Waste Management xxx (2017) xxx–xxx

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 xxxx

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/

Please cite this article in press as: Nigussie, A., et al. Earthworms change the quantity and composition of dissolved organic carbon and reduce greenhouse gas emissions during composting. Waste Management (2017), http://dx.doi.org/10.1016/j.wasman.2017.02.009

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)

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