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Delayed addition of nitrogen-rich substrates during composting of municipal waste: Effects on nitrogen loss, greenhouse gas emissions and compost stability



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HIGHLIGHTS

• Nitrogen rich (N-rich) substrate was added at different times during composting.

• Delayed addition of an N-rich substrate reduced nitrogen loss from compost.

 \bullet The timing of the addition of an N-rich substrate affected N_2O and CH_4 emissions.

• Delayed addition of N-rich substrate resulted in stable compost as early addition.

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ABSTRACT

Municipal waste is usually composted with an N-rich substrate, such as manure, to increase the N content of the product. This means that a significant amount of nitrogen can be lost during composting. The objectives of this study were (i) to investigate the effect of split addition of a nitrogen-rich substrate (poultry manure) on nitrogen losses and greenhouse gas emissions during composting and to link this effect to different bulking agents (coffee husks and sawdust), and (ii) to assess the effect of split addition of a nitrogen-rich substrate on compost stability and sanitisation. The results showed that split addition of the nitrogen-rich substrate reduced nitrogen losses by 9% when sawdust was used and 20% when coffee husks were used as the bulking agent. Depending on the bulking agent used, split addition increased cumulative N₂O emissions by 400-600% compared to single addition. In contrast, single addition increased methane emissions by up to 50% compared to split addition of the substrate. Hence, the timing of the addition of the N-rich substrate had only a marginal effect on total non-CO2 greenhouse gas emissions. Split addition of the N-rich substrate resulted in compost that was just as stable and effective at completely eradicating weed seeds as single addition. These findings therefore show that split addition of a nitrogen-rich substrate could be an option for increasing the fertilising value of municipal waste compost without having a significant effect on total greenhouse gas emissions or compost stability.

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1. Introduction

A large amount of municipal waste is generated globally due to rapid urban population growth (https://paperpile.com/c/klQWBi/ OYHNScarlat et al., 2015https://paperpile.com/c/klQWBi/ltBc). In many developing countries, over 80% of municipal waste ends up in landfills or incineration sites (Scarlat et al., 2015). Studies have shown that biodegradable materials constitute more than 50% of municipal waste in developing countries (Getahun et al., 2012a) and therefore composting has been suggested as a method to reduce the amount of municipal waste in landfill sites. More importantly, composting of municipal waste can be used to recycle nutrients from urban areas to agricultural land, thereby closing the rural-urban nutrient loop (Nigussie et al., 2015) and contributing to a circular economy.

Municipal waste compost contains a high amount of organic matter, and incorporation in soil may therefore increase soil aggregate stability, reduce the draught force for soil tillage (Peltre et al., 2015), promote soil microbial activity and enhance soil



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chemical properties (Hargreaves et al., 2008). No adverse effects of the application of municipal waste compost on croplands have been reported (https://paperpile.com/c/klQWBi/uXtuPoulsen et al., 2013).

However, municipal waste composts, particularly in many (sub) tropical countries, contain low levels of nitrogen (N), commonly < 1% of dry matter (Getahun et al., 2012b). In addition, less than 10% of total N is mineralised from municipal waste compost in the first year after soil application (Zhang et al., 2006). This means that the compost will also contain relatively little mineralisable nitrogen, therefore relatively large amounts (40–50 Mg ha⁻¹) of compost have to be applied to satisfy plant requirements (Hargreaves et al., 2008). However, high input of municipal waste compost might result in losses of other nutrients and the accumulation of heavy metals in soils and plant tissues, thus impeding the ecological and environmental benefits of the compost. The optimisation of municipal waste composting should therefore place an emphasis on increasing the nitrogen concentration in the compost while reducing losses of other nutrients.

The composting of municipal waste and N-rich substrates such as poultry manure generally increases the N concentration in the compost (Lhadi et al., 2006; Rizzo et al., 2015). Poultry manure has a total N content greater than 3% dry matter, but contains pathogens such as *E. coli* and *Salmonella* and weed seeds, implying that poultry manure should be treated (composted) prior to soil application. Composting of municipal waste with poultry manure is therefore a strategy for increasing the N concentration in compost and sanitising poultry manure.

Temperatures above 45 °C during composting. *i.e.* the thermophilic phase, are essential for compost sanitisation. High temperatures (>45 °C) shift the NH₄⁺ \leftrightarrow NH₃ equilibrium towards ammonia (Pagans et al., 2006) and inhibit nitrification, both of which increase ammonia volatilisation. Poultry manure has a high NH₄⁺ concentration, implying that composting of municipal waste with poultry manure may increase ammonia volatilisation, and thereby result in high N losses. High N loss has also been observed in experiments. For example Dias et al. (2010) and Rizzo et al. (2015) report over 65% N loss during the thermophilic phase of poultry manure and crop residue composting. Minimising N losses is therefore essential for increasing the agronomic value of the final product and reducing environmental problems. Split addition of an N-rich substrate (i.e. poultry manure) has been suggested as a way of reducing N losses from composting (Bryndum, 2014; Dresbøll and Thorup-Kristensen, 2005). During split addition of an N-rich substrate, the first part is added at the beginning of composting in order to support the turnover of carbon (C). The remaining part is then added later (i.e. after the thermophilic phase) in order to increase the N concentration in the compost. It is hypothesised that split application of an N-rich substrate reduces N losses via ammonia volatilisation.

C-rich materials (bulking agents), such as crop residues, are commonly added to compost mixtures to adjust the C/N ratio so that immobilisation prevents losses and to adjust the moisture content and allow air movement through the compost pile (Yang et al., 2013). These C-rich materials influence the composting temperature (Santos et al., 2016) and N transformations such as mineralisation, immobilisation and volatilisation (Chowdhury et al., 2014). However, it is not known how the timing of the addition of an N-rich substrate affects N losses under different C-rich materials.

In addition to high ammonia loss, composting produces a significant amount of greenhouse gases in the form of methane and nitrous oxide (Chowdhury et al., 2014). Inorganic N concentration (Cayuela et al., 2012), C quality (Chowdhury et al., 2014) and temperature are among the key factors that influence greenhouse gas (GHG) emissions during composting. Methane (CH₄) is mainly produced in the thermophilic phase (Sánchez-Monedero et al., 2010) due to the presence of labile carbon compounds in conjunction with anoxic microsites. Nitrous oxide (N₂O) is emitted after the thermophilic stage because nitrifying and denitrifying bacteria are mesophilic (Vu et al., 2015). Hence, nitrification and denitrification, which are the major N₂O-producing processes. hardly occur during the thermophilic stage of composting. The timing of the addition of an N-rich substrate might therefore influence GHG emissions from composting. For example, additions of an N-rich substrate after the thermophilic phase might increase N₂O emissions by increasing the bioavailability of mineral N for nitrifying and denitrifying bacteria. On the other hand, the single addition of an N-rich substrate could trigger CH₄ emissions due to the occurrence of anaerobic sites, high temperatures and bioavailable C and/or N for methanogenic bacteria. Studies also claim that high NH⁺₄ concentrations reduce methane oxidation due to the enzymatic similarity between methane and ammonium oxidations (Wei et al., 2016). However, no studies have been conducted to investigate the relationship between the timing of N-rich substrate addition and GHG emissions from composting.

Compost should be free from pathogens and weed seeds, and must have a high level of stability prior to soil application. Compost is considered stable when the rate of organic matter decomposition is reduced to an insignificant level. Application of unstable compost leads to oxygen depletion in the root zone, and may impede plant growth owing to its content of phytotoxic compounds (Wichuk and McCartney, 2010). Split addition of an N-rich substrate might be less efficient at eradicating weed seeds and producing stable compost than single addition because the newly added substrate will have less time to decompose. However, no studies have been conducted to evaluate the effect of split addition on compost stability and sanitisation.

The objectives of the study were (i) to investigate the effectiveness of split addition of an N-rich substrate on N losses and GHG emissions during composting using different bulking agents, and (ii) to assess the effect of split addition of an N-rich substrate on sanitisation and compost stability. It was hypothesised that (i) split application of an N-rich substrate reduces total N loss, with effects varying between bulking agents, (ii) split application of N-rich substrate decreases CH₄ emissions, but increases N₂O emissions from composting, and (iii) split application of an N-rich substrate is effective at eradicating weed seeds and is as efficient at producing stable compost as single addition.

2. Materials and methods

2.1. Experimental set-up

A composting experiment was carried out at the University of Jimma, Ethiopia for 90 d in 1 m \times 1 m x 1 m boxes made of wood (*Juniperus procera*). The study area is located at 7°41′N latitude, 36°50′E longitude and at an average altitude of 1780 m above sea level. The experiment was conducted in a greenhouse, and the room temperatures in the greenhouse ranged between 20 °C and 33 °C throughout the experimental period.

Biodegradable waste containing vegetables was collected from Jimma University's cafeteria. The vegetable mixture had 85% moisture, total carbon content of 410 g kg⁻¹ dry matter and total nitrogen content of 15 g kg⁻¹ dry matter. The vegetable wastes were mixed thoroughly with one of two bulking agents on a wet basis ratio of 3:1 (vegetable: bulking agent). The two bulking agents were coffee husks and sawdust – both locally available C-rich materials. These materials are also used as bulking agents for composting in many coffee-growing countries (Dias et al., 2010).

The coffee husks had a moisture content of 4.6%, a total carbon content of 411 g kg⁻¹ dry matter and a total nitrogen content of 12.2 g kg⁻¹ dry matter. The sawdust had 5% moisture, 457 g kg⁻¹ dry matter total carbon and 10.6 g kg⁻¹ dry matter total nitrogen. Fresh poultry manure was collected from Jimma University's experimental farm. The poultry manure had 6.4% moisture, total carbon content of 244 g kg⁻¹ dry matter and total nitrogen content of 37.6 g kg⁻¹ dry matter. The poultry manure was added to the vegetable waste and bulking agent mixture on a wet basis ratio of 3:3:1 (poultry manure: vegetable waste: bulking agent). The composting mixture containing coffee husk had a total carbon content of 390 g kg⁻¹ dry matter and a total nitrogen content of 27 g kg⁻¹ dry matter; whereas, the mixture containing sawdust had a total carbon content of 420 g kg⁻¹ dry matter and a total nitrogen content of 22 g kg⁻¹ dry matter.

The poultry manure was added to the composting mixtures in three different ways: (i) all poultry manure was applied at the beginning of composting, (ii) 80% of the poultry manure was applied at the beginning of composting and the remaining 20% added after the thermophilic phase, or (iii) 20% of the poultry manure was applied at the beginning of composting and the remaining 80% was added after the thermophilic phase. The experiment therefore had two factors: (i) two different bulking agents (i.e. coffee husks and sawdust), and (ii) three distributions of poultry manure application. The experimental design was a completely randomised design arranged as a 3×2 two-way factorial experiment with three replicates. Details of all treatments are presented in Table 1. The compost was turned every two weeks. The moisture content of the pile was adjusted to 60-65% by spraying water on top. The temperatures were recorded every 2 h using a temperature data logger (Tinytag View 2-TV-4020, United Kingdom). One data logger was placed in each box at the centre of the pile. In addition to this, the temperature was measured twice a day at different points in the pile. The temperature profile presented in Fig. 1 is the mean values of the three replicates.

2.2. Chemical analyses

Compost samples were collected at the beginning and end of the experiment for analysis of total carbon, total nitrogen, NO3 and NH⁴. The sampling was carried out from five different locations after turning the pile thoroughly. The pile was turned manually to ensure representative sampling. The five samples were then mixed to make a composite sample. Half of the samples were dried $(40 \circ C)$ and sent to the University of Copenhagen, Denmark, for analysis of total carbon and total nitrogen. Total carbon and total nitrogen were determined using an elemental analyser coupled to an isotope ratio mass spectrometer (PYRO Cube from Elementar, Germany coupled to an Isoprime100 IRMS, UK). The remaining samples were stored at -18 °C for NH⁺₄ and NO⁻₃ measurements. The NH⁺₄ and NO₃ concentrations were measured as described by Bryndum (2014). Briefly, compost samples were mixed with 1 M KCl in a 1:100 compost:solution ratio (w/v) and shaken for 1 h. The extracts were then analysed for NH_4^+ and NO_3^- concentrations using a flow injection analyser (FIA star TM 5000 analyser, Denmark). Waterextractable C was determined 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 1 h on the horizontal shaker and filtered through 0.45 µm (Whatman ™). Due to the heterogeneity of the compost samples, each sample was replicated four times and the replicates were finally pooled together after the extracts had been filtered through 0.45 μ m. A sub-sample (5 ml) was then taken and analysed for DOC quantity using San⁺⁺ channel SFA (SKALAR, The Netherlands). The final and initial total N concentrations were used to calculate the N loss during composting as:

Fractional nitrogen loss =
$$\frac{(Q_iCi - QfCf)}{QiCi} \times 100$$
 (1)

where *Qi* and *Qf* are total dry mass (kg) of the vegetable wastebulking material-manure mixture that was added during the experiment and remained at the end of the experiment respectively, and *Ci* and *Cf* are nitrogen concentrations that were added during the experiment and remained at the end of the experiment respectively.

2.3. Gas sampling

The static chamber method was used to collect gas samples (Cayuela et al., 2012). For gas sampling, a 20 L polyethylene gas chamber equipped with a rubber stopper was inserted 2 cm into the composting materials. The rim of the chamber was then covered in the composting material and water applied around it to provide a good air seal. Two mini fans (12 V) were installed in the polyethylene chamber to ensure homogeneous distribution of air in the headspace. Gas samples were collected by syringe every 2 d for the first week after the addition of substrates, and then once a week until the end of the experiment. Gas samples were collected at three time points (0, 20, 40 min) and injected into 3 ml screwcapped evacuated Exetainer[®] vials. Gas samples were also collected at 0, 20, 40, 60, 80 and 100 min every month in order to check the linearity assumption. The gas samples were analysed at the University of Copenhagen using gas chromatography (Bruker 450- GC 2011, United Kingdom). The emissions rate in mg kg^{-1} initial dry matter d⁻¹ was calculated as:

Emission rate =
$$\left(\frac{\Delta C}{\Delta t}\right) * \left(\frac{V}{W}\right) * \left(\frac{M}{Vs}\right) * \left(\frac{P}{Po}\right) * \left(\frac{273}{T}\right) * \left(\frac{A1}{A2}\right)$$
(2)

where ΔC is the change in concentration of gas (ppm) during time interval Δt in d, V is the headspace volume (L), M is the molecular mass of the gas of interest (44, 16 and 44 g for CO₂, CH₄ and N₂O, respectively), Vs is the volume occupied by 1 M 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, A1 and A2 are the area (m²) of the composting container and chamber respectively, and W is the initial dry mass of the composting material (kg).

The cumulative emissions were calculated using the trapezoid formula (Equation (3)) (Ly et al., 2013):

$$A_{t(ab)} = \frac{(t_b - t_a).(F_{ta} + F_{tb})}{2}$$
(3)

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

Therefore, the total cumulative emission was calculated as the sum of cumulative emissions on each day using Equation (4):

$$Total \ cumulative \ emission = \sum A_{t(ab)}$$
(4)

The global warming potential (CO₂-equivalents) of each treatment was calculated by multiplying total cumulative CH₄ and N₂O emissions by 34 and 298 respectively (IPCC, 2013). The CO₂ emission from composting is considered a biogenic gas (Vu et al., 2015) and hence is not included in the global warming potential of the

Table 1				
Codes and	detailed	descriptions	of all	treatments.

Code	Descriptions of the treatments
C-100	All poultry manure was applied at the beginning of composting and the bulking agent was coffee husks.
C-80/	80% of the poultry manure was applied at the beginning of composting and the remaining 20% was applied after the thermophilic phase. The bulking agent was
20	coffee husks.
C-20/	20% of the poultry manure was applied at the beginning of composting and the remaining 80% was applied after the thermophilic phase. The bulking agent was
80	coffee husks.
S-100	All poultry manure was applied at the beginning of composting and the bulking agent was sawdust.
S-80/	80% of the poultry manure was applied at the beginning of composting and the remaining 20% was applied after the thermophilic phase. The bulking agent was
20	sawdust.
S-20/	20% of the poultry manure was applied at the beginning of composting and the remaining 80% was applied after the thermophilic phase. The bulking agent was
80	sawdust.



Fig. 1. Temperature profiles during the composting of household waste and poultry manure. (a) Substrates with coffee husks, and (b) substrates with sawdust; C = coffee husks, S = sawdust; C-100 and/or S-100 = all poultry manure was applied at the beginning of composting; C-80/20 and/or S-80/20 = 80% of the poultry manure was applied at the beginning of composting and the remaining 20% was added after the thermophilic phase; C-20/80 and/or S-20/80 = 20% of the poultry manure was applied at the beginning of composting and the remaining 80% was added after the thermophilic phase; arrows indicate the time of the second substrate addition.

process (Yang et al., 2013).

2.4. Seed germination

Seeds of four weed species, namely *Parthenium hysterophorus, Digitaria abyssinica, Lantana camara* and *Solanum marginatum*, were used. These weeds are common in many parts of the tropics and subtropics. Small nylon-mesh bags ($4 \text{ cm} \times 4 \text{ cm}$) were prepared and 20 seeds of each species placed in separate bags. The nylon bags were then put deep in the compost piles. At the end of the experiment, the nylon bags were retrieved and the seeds tested for

germination. For germination tests, the seeds were placed in 90 mm Petri dishes with moistened filter paper. The Petri dishes were sealed using paraffin film to reduce moisture loss and then placed in an incubator at 25 °C. Germination was periodically recorded for three weeks, after which time 5 ml of 0.2% KNO₃ was added to promote further germination. The seeds were kept in an incubator for a further two weeks.

2.5. Phytotoxicity assay

The germination test was conducted in three replicates to assess

the phytotoxicity of the composts. Twenty-five barley (Hordeum vulgare L.) seeds were placed in 90 mm Petri dishes, moistened with 5 ml of the compost extracts and incubated at 20 °C for 3 d. The extract was prepared from fresh compost using ultra-pure milli-Q water at a 1:10 ratio (w/v), shaken for 1 h on the horizontal shaker and filtered through 0.45 µm (Whatman TM). Similarly, a control was prepared from ultra-pure milli-Q water. The germination test was then calculated as:

Relative seed germination (%) = $\left[\frac{(Ne)}{Ne}\right]$

$$\frac{10. \text{ of seeds germinated in compost extract}}{(\text{No. of seeds germinated in control})} \times 100$$

2.6. Design of the incubation experiment

The incubation experiment was undertaken to determine compost stability and N mineralisation after soil application of the composts. Soils were collected from the plough layer of agricultural fields (Tasstrup, Denmark) which for over 30 years had only received mineral fertilisers. The soil samples were air-dried and sieved to ≤ 2 mm. The soil was a sandy clay loam (clay 15%, silt 18%, sand 65%) with a C content of 1.15%, total N content of 0.13% and a pH (CaCl₂) value of 5.6. Fifty grams of soil were added to 100 ml polyethylene cups, wetted to 60% water-holding capacity (WHC) and pre-incubated for two weeks. After that time, the composts – produced with different timings of substrate addition (Table 1) were applied to the incubated soils at a rate of 5% (dry weight basis) and mixed thoroughly. The treatments were replicated three times. The soil and compost mixtures were packed to 1.2 g cm^{-3} and the moisture content was adjusted to 60% WHC using milli-Q water. Soil without compost was packed to the same density and moisture content, and then used as a control. The experiment was conducted in the dark at a temperature of 15 °C. The weight of the cups was checked every week, and the water loss was adjusted by adding milli-Q water.

During gas sampling, the cups were placed in an airtight 0.9 L Kilner jar equipped with a rubber stopper and 5 ml gas samples were collected after 0, 60 and 120 min using an air-tight syringe and injected into 3 ml screw-capped Exetainer[®] vials. The samples were analysed for CO₂ concentration using gas chromatography (Bruker 450-GC 2011, United Kingdom). The gas samples were collected every 2 d for the first week and then once a week until the end of the experiment. The CO₂ emissions were calculated from the increase in CO₂ concentration inside the Kilner jars, assuming a linear increase in CO₂ concentration during sampling. The linearity assumption was checked once a month by collecting gas samples at 0, 20, 40, 60, 80 and 100 min.

Two destructive soil samples were collected at 21 and 60 d from the sub-set of each treatment. The NH_4^+ and NO_3^- concentrations were extracted using 1 M KCl solution. Briefly, 20 g of sample (dry matter basis) was mixed with 80 ml of 1 M KCl, shaken for 1 h, filtered and stored at -18 °C. The NH₄⁺ and NO₃⁻ concentrations were analysed from the extract using flow injection analysis (FIA star TM 5000 analyser, Denmark).

2.7. Statistical analyses

All treatments (*i.e.* both the composting and incubation

experiments) were arranged in a completely randomised design, and two-way analysis of variance (ANOVA) was performed. Tukey tests were used to compare the treatment means if the factors' effect was significant at P < 0.05. All statistical analyses were carried out using SAS version 9.2 statistical package.

(5)

3. Results

3.1. Temperature profile

The temperature throughout the composting period is shown in Fig. 1. The thermophilic phase was reached within one week for all treatments and lasted for more than three weeks. The peak temperatures were between 55 and 63 °C and between 52 and 55 °C respectively when coffee husks and sawdust were used as the bulking agent. The timing of the addition of the N-rich substrate did not affect the temperature in the first active phase of the composting, but split addition resulted in a second temperature peak, which lasted for less than two weeks.

3.2. Nitrogen loss and mineralisation during composting

Total N loss varied depending on the timing of the addition of the N-rich substrate (P = 0.003) and on the bulking agent (P < 0.001), whereas the interaction was marginally significant (P = 0.06; Table 2). The highest N losses were observed when all of the N-rich substrate was added at the beginning of composting, irrespective of the bulking agent. Split addition of the N-rich substrate reduced total N loss by up to 20% with coffee husks containing substrates, and by up to 9% with sawdust containing substrates (Fig. 2). The NO₃ concentration after 90 d of composting period was affected by the timing of the N-rich substrate addition (P = 0.01) and bulking agent (P < 0.001) (Table 3). When 20% of Nrich substrate was applied at first application (i.e. the 20/80 treatments), the NO_3^- concentration increased by 60% and 123% with coffee husk and sawdust-containing substrates respectively. Timing of the N-rich substrate addition had a marginal effect on the NH⁺₄ concentration of the final product (P = 0.06), but the NH₄⁺ concentration differed between bulking agents (P < 0.001) and there was a significant interaction between bulking agent and the timing of the addition of the N-rich substrate (P = 0.03).

3.3. Greenhouse gas emissions

The effect of split addition of the N-rich substrate on cumulative GHG emissions is presented in Fig. 3. Analysis of variance showed that cumulative CO₂ emissions were significantly affected by the timing of the N-rich substrate addition (P = 0.01) and bulking agent (P = 0.02), and that there was a significant interaction between the two factors (P = 0.004). Application of all the N-rich substrate at the beginning resulted in higher cumulative CO₂ emissions compared with split application, and this effect was more pronounced with the substrates containing coffee husks (Fig. 3a). Split addition of Nrich substrates affected total cumulative CH_4 emissions (P = 0.05)

Table 2
Carbon and nitrogen mass balances after 90 d of composing period (mean + standard error of the mean: $n - 3$)

Treatment	Total carbon loss	Carbon loss (CO ₂ -C)	Carbon loss (CH ₄ -C)	Carbon unaccounted for	Total N retained	N loss (N ₂ O-N)	N loss (NH ₃ , N ₂ , NO _x)
	% Initial carbon				% Initial nitrogen		
C_100	65.5 ± 0.6	48.9 ± 1.6	0.08 ± 0.03	16.6 ± 2.0	36.3 ± 2.4	0.02 ± 0.01	63.7 ± 2.4
C_80/20	59.2 ± 3.0	36.1 ± 5.9	0.10 ± 0.03	23.0 ± 4.8	42.1 ± 2.2	0.13 ± 0.04	57.8 ± 2.2
C_20/80	57.3 ± 3.3	31.0 ± 4.2	0.07 ± 0.01	26.2 ± 1.0	50.4 ± 1.2	0.18 ± 0.03	49.4 ± 1.2
S_100	54.3 ± 2.3	33.8 ± 4.7	0.14 ± 0.02	20.3 ± 3.9	57.8 ± 3.2	0.32 ± 0.07	41.9 ± 3.1
S_80/20	54.7 ± 3.1	29.6 ± 1.0	0.20 ± 0.01	24.7 ± 3.7	52.2 ± 1.4	0.34 ± 0.03	47.5 ± 1.4
S_20/80	52.9 ± 2.7	31.5 ± 1.0	0.05 ± 0.02	21.4 ± 2.2	61.6 ± 3.1	0.40 ± 0.03	38.0 ± 3.1
ANOVA							
Т	0.23	0.01	<0.001	0.54	0.003	0.04	0.003
В	0.01	0.01	0.01	0.68	< 0.001	< 0.001	< 0.001
T*B	0.38	0.01	0.01	0.62	0.06	0.55	0.06

C = coffee husks, S = sawdust; C-100 and/or S-100 = all poultry manure was applied at the beginning of composting; C-80/20 and/or S-80/20 = 80% of the poultry manure was applied at the beginning of composting and the remaining 20% was added after the thermophilic phase; C-20/80 and/or S-20/80 = 20% of the poultry manure was applied at the beginning of composting and the remaining 80% was added after the thermophilic phase; T = timing of N-rich substrate addition; B = bulking agent; T*B = treatments interaction effect.



Fig. 2. Total nitrogen loss after 90 d of composting period. C = coffee husks, S = sawdust; C-100 and/or S-100 = all poultry manure was applied at the beginning of composting; C-80/20 and/or S-80/20 = 80% of poultry manure was applied at the beginning of composting and the remaining 20% was added after the thermophilic phase; C-20/80 and/or S-20/80 = 20% of the poultry manure was applied at the beginning of composting and the remaining 80% was added after the thermophilic phase.

Table 3

Chemical properties of the composts after 90 d of composting period (mean \pm standard error of the mean; n = 3).

Treatment	Total carbon	Total nitrogen	NO_3^-	$\rm NH_4^+$	Cw	G
	g kg ⁻¹ dry matter					% Control
C_100	323.4 ± 5.3	23.0 ± 0.14	0.6 ± 0.06	0.7 ± 0.02	10.52 ± 0.40	98
C_80/20	319.2 ± 4.6	26.4 ± 0.15	0.8 ± 0.03	0.6 ± 0.03	11.06 ± 0.56	99
C_20/80	349.5 ± 23.2	29.7 ± 0.10	1.2 ± 0.04	1.1 ± 0.01	12.74 ± 2.77	93
S_100	337.9 ± 15.8	21.9 ± 0.13	0.1 ± 0.02	0.08 ± 0.01	6.99 ± 0.17	96
S_80/20	351.4 ± 24.0	23.1 ± 0.06	0.1 ± 0.01	0.08 ± 0.01	7.18 ± 1.24	93
S_20/80	363.3 ± 18.7	24.8 ± 0.13	0.6 ± 0.07	0.1 ± 0.01	7.87 ± 1.12	99
ANOVA						
Т	0.23	0.01	0.01	0.06	0.54	0.28
В	0.75	0.01	<0.001	< 0.001	0.01	0.94
T*B	0.67	0.10	0.35	0.03	0.90	0.89

T = timing of N-rich substrate addition; B = bulking agent; T*B = interaction between timing of N-rich substrate addition and bulking agent; C = coffee husks, S = sawdust; C-100 and/or S-100 = all poultry manure was applied at the beginning of composting and the remaining 20% was added after the thermophilic phase; C-20/80 and/or S-20/ 80 = 20% of the poultry manure was applied at the beginning of composting and the remaining 30% was added after the thermophilic phase; Cw = water extractable organic C; G = Relative seed germination; T = timing of N-rich substrate addition; B = bulking agent; T*B = treatments interaction effect.

and there was a significant interaction between the timing of the addition of the N-rich substrate and the bulking agent (P = 0.05). Regardless of the bulking agent used, the highest CH₄ emissions were observed when 80% of the N-rich substrate was added at the beginning of composting (*i.e.* 80/20 treatments) (Fig. 3b). The

timing of N-rich substrate addition and bulking agents affected cumulative N₂O emissions (P = 0.03 and P < 0.001 respectively). The 20/80 treatments increased cumulative N₂O emissions by 600% with coffee husks as the bulking agent, and by 25% with sawdust as the bulking agent (Fig. 3c) compared to single addition. The total

GHG budget (*i.e.* non-CO₂ GHG) (Fig. 3d) varied between the bulking agents (P = 0.001), but the timing of substrate addition had a marginal effect (P = 0.06). Total greenhouse gas emissions were higher with coffee husks than with sawdust. When CO₂ was accounted for in the total GHG budget, the total GHG emissions varied between the timing of substrate addition (P = 0.01) and the treatment interactions (P = 0.05), but the effect of bulking agent was non-significance (P = 0.16) (Fig. 3e). In this scenario, early addition of N-rich substrate increased the total GHG budget compared with delayed addition.

3.4. C and N mineralisation after soil application

CO₂ evolution from the composts after soil application is presented in Fig. 4. The timing of N-rich substrate addition and bulking agent affected C mineralisation (P = 0.03 and P = 0.002 respectively). Postponing the addition of N-rich substrate increased CO₂ emissions after soil application, irrespective of the bulking agent used, indicating lower compost maturity. The NO₃ concentration differed between soils incubated with compost produced with a different timing of N-rich substrate addition (P = 005) and bulking agent (P < 0.001), and there was also a significant interaction (P = 0.02) (Supplementary Fig. 1). After 60 d of incubation, late



Fig. 3. Total cumulative GHG emissions after 90 d of composting period. (a) CO_2-C , (b) CH_4-C , (c) N_2O-N (d) total GHG emissions excluding CO_2 , (e) total GHG emissions including CO_2 ; C = coffee husks, S = sawdust; C-100 and/or S-100 = all poultry manure was applied at the beginning of composting; C-80/20 = 80% of the poultry manure was applied at the beginning of composting and the remaining 20% was added after the thermophilic phase; C-20/80 = 20% of the poultry manure was applied at the beginning of composting and the remaining 80% was added after the thermophilic phase; $*^*$ indicates non- CO_2 GHG emissions.

addition of the N-rich substrate increased the NO_3^- concentration by 20% and 74% with coffee husks and sawdust respectively compared to single addition. The NH_4^+ concentration, however, was not affected by the treatments.

3.5. Weed seed germination

The seeds of *Parthenium hysterophorus* and *Digitaria abyssinica* had decomposed completely after 60 d of composting. Some *Lantana camara* and *Solanum marginatum* seeds were recovered, even after 90 d of composting, however the seeds did not germinate. Thus all of the treatments prevented weed emergence after 90 d of composting.

4. Discussion

4.1. Relative nitrogen loss

Postponing the addition of the N-rich substrate reduced total N loss irrespective of the bulking agent, as hypothesised. The total N loss observed in this study ranged from 40% to 60% of the initial N content, and was comparable with earlier studies (Bernal et al., 2009: Steiner et al., 2010), but higher than other studies (Yang et al., 2013). The high N losses in this study could be explained by the high temperatures, the long duration of the thermophilic phase (Fig. 1), the high NH_4^+ concentration and C loss (Tables 2 and 3) and the relatively open design of the composting containers. The N loss as N₂O was less than 1% for all treatments (Table 2), and N loss via leaching was minimised by controlling the moisture content throughout the experimental period. The majority of N losses could therefore be explained by ammonia emissions and N₂ losses (Table 2). It is well known that temperatures above 45 °C increase ammonia volatilisation dramatically (Pagans et al., 2006). In this experiment, the thermophilic phase (>45 °C) lasted for more than three weeks (Fig. 1) and the NH_4^+ concentration was >1000 mg kg⁻¹ dry matter for all treatments, implying the presence of optimal conditions for ammonia volatilisation. The high initial concentration of NH₄⁺ when all the N-rich substrate was added at the beginning was therefore more prone to NH₃ volatilisation than the split treatments. Split addition of N-rich substrate resulted in a second temperature peak, but this temperature lasted for less than two weeks. Consequently, when the addition of the N-rich substrate was postponed, the majority of N added was exposed to a lesser extent to conditions that are conducive to volatilisation. In agreement with these findings. Bryndum (2014) reported a 33% reduction in N loss due to the delayed addition of N-rich substrates (*i.e.* poultry manure) from composting of sugarcane bagasse and filter cake. Dresbøll and Thorup-Kristensen (2005), however, did not observe a significant effect of split addition of clover on N loss from composting of wheat straw and clover. These contradictory results are probably due to the differences in NH⁺₄ concentration, temperature and materials used for composting. For instance, there are higher levels of ammonium and easily mineralisable nitrogen compounds in poultry manure than in clover grass, which contains mainly organic nitrogen. The NH₄⁺ concentration was $<400 \text{ mg kg}^{-1}$ dry matter during the earlier study (Dresbøll and Thorup-Kristensen, 2005). Hence, the effect of split application is less in clover because nitrogen has to be mineralised before it can volatilise. Furthermore, the thermophilic phase only lasted for two weeks in the previous study (Dresbøll and Thorup-Kristensen, 2005), which could have resulted in less ammonia volatilisation compared to the present experiment. The effect of delayed addition of N-rich substrate on N loss was not as great as expected and/or reported by Bryndum (2014). This variation is explained by the second thermophilic phase, which was observed in the present study, but not by Bryndum (2014). The second thermophilic phase might result in high N losses, but these high temperatures are crucial for compost sanitisation. It is hypothesised that splitting the N-rich substrate more than twice might further reduce total N loss. and this hypothesis should be investigated.

The effect of split addition on N loss varied between both bulking materials (P < 0.001). These variations could be explained by the higher temperatures (55–63 °C) when coffee husks rather than sawdust were used (52–55 °C) (Fig. 1), which subsequently led to higher ammonia volatilisation. High N immobilisation could be another explanation for the minimum effect of split addition with the substrates containing sawdust because the higher C/N ratio of this mixture may have efficiently immobilised ammonium. The total N loss was correlated with organic matter and/or C loss during the composting process ($r^2 = 0.78$; P < 0.001). The higher C



Fig. 4. Cumulative CO_2-C emissions from soil during a 90 d of incubation period. C = coffee husks, S = sawdust; C-100 and/or S-100 = all poultry manure was applied at the beginning of composting; C-80/20 = a0% of the poultry manure was applied at the beginning of composting and the remaining 20% was added after the thermophilic phase; C-20/80 = 20% of the poultry manure was applied at the beginning of composting and the remaining 80% was added after the thermophilic phase.

loss with coffee husk substrate compared to sawdust substrate (Table 2) could therefore be an explanation for the observed differences in total N losses between the two bulking materials. In agreement with these findings, Dias et al. (2010) observed N loss with substrate containing coffee husks that was three times higher than with sawdust. To the authors' knowledge, this is the first study that has investigated the interactions between the timing of N-rich substrate addition and bulking agent on N loss. Further studies are needed to investigate the effectiveness of split substrate addition under different composting conditions, such as substrate quality, moisture content, aeration and other variables. Split addition of Nrich substrates also increased mineral N content in the final product after soil application, as hypothesised. Immobilisation and ammonia loss explained the observed variations between the treatments in mineral N concentrations. The mineral N concentrations in the soil after 21 and 60 d of incubation agreed with the inorganic N concentration of the composts (Table 3).

4.2. Greenhouse gas emissions

The hypothesis that split addition of an N-rich substrate increases N₂O emissions was confirmed. Both nitrifying and denitrifying bacteria are mesophilic, and high temperatures effectively reduce N₂O production (Vu et al., 2015). In the present experiments, over 95% of N₂O emissions were observed after the thermophilic phase for all treatments. Postponing the addition of N-rich substrate increases the accessibility of inorganic N (NH[‡] and NO³) for nitrifying and denitrifying bacteria and consequently increases N₂O production. The cumulative N₂O emissions were higher than those in previous reports on composting (Chowdhury et al., 2014; Vu et al., 2015). High NH[‡] and NO³ concentrations in poultry manure may explain the high N₂O emissions in this experiment.

Early additions of N-rich substrate (i.e. 100/0 and 80/20 treatments) increased the cumulative CH₄ emissions from composting, as hypothesised, and the effect was more pronounced with the substrates containing sawdust. The high CH₄ production during early additions of the N-rich substrate could be explained by (i) the high load of materials which results in more anaerobic pockets in the pile, (ii) the presence of high temperatures in conjunction with high available C and N for the methanogens which increases CH₄ production, and (iii) low methane oxidation due to the similarity between enzymes involved in methane and ammonium oxidation (Wei et al., 2016). Methanotrophs can switch substrates from CH_4 to NH_4^+ and result in low CH_4 oxidation. Regardless of the bulking agent used, cumulative CH₄ emissions were lower during single addition of the N-rich substrate than when 80% of the N-rich substrate was added at the beginning of composting (Fig. 3). This observation is attributed to the high NH⁺₄ concentration and ammonia volatilisation during single addition of the N-rich substrate because methanogenic activity decreases as ammonia volatilisation and NH⁺₄ concentration increase (Hao et al., 2005). The present findings are in agreement with the results of previous studies reporting on the response of CH₄ production from soil to different nitrogen fertilisers (Wei et al., 2016). To the authors' knowledge, this is the first report on the relationship between split addition of an N-rich substrate and GHG emissions during composting. Further investigations are needed to reveal the mechanisms of how the timing of substrate addition influences the GHG budget. For both greenhouse gases (N₂O and CH₄), the cumulative emissions were higher with the substrates containing sawdust than those containing coffee husks. The likely explanation is that the bulkier nature of coffee husks allows more air movement through the compost pile than sawdust does. We used two different scenarios to assess the GHG budget from the treatments. The first scenario exclude CO_2 from the total GHG budget because higher CO_2 evolution indicates a greater degree of stabilisation of the compost. Moreover, the less stable composts produce more CO_2 during soil application (Fig. 4); hence, the total CO_2 emissions would eventually converge to the same value for all composts (Supplementary Fig. 2). Under this scenario, the total GHG budget was not affected by the timing of N-rich substrate addition. Early addition of N-rich substrate, however, increased the total GHG budget when CO_2 was included in the GHG budget. The obvious explanation is that early addition produce higher CO_2 than delay addition (Fig. 3a), and CO_2 contributed over 85% of the total GHG budget in all treatments.

4.3. CO₂ evolution and compost stability

The hypothesis that split application of an N-rich substrate increases C mineralisation after soil application was confirmed. The CO₂ evolution from the composts after soil application correlated with the cumulative CO_2 emissions during composting (Fig. 3) and C loss (Table 2). Higher total C loss and CO₂ emissions during composting (Fig. 3 and Table 2) and the subsequent low CO₂ emissions after soil application (Fig. 4) indicated that composts produced by early additions were at a more advanced stage of decomposition compared to split addition. An obvious explanation for this is that the newly added N-rich substrate had less time to decompose. The CO₂ emissions after soil application were higher with substrates containing coffee husks, implying that the amount of the easily degradable compound was higher in coffee husks than in sawdust. The C loss observed in this experiment (Table 2) was comparable with previous studies on composting (Dias et al., 2010). The unaccounted for C ranged between 16% and 26% (Table 2) and indicated C that was not measured between sampling dates (Vu et al., 2015; Nigussie et al., 2016) and/or C loss via volatile compounds (Chowdhury et al., 2014). The sampling frequency might be too infrequent, so that not all the CO₂ emissions were captured, particularly from split addition of the substrate when intensive decomposition occurs in the later stages of composting. The unaccounted for C reported in this experiment is, however, comparable with Chowdhury et al. (2014) and Sommer and Dahl (1999) who reported unaccounted for C of between 17% and 36%.

Postponing the addition of N-rich substrates killed seeds of four different and unrelated weed species, as hypothesised. Temperatures above 50 °C for a minimum of three days are sufficient to destroy most weed seeds and pathogens (Ryckeboer et al., 2003). The temperatures in all treatments (Fig. 1) and even the second thermophilic phase during split addition therefore probably destroyed the weed seeds. The temperature profile suggests that a small amount of N could be enough to generate the thermophilic phase and kill weed seeds and potentially also human pathogens from composting.

The hypothesis that split application of N-rich substrate produces stable compost was confirmed. Combinations of different indices were used to test compost stability since it is difficult to test stability using a single index (Wichuk and McCartney, 2010). Temperature during the composting process is a simple method for evaluating compost stability. Compost temperatures are affected by aeration, pile size, moisture content, degree of insulation and other parameters. Compost temperatures alone therefore provide misleading evidence about compost stability. CO₂ evolution is a more reliable indicator of compost stability (Aslam and Vander-Gheynst, 2008). Less than 30% mineralisable-C in 70 d is used as

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an indication of a stable compost (Bernal et al., 2009), confirming that all of the treatments produced stable compost (Fig. 4). Waterdissolved organic carbon (C_w) < 17 g kg⁻¹ dry matter (Bernal et al., 2009) and a Cw:total nitrogen ratio < 0.7 (Santos et al., 2016) are good indicators of compost maturity, indicating that all of the treatments produced mature compost (Table 3). The decrease in NH[‡] concentration after composting, C:N ratios <15:1 (Table 3) and eradication of weed seeds could also be indicators of compost stability and/or maturity. An NH[‡]:NO³ ratio below 0.16 is often used to indicate compost maturity (Bernal et al., 2009), but none of the treatments did satisfy this criterion due to the high concentration of NH[‡] in the poultry manure. The NH₄:NO₃ ratio was, however, in the acceptable range (*i.e.* 0.5–3.0) (Bernal et al., 2009). The relative seed germination was >90% (Table 3), confirming that the composts were mature.

4.4. Implications

Nitrogen loss during composting reduces the agronomic value of compost and contributes to greenhouse gas emissions and unpleasant odours. Different strategies have been identified for reducing N losses from composting, but existing technologies such as controlling the pH (Pagans et al., 2006), controlling the aeration rate (Chowdhury et al., 2014) and the use of absorbents (i.e. active carbon, biochar and zeolite) (Khan et al., 2016; Steiner et al., 2010) are expensive and difficult to scale up to field-scale conditions. The addition of crop residues to adjust the C:N ratio is another effective method for reducing N losses (Dias et al., 2010), but there are competing uses of crop residues for animal feed and fuel in smallholding farming systems in developing countries (Nigussie et al., 2015). It is therefore suggested that split addition of N-rich substrate is a less expensive method than the existing solutions for reducing N losses from composting and produces compost with a high fertilising value. Many smallholder farmers prepare compost by continuously adding organic materials to a compost pile, implying that the present experiment corresponds to the activity of smallholder farmers in developing countries. In practice, however, it is not only nitrogen-rich substrates that are added continuously to a compost pile, but different qualities of materials as well. Further studies, therefore, are needed to determine the effect of split application of different quality substrates on the composting process.

5. Conclusions

Irrespective of the bulking agent used, split addition of the nitrogen-rich substrate reduced nitrogen loss without having a significant effect on total greenhouse gas emissions, compost stability and sanitisation. Split addition of the N-rich substrate increased N mineralisation after soil application, implying an increase in the fertilising value of the end product. This study demonstrated that the timing of substrate addition affected CH₄ and N₂O emissions, but not the total GHG budget. Split addition of N-rich substrates did not affect the temperature profile during the thermophilic phase and was effective at eradicating a wide range of weed seeds. This study provides essential information with regard to the timing of substrate addition to produce compost with a high fertilising value and avoid emissions of greenhouse gases. However, further studies are needed to scale up this technique to field conditions. Experiments in the presence of plants are also recommended in order to test the effect of split addition of an N-rich substrate on the agronomic value of the compost.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.09.123.

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