01$) decreased DOC concentration compared with the higher ratio.

The percentage of DOC retrieved throughout the fractionation procedure was 85–98%, which was comparable with the previous study on compost (Straathof and Comans, 2015). Concentrations of hydrophilic and hydrophobic (HA, FA, HON) fractions of DOC varied between treatments and there was a significant earthworm x substrate interaction (Table 1). FA was the dominant proportion of DOC (>45%) while HON contributed the lowest proportion (<14%), irrespective of the treatments (Supplementary Fig. 1). Analysis of variance showed that the concentrations of HA, FA, HON and Hi were affected by substrate quality ($P = 0.001$, $P = 0.001$, $P < 0.001$ and $P < 0.001$ respectively), but the presence of earthworms only had a significant effect on HA and FA ($P = 0.001$ and $P = 0.001$ respectively). Substrates that underwent the longest pre-composting period (*i.e.* substrate_1) had the lowest concentrations of HA, FA, HON and Hi. The relative proportions of each fraction to total DOC are presented in Supplementary Fig. 1. The presence of earthworms decreased the relative proportion of HA to total DOC ($P = 0.02$), but the relative proportions of FA, HON and Hi were not significantly affected by the earthworms. Similarly, feeding ratio did not affect the proportion of each fraction to total DOC. Linear regression between the last week of CO_2 evolution and the concentration of DOC and its different fractions (Fig. 2) showed a significantly positive relationship for all components ($P < 0.001$ in all cases). The stability of the various com-

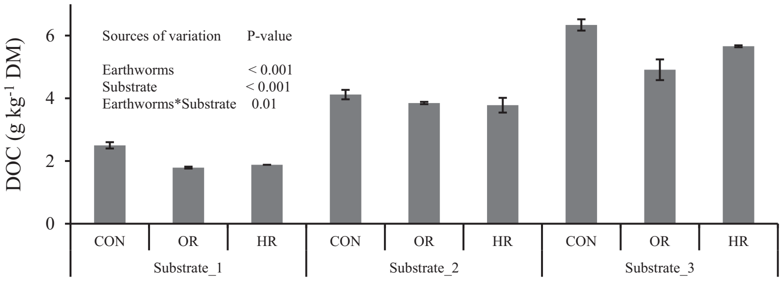

Fig. 1. DOC concentration of the composts after 60 days of composting (mean + standard error of the mean; n = 3). CON = without earthworms, OR = optimal substrate-to-earthworm ratio, HR = higher substrate-to-earthworm, DM = dry matter.

Table 1
Absolute concentration (mean + SEM) of the different DOC fractions after 60 days of composting.

Substrate		DOC fractions (g kg^{-1} DM)			
		HA	FA	HON	Hi
Substrate_1	CON	0.34 ± 0.07	1.13 ± 0.10	0.37 ± 0.03	0.38 ± 0.03
	OR	0.20 ± 0.10	0.90 ± 0.10	0.23 ± 0.12	0.32 ± 0.01
	HR	0.22 ± 0.02	0.94 ± 0.17	0.22 ± 0.18	0.31 ± 0.01
Substrate_2	CON	0.61 + 0.06	2.02 ± 0.09	0.41 ± 0.04	0.80 ± 0.07
	OR	0.53 ± 0.09	1.75 ± 0.01	0.56 ± 0.04	0.67 ± 0.03
	HR	0.52 ± 0.11	1.61 ± 0.01	0.60 ± 0.12	0.78 ± 0.04
Substrate_3	CON	1.15 ± 0.07	2.97 ± 0.23	0.62 ± 0.01	0.99 ± 0.10
	OR	0.57 ± 0.10	2.79 ± 0.27	0.52 ± 0.06	0.95 ± 0.08
	HR	0.82 ± 0.06	2.76 ± 0.11	0.62 ± 0.05	1.06 ± 0.04
ANOVA					
Earthworm (E)		<0.001	<0.001	0.42	0.49
Substrate (S)		<0.001	<0.001	<0.001	<0.001
Earthworm * Substrate		0.007	0.18	0.01	0.03

CON = without earthworms, OR = optimal substrate-to-earthworm ratio, HR = high substrate -to-earthworm ratio, HA = humic acid, FA = fulvic acid, HON = hydrophobic neutral, Hi = hydrophilic compounds, SEM = standard error of the mean, DM = dry matter.

pounds, as judged from the slope for the various compounds, was different. With a 50% reduction of the final CO_2 flux, HA was most reduced (to 40%), followed by DOC, FA and Hi (51–53%), while HON was most stable (reduced to 59%).

3.2. GHG emissions

The earthworm treatments and substrate quality influenced GHG emissions during composting (Fig. 3). Total cumulative CO_2 emissions differed between the earthworm treatments ($P < 0.001$) and substrate quality ($P < 0.001$). There was also an interaction between the two factors ($P < 0.001$). The presence of earthworms increased CO_2 production mainly from substrate_2 and substrate_3. Similarly, the optimal feeding ratio increased CO_2 emissions from vermicomposting compared with the higher ratio ($P < 0.001$). N_2O emissions were affected by the earthworm treatments ($P = 0.001$) and substrate quality ($P < 0.001$). The high feeding ratio increased cumulative N_2O emissions compared with the optimal ratio ($P < 0.001$), but no difference was observed between high feeding ratio and composting without worms. CH_4 production was very small in all treatments, and its contribution to the GHG budget was negligible and there was no significant effect of the treatments (data not shown). When CO_2 was excluded from the total GHG emissions, the optimum feeding ratio decreased total GHG emissions compared to the high feeding ratio and non-earthworm treatment. However, both earthworm treatments had a higher total GHG budget than non-earthworm treatments ($P < 0.001$) when CO_2 was included in the total GHG budget. Similarly, the optimum feeding ratio had higher total GHG emissions than the high feeding ratio ($P < 0.001$) when CO_2 was accounted for in the total GHG budget.

3.3. Chemical properties

The NO_3^- concentration differed between the earthworm treatments ($P < 0.001$), substrate quality ($P < 0.001$) and there was also a significant interaction ($P = 0.02$). The presence of earthworms increased NO_3^- concentration by up to 400% compared with the non-earthworm treatments. NH_4^+ concentration was not, however, affected by the earthworm treatments ($P = 0.49$), substrate quality ($P = 0.51$) and there was no interaction effect ($P = 0.71$). The NH_4^+: NO_3^- ratio varied between the earthworm treatments ($P < 0.001$), substrate quality ($P = 0.02$) and there was also a significant interaction ($P = 0.03$). The earthworm treatments reduced the NH_4^+: NO_3^- ratio by up to 50–80%. The pH was affected by the earthworm treatments ($P < 0.001$) and substrate ($P = 0.01$). The presence of earthworms reduced pH compared with non-earthworm treatments, irrespective of the substrate quality. The optimal feeding ratio resulted in lower pH compared to the high feeding ratio ($P < 0.01$).

3.4. Earthworm biomass

The change in earthworm biomass was significantly affected by substrate quality ($P = 0.01$), but not by feeding ratio ($P = 0.03$) or the interaction ($P = 0.24$). The substrate that pre-decomposed for nearly 1½ months resulted in the highest increase in earthworm biomass (Fig. 5).

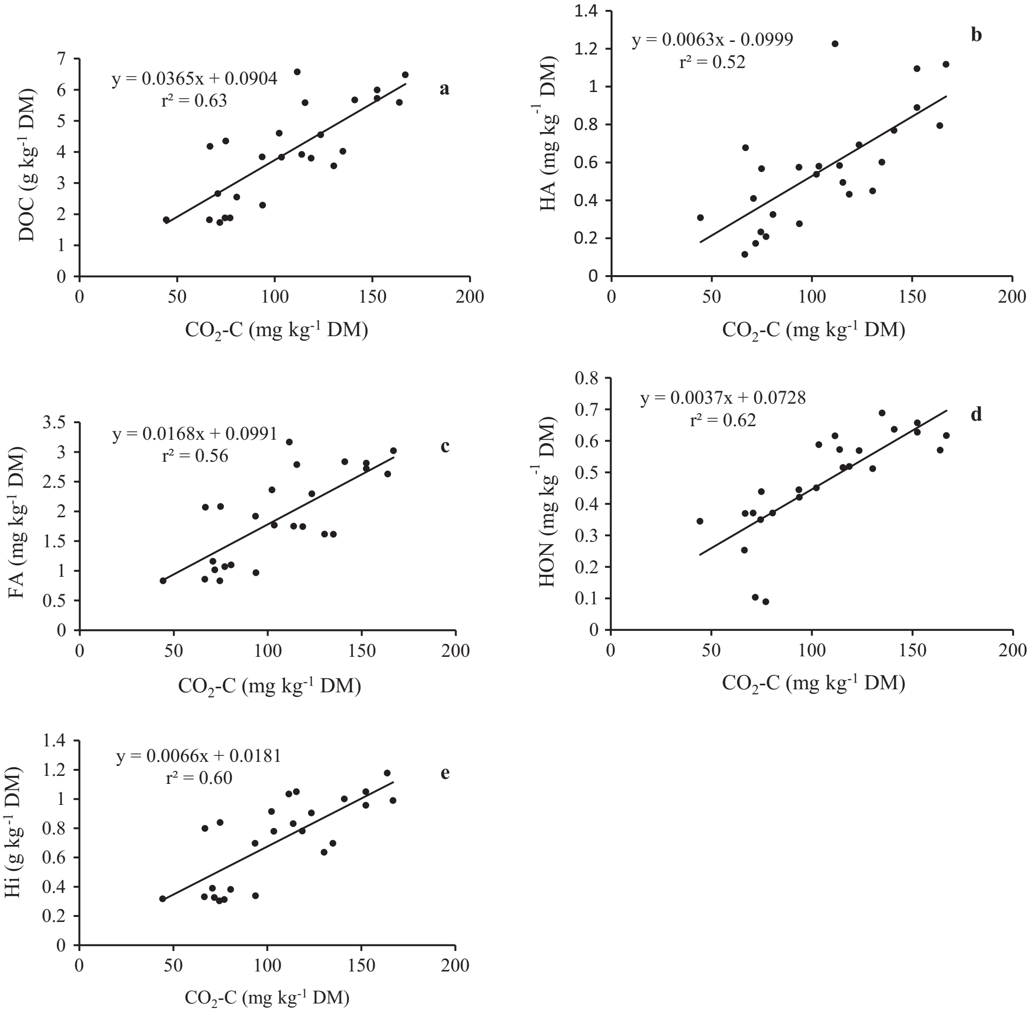

Fig. 2. Linear regression between CO_2-C emissions at the end of experiment and different dissolved organic carbon pools at the end of experiment: (a) total DOC, (B) humic acid, (c) fulvic acid, (d) hydrophobic neutral, (e) hydrophilic compounds. DM = dry matter.

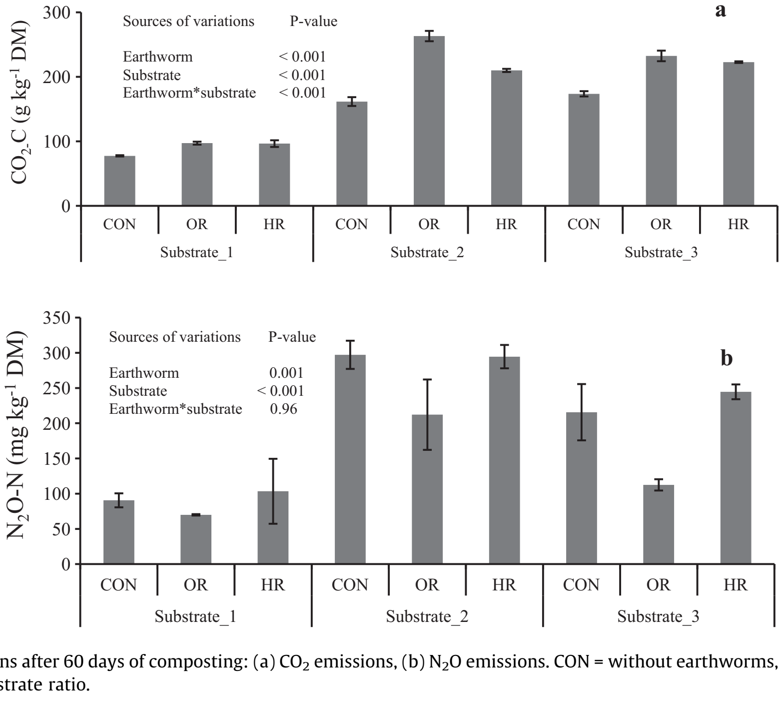

Fig. 3. Total cumulative GHG emissions after 60 days of composting: (a) CO_2 emissions, (b) N_2O emissions. CON = without earthworms, OR = optimal earthworm-to-substrate ratio, HR = higher earthworm-to-substrate ratio.

4. Discussion

4.1. DOC quantity and composition

The hypothesis that earthworms decreased the concentration of DOC compared with the non-earthworm treatment was confirmed. The result was consistent with the increase in C mineralisation caused by the earthworms (Figs. 2a & 3a). In agreement with our findings, Hanc and Dreslova (2016) and Lazcano et al. (2008) found a lower DOC concentration after vermicomposting than after thermophilic composting. A new vermicomposting parameter (*i.e.* feeding ratio) was considered in the present study, and demonstrated to have a significant effect on the DOC concentration (Fig. 1). The optimum feeding ratio decreased the DOC concentration by 5–14% compared with the high feeding ratio. The effect of feeding ratio on DOC was largest in the substrate that had undergone a shorter pre-composting period (substrate_3), and this is mainly due to the high DOC content in the manure (17 g kg^{-1} dry matter) (data not shown). DOC contains easily degradable compounds and its concentration declines consistently with composting time. Similarly, it was found that composts that had undergone a longer pre-composting period (substrate_1) had a lower DOC content. The significant correlation between DOC concentration and CO_2 emissions from the last week of composting ($r^2 = 0.63$; $P = 0.001$) (Fig. 2a) confirmed active decomposition in the substrates with a high DOC content. The DOC concentration of compost influences microbial activities and C mineralisation after soil application (Straathof et al., 2014) and hence it is used as an indicator of compost stability. Composts with a low DOC content (less than 4 g kg^{-1} dry matter) are considered more stable (Bernal et al., 2009), implying that the earthworms, especially at optimum feeding ratios, stabilise compost more quickly than non-earthworm treatments. The possible explanation for the higher stabilisation caused by earthworms is that they enhance the decomposition process through their interaction with microorganisms. Substrate_3 was not fully stable because it had high total DOC content (5–6 g kg^{-1} dry matter), and this is mainly due to the presence of manure that underwent pre-decomposition for several days. The DOC concentration observed in this study was comparable with the earlier composting studies (Hanc and Dreslova, 2016), which reported a DOC concentration in the range 3–6 g kg^{-1} dry matter. However, Straathof and Comans (2015) reported a low DOC content (<0.5–1.0 g kg^{-1}) in some composts, although their municipal-waste compost also contained high amounts of DOC (5–7 g kg^{-1} dry matter). Their values cannot be directly compared with our results, as in their composts organic materials were mixed with soil prior to composting. After correcting for non-organic (mineral) soil components, their compost contained between 2–3 g kg^{-1} dry organic matter (compost with woody material or forest leaf litter) up to 19 g kg^{-1} dry matter (for municipal organic waste). Current literature often reports the DOC concentration per dry matter without considering the mineral fractions of compost materials (i.e. soils). Hence, we recommend that DOC be expressed per weight of organic materials after discounting mineral content in order to assess compost stability. In our study, however, only organic materials were used and hence the DOC values could be used directly to indicate compost stability.

The concentrations of different DOC compositions (FA, HA, HON and Hi) was affected by the earthworm treatments, substrate quality and their interaction (Table 1). The presence of earthworms decreased concentrations of FA and HA, whereas the concentrations of all four components of DOC were lower in vermicompost than in compost in the absence of worms. The low concentration of the DOC fractions in the earthworm treatments coincided with the decrease in the total DOC concentration (Fig. 1). Therefore, the relative proportion of each fraction to total DOC (Supplementary Fig. 1) could provide more reliable information on the earthworms' effect on DOC composition and the turnover of the fractions during composting.

Straathof and Comans (2015) reported the DOC composition of composts produced from different input materials and processing conditions and noted significant variation in the relative contribution of HA, FA, HON and Hi in the various composts. Our study showed only small effects of treatments and substrate on the fractional contribution to total DOC. One major explanation for the discrepancy is that these authors used a larger range of input material (from wood and forest leaf litter to municipal waste) and there were also large differences between composting practices (temperature, time).

We hypothesised, in agreement with Straathof and Comans (2015) that during the composting process, Hi compounds are depleted more quickly than the three hydrophobic compounds because the hydrophilic fraction consists of low-molecular-weight sugars and amino acids, which are readily available C sources for microorganisms. Straathof and Comans (2015) reported a lower Hi proportion, but higher proportions of HA, FA and HON in composts that had undergone a longer composting period and high temperature (>70 °C) compared with composts produced at a lower temperature with a composting period of <28 days (*i.e.* unstable compost). They suggested that the fractional contribution of Hi to DOC may be a better indicator for the contribution of compost on microbial activity. However, our data contradict this hypothesis. In fact, the fractional contribution of HA declined with increasing stability; and a ranking of the compounds with increasing stability was $HA > DOC \approx FA \approx Hi > HON$. We suggest two mechanisms to explain why HA turned out to be the least stable component. First, decline of HA (and FA to a smaller extent) is likely indicative for ligninolysis, whereas decline of Hi is indicative for cellulolysis. While it has for a long time been taken for granted that degradation of lignin proceeds at a slower rate than degradation of cellulose, recent data have actually indicated a faster decomposition of lignin than cellulose (Klotzbücher et al., 2011). Secondly, but equally important, changes in the various pools are driven both by depletion of the compound during decomposition but also by novel production during degradation and/or changes in solubility of compounds. Van Zomeren and Comans (2007) put their study in the framework of a novel perspective on humic substances as supramolecular associations of compounds with relatively small mass, and observed that at declining HA concentrations the chances increased that part of the HA fraction actually showed up in the FA fraction. Further research is needed to evaluate both potential mechanisms. Considering that the concentrations of HA and FA, but not those of HON and Hi, were lower in the vermicompost than in the non-worm compost, independent of the total amounts of DOC, we hypothesise a role of earthworms and their associated microbes in the breakdown of aromatic polymers. Earthworms also secrete mucus, and these compounds may be rich in carbohydrates and proteins (Pan et al., 2010) that may also preferentially end up in the Hi fraction. The HON fraction was not affected by the presence of earthworms and/or feeding ratio, and it is mainly because this fraction contains aliphatic compounds, including quinones (Straathof and Comans, 2015), which are resistant against microbial degradation. Next to the fractional contribution of individual compounds, the ratio of hydrophilic to hydrophobic substances (HA + FA + HON) may provide indications of compost stability. Our compost showed ratios between 0.20 and 0.30, while the study of Straathof and Comans (2015) indicated ratios of 0.66–0.79 for unstable composts.

To our knowledge, no studies have been conducted to assess the effect of earthworms on DOC composition. Further studies are recommended to advance understanding of the effect of earthworms on the DOC pools using different parameters such as substrate quality (*i.e.* fresh organic material), earthworm species and earthworm density.

4.2. Greenhouse gas emissions

As hypothesised, earthworms increased CO_2 emissions compared with non-earthworm treatments, and the results were comparable with earlier studies (Chan et al., 2010; Nigussie et al., 2016). Similarly, the CO_2 emissions from vermicomposting were higher at optimum feeding ratio than at the high ratio, as hypothesised. Higher cumulative CO_2 emissions indicated a greater stability of compost and confirmed that the earthworms resulted in compost that was at a more advanced stage of stabilisation. The CO_2 results were in agreement with the observation on DOC quantity (Fig. 1).

The hypothesis that the high feeding ratio increases N_2O emissions from vermicomposting was confirmed. As compared to the non-earthworm treatment, earthworms decreased N_2O emissions by 23–48% at the optimum feeding ratio. At the higher ratio, however, earthworms had no significant effect on N_2O emissions compared with the non-earthworm treatments. The low N_2O production in the earthworm treatments could be explained by: (i) continuous turning of substrates by earthworms which subsequently increases aeration (Chan et al., 2010; Nigussie et al., 2016) and (ii) high substrate stability after it has passed through the earthworms' gut (Luth et al., 2011). Hence, the low DOC concentration as evidence for compost stability (Fig. 1) could explain the low N_2O emissions in the earthworm treatments. At the higher feeding ratio, however, the presence of anaerobic patches and the lower degree of stabilisation (Fig. 1) reduced the mitigation effect of earthworms on N_2O emissions.

Reports on the effect of earthworms on N_2O emissions are contradictory. For instance, Hobson et al. (2005) and Lubbers et al. (2012) reported earthworm-induced N_2O emissions. In contrast, Chan et al. (2010), Nigussie et al. (2016) and Wang et al. (2014) found that earthworms decreased N_2O emissions during composting. The contrasting results can be explained by differences in earthworm species (*i.e.* difference in their feeding and burrowing behaviours) (Lubbers et al., 2013), substrate quality (*i.e.* carbon quality and nitrogen content) (Luth et al., 2011; Nigussie et al., 2016), temperature (Nigussie et al., 2016) and the scale of the experiment (Chan et al., 2010). In our study the decreasing effect of earthworms on N_2O emissions occurred at optimum feeding ratio, while at a high feeding ratio earthworms did not decrease N_2O emission, implying that feeding ratio is an important parameter for consideration when assessing the earthworms' effect on GHG emissions during composting. Denitrification in the earthworm gut was the main process contributing to N_2O emissions in the case of anecic earthworms (Lubbers et al., 2013). Composting materials mostly contain high levels of nitrogen, hence the contribution of denitrification occurring in the earthworm gut to total N_2O emissions was smaller than the contribution from denitrification in the environment around the worms. Furthermore, the feeding and burrowing behaviours of earthworms mostly used in soil experiments (i.e. *anecic* earthworms) are different from compost worms.

Two scenarios were used to assess the effect of earthworm treatments on total GHG emissions. The first scenario excluded CO_2 because higher CO_2 emissions indicate a greater stability of the material. Including CO_2 in the GHG balance would therefore privilege composts that are not stabilised over the course of the experiment, and therefore provide a biased assessment how earthworms affect the GHG balance (Chowdhury et al., 2014). Under this scenario, the effect of earthworm treatments on the total GHG emissions was similar to their effect on N_2O emissions since the contribution of CH_4 was negligible. However, when CO_2 was included in the total GHG budget, the earthworm treatments increased total GHG emissions compared with the non-earthworm treatments. This variation was explained by the high CO_2 emissions in the earthworm treatments (Fig. 3a), and the large contribution of CO_2 to the total GHG budget in all treatments (>80%). These results are comparable with previous studies on composting (Andersen et al., 2010; Nigussie et al., 2016).

4.3. Properties of compost

Irrespective of substrate quality, the presence of earthworms and optimum feeding ratio decreased pH, but increased the NO_3^- concentration. The high NO_3^- concentration in the earthworm treatments and optimum feeding ratio was explained by the mineralisation of N from the organic materials and/or reduced N_2 losses through denitrification (Fig. 3b). The NH_4^+ concentration decreases during compositing due to ammonia volatilisation and nitrification processes, while the NO_3^- concentration increases towards the end of the composting period. Hence, the NH_4^+: NO_3^- ratio has been used to assess the maturity of compost (Bernal et al., 2009), and the threshold value of <0.16 is an indicator of mature compost, implying that all the composts were mature. Still the earthworm treatments produced more mature compost than the non-earthworm treatments. We used the total DOC content and NH_4^+: NO_3^- ratio to estimate compost stability, and both indices yield the same result except for substrate_3. The presence of manure increased the NO_3^- concentration in substrate_3, consequently reduced the NH_4^+: NO_3^- ratio, suggesting that NH_4^+: NO_3^- ratio depends on the substrate quality and hence it may not be a reliable index compared with DOC. The decrease in pH values in the earthworm treatments at optimum feeding ratio could be due high nitrification in these treatments (Fig. 4a) and the production of organic acids as a result of greater decomposition (Figs. 1 & 3a) (Lazcano et al., 2008).

The data presented here confirmed a significant effect of feeding ratio on the stabilisation of organic material (Fig. 1), GHG emissions (Fig. 3) and properties of the end product (Fig. 4), however (applied) researchers on vermicomposting have paid little attention to feeding ratio. Feeding ratio influences earthworm growth (Fig. 5: Luth et al., 2011), aeration and temperature, subsequently affecting decomposition, mineralisation, nutrient losses and GHG emissions. Hence, it is suggested that this ratio be considered an equally important parameter when evaluating the effectiveness of earthworms in the stabilisation of organic materials to parameters such as earthworm species, and substrate quality.

5. Conclusions

Earthworms accelerated the stabilisation of organic materials compared with the non-earthworm treatments, as confirmed by CO_2 production, DOC concentration, DOC composition and NH_4^+: NO_3^- ratio. Earthworms decreased the total DOC concentration, but they did not affect the relative composition of hydrophilic, fulvic acid and hydrophobic neutral fractions. The relative contribution by humic acid decreased by 15–45% in the earthworm treatments, implying that the humic acid fraction was less recalcitrant than commonly assumed, and was likely used as a substrate by microorganisms. The hydrophilic-to-hydrophobic ratio was consistent between the different stable composts, despite differences between input materials and processing conditions, and this ratio could be used as an additional criterion to assess compost stability. This ratio is much higher unstable composts, implying that it can be used as an indicator of compost stability. Fractionation of DOC is therefore important for understanding the stabilisation of organic waste. A higher (supra-optimal) feeding ratio reduced the

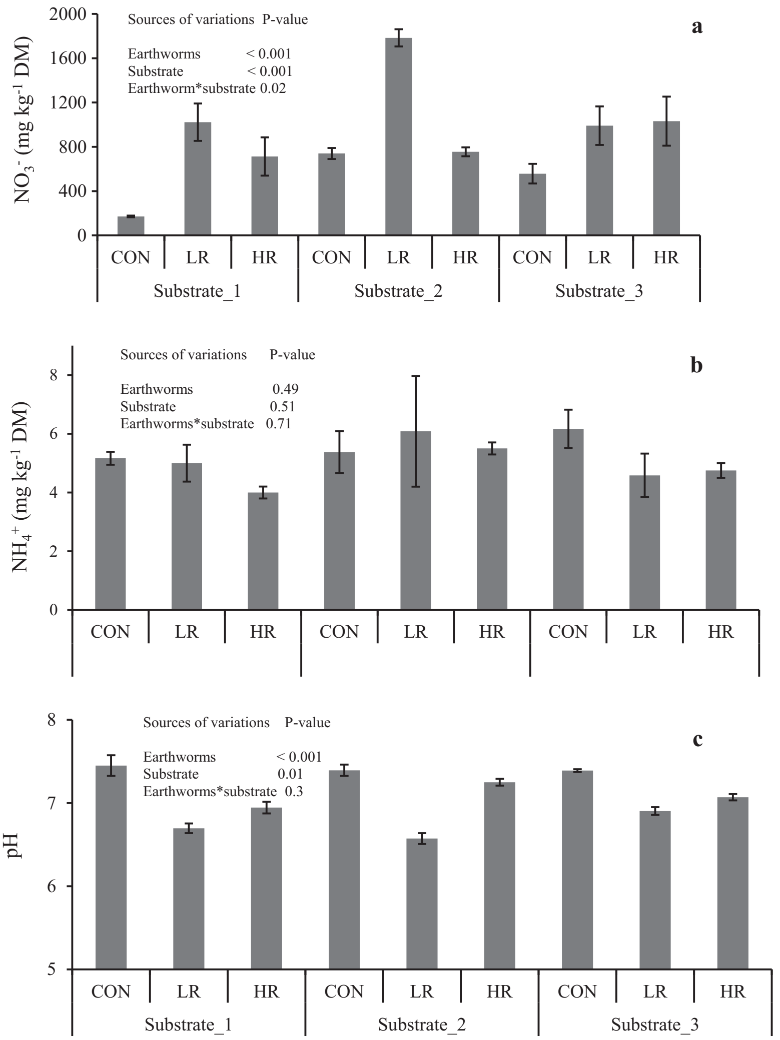

Fig. 4. Chemical properties of the composts after 60 days of composting (mean ± standard error of the mean; n = 3). (a) NO_3^- (b) NH_4^+(c) pH. CON = without earthworms, OR = optimal substrate-to-earthworm ratio, HR = high substrate-to-earthworm ratio.

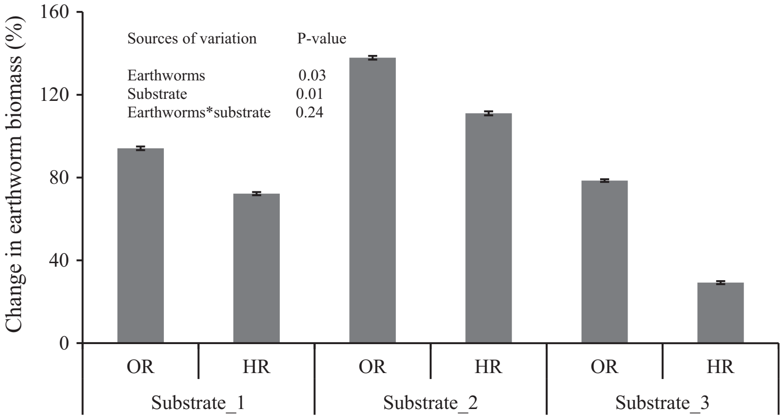

Fig. 5. Change in earthworm biomass after 60 days of vermicomposting. LR = optimal substrate-to-earthworm ratio, HR = high substrate-to-earthworm ratio.

stabilisation process and increased N_2O emissions by 30–50% compared with the optimum feeding ratio. Hence feeding ratio should be considered as an important parameter when assessing the earthworms' effect on the stabilisation of organic materials and GHG emissions during composting.

Acknowledgements

This study was conducted as part of a PhD thesis project supported by the Agricultural Transformation by Innovation (AgTraIn) Erasmus Mundus Joint Doctorate Programme, funded by the EACEA

(Education, Audio-visual and Culture Executive Agency) of the European Commission. The authors would like to thank Mr Jaap Nelemans for laboratory assistance, Mr. Lou Derks from De Polderworm for providing the earthworms and technical advice, prof. Rob Comans for helpful discussion on DOC fractionation and the interpretation of the various fractions, and three anonymous reviewers for constructive criticism on an earlier version of the manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.wasman.2017.02.009.

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