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Reduced turning frequency and delayed poultry manure addition reduces N loss from sugarcane compost

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ABSTRACT

Composting is an effective method to recycle biodegradable waste as soil amendment in smallholder farming systems. Although all essential plant nutrients are found in compost, a substantial amount of nitrogen is lost during composting. This study therefore investigated the potential of reducing N losses by (i) delaying the addition of nitrogen-rich substrates (i.e. poultry manure), and (ii) reducing the turning frequency during composting. Furthermore, we tested the effect of compost application method on nitrogen mineralization. Sugarcane-waste was composted for 54 days with addition of poultry manure at the beginning (i.e. early addition) or after 21 days of composting (delayed addition). The compost pile was then turned either every three or nine days. Composts were subsequently applied to soil as (i) homogeneously mixed, or (ii) stratified, and incubated for 28 days to test the effect of compost application on nitrogen mineralization. The results showed that delayed addition of poultry manure reduced total nitrogen loss by 33% and increased mineral nitrogen content by >200% compared with early addition. Similarly, less frequent turning reduced total N loss by 12% compared with frequent turning. Stratified placement of compost did not enhance N mineralization compared to a homogeneous mixing. Our results suggested that simple modifications of the composting process (i.e. delayed addition and/or turning frequency) could significantly reduce N losses and improve the plant-nutritional value of compost.

1. Introduction

Sugarcane is cultivated commercially in >109 countries (Dotaniya et al., 2016), and its production is expected to expand in the future due to the increase in demand for ethanol. Currently, sugarcane processing generates a large amount of biomass by-products. About 23% and 3% sugarcane harvest ended as bagasse and filter cake respectively (George et al., 2010). In 2012 alone, for example, >4 million metric tons of sugarcane were produced (FAOSTAT, 2014) in Costa Rica (where this study was conducted) which is estimated to generate about 1.3 million metric tons of bagasse, and 0.2 million metric tons of filter cake. In many countries, the major part of bagasse is commonly combusted for power generation to run the sugar mills (Dotaniya et al., 2016). Filter cake, on the other hand, has no direct use and is mostly piled out in the open-air. In most cases, filter cake is applied as soil amendment with little or without processing. Globally, the management of sugarcane by products is very limited, leading to widespread environmental concerns and suboptimal resource utilization (Chandel et al., 2012; Pandey et al., 2000). Hence composting could be one solution to alleviate the environmental problems caused by sugarcane by-products as well as to enhance crop production.

Composting is a well-known processing method for the bioconversion of organic waste into soil amendments. Composting is effective in decreasing the volume of organic waste and thereby eases transportation of waste (Cook et al., 2015). Application of compost on cropland increases or maintains soil organic matter content and thereby contributes to long-term agricultural sustainability. Studies have shown that sugarcane-waste compost has a potential to be used as organic amendment (Franca et al., 2016; Prado et al., 2013; Stoffella and Graetz, 2000). However, it is still necessary to optimize the sugarcane-waste compost for rapid and efficient release of nutrients. It is also essential to reduce nutrient losses and thereby produce composts with higher agronomic value.

Nutrient content of compost depends mainly on the quality of feedstock materials. In addition to this, proper compost management is required to reduce nutrient losses. This includes: (i) adjusting the initial compost properties such as the C:N ratio, structure and moisture of the feedstock, and (ii) maintaining optimal composting conditions such as oxygen, temperature, and moisture con-

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tent (Bernal et al., 2009). Uncontrolled composting processes result in nutrient losses *via* leaching and gaseous emissions. Nitrogen (N) loss, in particular, is extensive in poorly managed composting piles (Chowdhury et al., 2014a; Vu et al., 2015). The total N losses during composting can account for 40–70% of initial N (Nigussie et al., 2017). The major N loss is ammonia (NH_3), which contributes over 70% of the total N losses (Chowdhury et al., 2014a). High N loss reduces the agronomic value of compost and contributes to greenhouse gas emissions.

Many efforts have been made to reduce N losses mainly by manipulating various physical and chemical parameters (Chowdhury et al., 2014a,b). Addition of carbon-rich materials (bulking agents) is a well-established approach to reduce N losses because these materials increase the C:N ratio of the composting mixtures (Chowdhury et al., 2014a; Khan et al., 2014). For example, the addition of bagasse to filter cake compost increased the C:N ratio from 14 to 22, consequently the total N loss decreased from 14% to 10% (Meunchang et al., 2005). Similarly, N-rich substrates (i.e. poultry manure) are commonly added to lignocellulosic materials (i.e. sugarcane by-products) in order to optimize the initial C:N ratio and/or increase the fertilizing-value (i.e. N content) of the final product (Adamtey et al., 2009). However, this practice increases N losses during composting because the co-existence of high concentrations of NH_4^+, easily mineralizable compounds and high temperatures (>45 °C) which favor ammonia volatilization during the initial phases of composting (Eklind et al., 2007). Delayed addition of N-rich material (i.e. addition of N-rich substrates after the thermophilic phase) is therefore suggested to reduce N losses during composting. To our knowledge, very few studies have been conducted on the effect delayed addition of N-rich substrate on N loss during composting (Dresboll and Thorup-Kristensen, 2005; Nigussie et al., 2017). Moreover, the existing literature is contradictory and hence further studies are required to elucidate the relationship between the timing of the addition of the N-rich substrate and N loss during composting.

Controlling aeration rate is another important parameter to minimize N losses during composting (Chowdhury et al., 2014a; Jiang et al., 2011). In developing countries (such as Costa Rica), most composting operations are carried out using low-tech technologies. Hence aeration is performed manually by turning compost piles (Getahun et al., 2012). Turning increases aeration; however, frequent turning could also result in high N losses via ammonia volatilization (Kalamdhad and Kazmi, 2009; Tirado and Michel, 2010). For example, Cook et al. (2015) observed that frequent turning of compost piles (i.e. three times per week) increased total N losses by >200% compared with less frequent turning (i.e. once a week) during composting of slurry-woodchip mixtures. In contrast, Ogunwande et al. (2008) observed that total N loss was not affected by turning frequency during composting of poultry manure and sawdust. Likely, differences in NH_4^+ concentration, temperature and scale of the experiment could explain this variation. These studies imply that the effect of turning frequency may depend on the characteristics of the composting material.

While a limited number of studies have been conducted to optimize the fertilizing-value of compost via delayed addition of poultry manure (Nigussie et al., 2017) and turning frequency (Cook et al., 2015; Getahun et al., 2012; Jiang-Ming, 2016), the combination of turning frequency and delayed addition of poultry manure has received even less attention. In this study, we examined the effect of the combination of turning frequency and delayed addition of poultry manure on N loss, N-mineralization and the composting process. Furthermore, we tested whether the distribution of compost in soil influences nitrogen mineralization. Concentrated placement in compost pockets rather than homogenously mixed compost may accelerate nitrogen mineralization because the soil microbial biomass will be less protected from predation in small soil pores, thus enhancing the rapid transformation and release of N (Breland, 1994). On the other hand, Magid et al. (2006) found that the accelerating effect of concentrated placement was dependent on the C:N ratio of materials, indicating that *high* C:N ratio materials would be less prone to immobilizing N from soil when concentrated, whereas *low* C:N materials would be less affected by concentrated placement. Therefore, the general objective of this study was to quantify processes which can increase the N fertilizer value of sugar cane waste compost. This was approached by investigating: (i) the effects of the time of poultry manure addition to compost, testing if late application could reduce N losses, (ii) the effects of compost-turning frequency on N losses and N availability in mature compost, and (iii) the effect of localized versus mixed placement of compost in soil on N release, testing if localized placement would lead to faster increase in soil mineral N.

2. Materials & methods

Two independent experiments were carried out in this study. The first experiment was conducted to investigate the effect of delayed addition of N-rich substrate (poultry manure) and turning frequency on total N loss and composting process. To achieve this objective, a meso-scale composting experiment was conducted at CATIE (Centro Agronómico Tropical de Investigación y Enseñanza), Costa Rica. The second experiment was conducted to assess the effect of compost application method on N mineralization, and consisted of a soil incubation study conducted at University of Copenhagen, Denmark. Details of both experiments are presented in Sections 2.1 and 2.2.

2.1. Composting experiment (Experiment 1)

2.1.1. Raw materials for the composts

Compost was made from sugarcane bagasse, filter cake and poultry manure in 1 m^3 piles. The physical and chemical characteristics of the composting materials are presented in Table 1. Bagasse and filter cake were provided fresh from two local sugar mills (Ingenio Attiro and Ingenio Juan Viñas, both in the region of Turrialba, CR). Poultry manure was provided by a regional processor (Rolando Guzman Villega in Grecia, CR). The poultry manure had been pre-composted for 22 months with sawdust and $CaCO_3$ before being used in this experiment, and had a higher total N and mineral N content, than the sugarcane materials. The experiment ran for 54 days in a mesh greenhouse with concrete floor.

2.1.2. Compost set-up

Filter cake and bagasse were mixed on wet basis ratio of 9:1 (filter cake: bagasse) to obtain a mixture (400 kg fresh weight) with a moisture content of 60–65% and C:N ratio of 20:1, considered suitable for composting. Moreover, the addition of bagasse (i.e. porous and fibrous material) increases the porosity of the mixture and facilitates airflow. In order to increase the nitrogen content of the bagasse and filter cake, poultry manure (40 kg fresh weight) was added to the mixture in two different manners: (i) at the beginning of composting, hereafter referred to as *early addition*, and (ii) after 27 days of composting, hereafter referred to as *late addition*. The manure mixing rate (10% on fresh weight basis) was chosen in order to mainly influence total and mineral N in the compost, but with limited impact on compost structure and porosity, to ensure comparability between treatments. In addition to differences in time of manure application, the compost piles were turned at two different intervals: (i) every three days, hereafter referred to as *frequent turning*, and (ii) every nine days, hereafter referred to as *less frequent* turning. Details of the treatments are presented in Table 2. The experimental design was therefore a

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Table 1
Chemical characteristics of sugarcane bagasse and filter cake and poultry manure before composting.

Parameter	Bagasse		Filter cake		Poultry manure	
pH (H_2O)	4.4	(0.12)	5.1	(0.03)	8.5	(0.32)
Moisture (%)	55.7	(0.35)	67.0	(0.22)	12.5	(1.03)
Total N (%)	0.44	(0.01)	2.01	(0.05)	2.09	(0.13)
C:N ratio	110		18		11	
NH_4-N (mg/kg)	11.9	(3.5)	594.7	(86.6)	1995.7	(778)
NO_3-N (mg/kg)	LoD		LoD		594.6	(576)
P (%)	0.04	(0.0)	1.39	(0.02)	1.49	(0.06)
K (%)	0.16	(0.0)	0.3	(0.01)	2.32	(0.16)
Ca (%)	0.11	(0.01)	3.34	(0.06)	13.1	(0.98)
Mg (%)	0.04	(0.0)	0.28	(0.01)	0.60	(0.02)
Cu (mg/kg)	10	(0.0)	109	(2)	77	(11)
Zn (mg/kg)	12	(1.0)	165	(7)	340	(2)
Mn (mg/kg)	52	(0.0)	1195	(9)	674	(148)
Fe (mg/kg)	1967	(46)	13,127	(768)	8625	(4649)

Values are means of three replicates and standard error of means (SE) are indicated in brackets. LoD indicates values below the Limit of Detection.

Table 2
Naming and management of the four compost treatments.

Name	Time of PM addition	Turning frequency (days)
Early 3	Day 0	3
Early 9	Day 0	9
Late 3	Day 27	3
Late 9	Day 27	9

PM: Poultry manure.

complete random design arranged in 2 × 2 factorial arrangements (timing of poultry manure addition × turning frequency), and the treatments were replicated three times. The moisture content was adjusted to 50–60% by adding water to the heaps during the second and fourth week of composting.

2.1.3. Monitoring temperature and compost sampling

Temperatures were measured every three days from three different points inside the pile using a Reotemp spear thermometer (30 cm). Total compost mass was determined by weighing the entire pile in a polyethylene “big bag”, and correcting for moisture content. Samples were collected at 9, 18, 27, 36, 45 and 54 days of composting for analysis. The sampling procedure was as follows: (i) the compost was mixed thoroughly and spread on a surface (i.e. approximately 4 m^2), (ii) 16 equal-sized squares were marked, (iii) one liter sample were collected from each square, and (iv) half of the sample weight was removed and the remaining half was mixed thoroughly. The last step was performed three times until only 2 L of sample was remained. Three samples of 100 g each were then collected from the last step. The first sets of samples were immediately used for determination of gravimetric moisture content. The second sets of sample were frozen for the subsequent chemical analysis, and the third sets of samples were freeze-dried to ensure stable storage until they were used for the incubation experiment (Experiment 2).

2.1.4. Physical and chemical analyses

Gravimetric moisture content was determined by oven drying at 105 °C for 24 h. For total C and N determination, samples were dried at 65 °C, finely ground and analyzed by gas chromatography (Thermo Finnigan, Flash EA 1112 series). Mineral nitrogen (NH_4^+-N and NO_3^--N) was extracted by shaking for 1 h in 2 M KCl (compost:solution ratio of 1:5 w/v) and filtered. The extracts had a dark color due to organic acids, which was cleared by shaking with active charcoal. Ammonia and nitrate concentrations were determined manually by distillation and titration. Compost pH was measured using MilliQ water in wet basis ratio of 1:5 (compost:water). For determination of other minerals, a complete digestion of samples (1:20 w/v) was performed using a mixture of nitric and perchloric acid (5:1 v/v). Total Cu, Zn, Mn, Fe, Ca, Mg and K content were determined by atomic absorption spectrophotometry (Perkin Elmer, Analyst 100). Total P was determined by molybdenum blue method (Thermo Spectronic, Helios alpha) using UV-V spectrophotometry. The N loss during composting was calculated with correction for total mass loss and changes in moisture content of the compost as:

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

where *Qi* and *Qf* are total dry mass (kg) of the composting material at initial and end of composting period respectively, and *Ci* and *Cf* are nitrogen contents at initial and end of composting period respectively.

2.2. Incubation experiment (Experiment 2)

2.2.1. Materials

For each of the four compost treatments (Table 2), compost sampled on day 27 and day 54 was used for the incubation, yielding eight compost materials (four compost treatments × two compost ages). To ensure homogeneity across compost replicates for the incubation, each compost variety material was sieved (>2 mm, >1.2 mm and <1.2 mm), the particle size distribution determined and portions of 2 g compost constructed matching the naturally occurring proportions of the three particle size fractions. The soil used for the incubation experiment, a loamy sand (69% sand, 16% silt, 12.5% clay and 1.3% C), originated from the experimental farm of the University of Copenhagen, 20 km west of Copenhagen, Denmark (55°400N, 12°180E). Since 2003, it had been cultivated with cereal crops and under-sown clover, but had otherwise been unfertilized. The soil was sieved through a 4 mm mesh and pre-incubated moist at 20 °C for four weeks to allow it to stabilize after the sieving disturbance. Prior to use it was rewetted to a moisture content of 15.4% (equal to 60% of its water-holding capacity).

2.2.2. Incubation set-up

Open-ended polyethylene (PE) pipes (d: 58 mm and h: 35 mm or 2 × 17.5 mm), with a cross of adhesive tape for support at the bottom, were used as incubation containers. Compost (2 g DW) was wetted to 50% moisture and either homogeneously mixed into or placed stratified in moist soil (100 g DW), which roughly corresponded to an application rate of 160–190 kg N ha^{-1}. Homogeneously mixed treatments were packed in the tall PE containers to a density of 1.3 g cm^{-3}, which corresponded to field density.

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Stratified treatments were made by sandwiching compost between two pre-packed slices of soil (each 50 g DW) in two short PE containers. The short PE containers were sealed vertically with adhesive tape and packed to 1.3 g cm^{-3}. In total, eleven treatments were made: eight treatments with homogeneously mixed compost, two stratified treatments using the two compost varieties that were assumed to have the largest N mineralization potential (type E9 age 27 and type L9 age 54) and one control treatment with only soil (Table 3). Each treatment had 16 replications (four replicates × four sampling times). The PE containers were incubated in the dark at 24 °C for 28 days.

2.2.3. Measurements and sampling

Destructive soil samples were collected at day 7, 14, 21 and 28. The stratified treatments were divided into two fractions, namely (i) a detritusphere fraction - the soil extending 7.5 mm to each side of the stratified compost, and (ii) the bulk fraction - consisting of the two remaining outer soil layers. The soil-compost column was sliced horizontally using razor blades. Each of the two soil fractions (*i.e.* detritusphere and bulk) were then homogenized before sampling.

2.2.4. Physical and chemical analyses

Gravimetric moisture content was determined by oven drying at 105 °C for 24 h. Mineral N (NH_4-N and NO_3-N) was determined using 1 M KCl. Briefly, the extract was prepared from soil:solution ratio of 1:4 (w/v), shaken for one hour, filtered and stored at −20 °C. Homogeneous and stratified treatment pairs were analyzed for soil microbial biomass nitrogen (SMB-N) by chloroform fumigation-extraction as illustrated by (Brookes et al., 1985). Samples were fumigated for 24 h at 25 °C and extracted in 1 M KCl as described for mineral N. The extracts of both fumigated and unfumigated soil/compost samples were subjected to digestion with sulfuric acid using the Kjeldahl N method. Total Kjeldahl N (TKN) and mineral N was measured using flow injection analysis (FIASTAR 5000). SMB-N was calculated as the difference in TKN content between the fumigated and unfumigated sample pairs, assuming that the excess of N obtained by fumigation represents 54% of the original SMB-N (Joergensen and Mueller, 1996).

2.3. Statistical analyses

Statistical analyses were performed using SAS 9.4 statistical package. The treatments were arranged in a completely randomized design, and analysis of variance (ANOVA) was performed. A repeated two-way ANOVA was used to compare the treatments' effect on temperature. The data were checked for the assumptions of ANOVA prior to data analyses. Levene and Shapiro-Wilk's tests were used to test for homogeneity of variance and normality respectively. Tukey test was used to compare the means if the factors' effect was found significant at P < 0.05.

Table 3
The naming and characteristics of the ten incubation treatments.

Name	Compost type	Compost placement in soil	Compost age (days)
E3-H-27	Early 3	Homogenized	27
E9-H-27	Early 9	Homogenized	27
E9-S-27	Early 9	Stratified	27
L3-H-27	Late 3	Homogenized	27
L9-H-27	Late 9	Homogenized	27
E3-H-54	Early 3	Homogenized	54
E9-H-54	Early 9	Homogenized	54
L3-H-54	Late 3	Homogenized	54
L9-H-54	Late 9	Homogenized	54
L9-S-54	Late 9	Stratified	54
Control	No addition	No addition	No addition

3. Results & discussion

3.1. Evolution of temperature, moisture and pH during composting

The temperature profile throughout the composting period is presented in Fig. 1. For all treatments, the peak temperature (i.e. 65–70 °C) was reached within two weeks, and lasted for more than one month. The two-way repeated ANOVA showed that turning frequency influenced temperature (P = 0.04); however, there was no significant effect of the timing of poultry manure addition (P = 0.56). Moreover, there was no significant interaction between the two factors (P = 0.73). Less frequent turning of piles (i.e. turning every nine days) delayed the peak temperatures compared with frequent turning (i.e. turning every three days). Literature reports varying effects of turning time on temperature development: in contrast to our findings, Cook et al. (2015) observed a slight delay of the thermophilic phase in frequently turned composting piles, while others (Getahun et al., 2012; Tiquia et al., 2002) observed a stimulation of turning on compost temperature. Our observed difference in the peak temperatures could be due to the development of anaerobic patches in less frequently turned piles, or the stimulatory effect of mixing active microbial hotspots with undecomposed material (Tiquia et al., 2002). Hence, during the peak temperature phase of this study, temperature loss by frequent turning was more than compensated for by the effects of improved aeration and mixing on the decomposition process. The temperatures reached ambient temperatures within 45 days in the frequently turned piles, and after 54 days in less frequently turned piles. Temperature is a simple method for evaluating compost maturity (Wichuk and McCartney, 2010) and composts that reach and remain at ambient temperature after turning are entering the maturation or curing stage. Even if in this experiment, an extended, post-thermophilic maturation stage was not included; our results imply, in agreement with others (Getahun et al., 2012; Tiquia et al., 2002), that frequent turning of compost pile can produce mature compost more quickly than less frequently turned piles.

While we found no effect of timing of poultry manure addition on temperature, earlier studies (Dresboll and Thorup-Kristensen, 2005; Nigussie et al., 2017) reported a second thermophilic phase after delayed addition of N-rich substrates. This difference could be

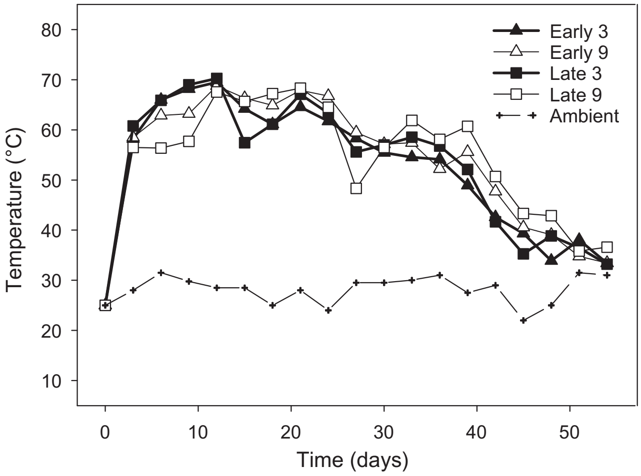

Fig. 1. Temperature development (°C) in the compost core during composting, measured before mixing. Ambient temperatures are midday temperatures in the greenhouse.

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explained by (i) the filter cake contained sufficient amounts of N and easily degradable C, hence the thermophilic phase lasted until the late addition of poultry manure, and (ii) we used pre-decomposed poultry manure which contained little labile C. Temperatures above 50 °C for a minimum of three days are considered sufficient to destroy most weed seeds and pathogens (Nigussie et al., 2017). In all treatments, the temperatures were above 55 °C for nearly one month (Fig. 1), implying that sanitation would not be a problem even with late addition of poultry manure.

Moisture content was affected by turning frequency. Frequent turning significantly ($P < 0.0001$), decreased the moisture content to below 50% from an initial value of 60–64% after the first nine days, compared to not turning during the first nine days which kept the moisture at around 55% (data not shown). Despite twice adjusting the moisture content to about 60–65% (calculation based on actual moisture measurements), the frequent turning decreased moisture to 35% in the final compost (54 days) compared to 45% for the treatments with a nine-day turning frequency. Considering that moisture should be in the range of 50–70% for optimal microbial activity and compost turnover (Jiang et al., 2011), the higher turning frequency probably did create a short period of sub-optimal conditions for compost microorganisms. Ambient temperatures in the greenhouse were around 30 °C at midday, favoring high moisture evaporation during turning. Since the turning of piles was an hour-long process, it was difficult to avoid high moisture loss under the frequent turning regime. The pH was not significantly affected by turning treatments at any time-points ($P = 0.06$) or timing of poultry manure addition ($P = 0.30$) and there was no significant interaction between the two factors on pH ($P = 0.27$). For all treatments, the pH increased from 5.7 to 8.3 during the thermophilic phase, and it decreased to around 7 after 45 days of composting (data not shown).

3.2. Changes in dry matter and carbon content during composting

The dry matter loss during the composting process is presented in Fig. 2. The analysis of variance showed that total dry matter loss varied between the two turning frequencies ($P = 0.02$). However, there was no significant effect of timing of poultry manure addition ($P = 0.19$) and the interaction between the two factors ($P = 0.35$) on total dry matter loss. At the end of composting, the total dry matter loss was 40% during frequent turning, and 44% during less frequent turning (Fig. 2). The dry mass loss in our study is comparable with the previous composting experiments (Santos et al., 2016). In contrast with the earlier studies (Cook et al., 2015; Getahun et al., 2012), we found lower dry matter loss in frequently turned piles compared with the less frequently ones. This observation is possibly related to the high moisture loss in the frequently turned piles which may have decreased the microbial activity and decomposition process. Dry matter was determined from the compost wet weight (before turning) and moisture content (after turning), which may have caused a systematic underestimation of DM owing to unaccounted moisture losses during turning. However this probably did not influence the overall conclusions as the daily variation was similar for all treatments and decreased towards the end of the experiment. In general, there was a high variation across replicates of the same treatment, which may have "masked" significant differences between treatments. High variation was particularly pronounced during the initial composting phase when the compost was very hot and moist and had not degraded very much, making representative sampling difficult. In agreement with our findings, large variation in dry matter were also reported by Dresboll and Thorup-Kristensen (2005).

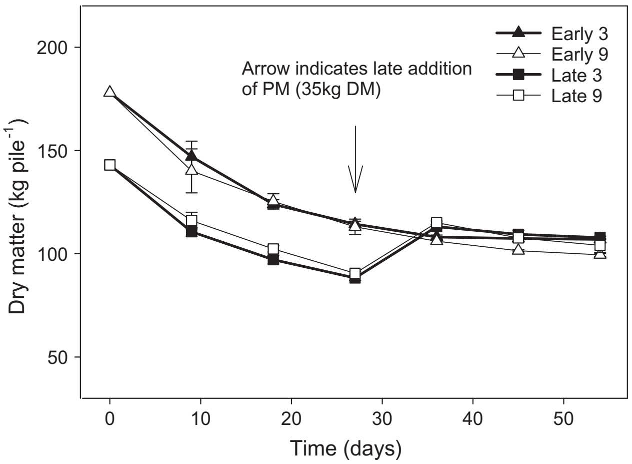

Fig. 2. Total dry matter (kg pile^{-1}) during 54 days of composting. Arrow indicates time of poultry manure (PM) addition to the treatments: Late 3 and Late 9. Bars indicate standard error of means ($n = 3$).

Total C content decreased from 39% to around 23% during composting. For all treatments, the greatest reduction in C content was observed during the first two weeks, which is in accordance with the rapid breakdown of easily degradable compounds in the initial phase of composting (Francou et al., 2005). The total carbon content after 54 days differed between the turning frequencies ($P = 0.02$) and the timing of poultry manure addition ($P = 0.001$). However, there was no a significant interaction between the two factors ($P = 0.25$). Delayed addition of poultry manure increased the total C content of the final compost by 6% compared with early addition of poultry manure, since the newly added poultry manure had less time to decompose.

3.3. Nitrogen dynamics during composting

The total N loss varied between the timing of poultry manure addition ($P = 0.02$); however, turning frequency had no a significant effect on total N loss ($P = 0.84$). In the present study, the total N loss ranged between 21 and 31% (Fig. 4), and was comparable with previously reported studies (Chowdhury et al., 2014a; Nigussie et al., 2017). The total N loss was however higher than Meunchang et al. (2005) who reported 15% N loss during composting of sugarcane filter cake and bagasse. A likely explanation for this difference is the addition of poultry manure with high mineral N content in our study. We found that delayed addition of poultry manure reduced the total N losses by up to 33% compared with early addition. In agrement with our findings, Nigussie et al. (2017) found that late addition of poultry manure decreased total N loss by 9–20% during composting of municipal waste. Similarly, Dresboll and Thorup-Kristensen (2005) reported that delayed addition of clover-grass reduced N losses by 26% during composting of wheat straw and clover-grass.

The N loss through leaching was minimized throughout the experimental period by controlling moisture content and avoiding precipitation on the heaps. Earlier studies (Chowdhury et al., 2014a; Nigussie et al., 2017; Vu et al., 2015) showed that N loss *via* N_2O emission is very low (<1%) during composting. Hence, the N losses in our study are likely from ammonia emissions and N_2 losses. A recent study of N_2O emissions, demonstrated a significant increase arising from delayed N addition, but that this was countered by a decrease in methane emissions (Nigussie et al., 2017). More studies on the effect of delayed addition on greenhouse gas emissions are warranted by these findings. The rapid decrease in NH_4^+ concentration after two weeks did not increase NO_3^- content (Fig. 3a and b), confirming the transformation of NH_4^+ to NH_3 during this period. Similarly, the highest N loss was observed during the first two weeks for all treatments. The peak

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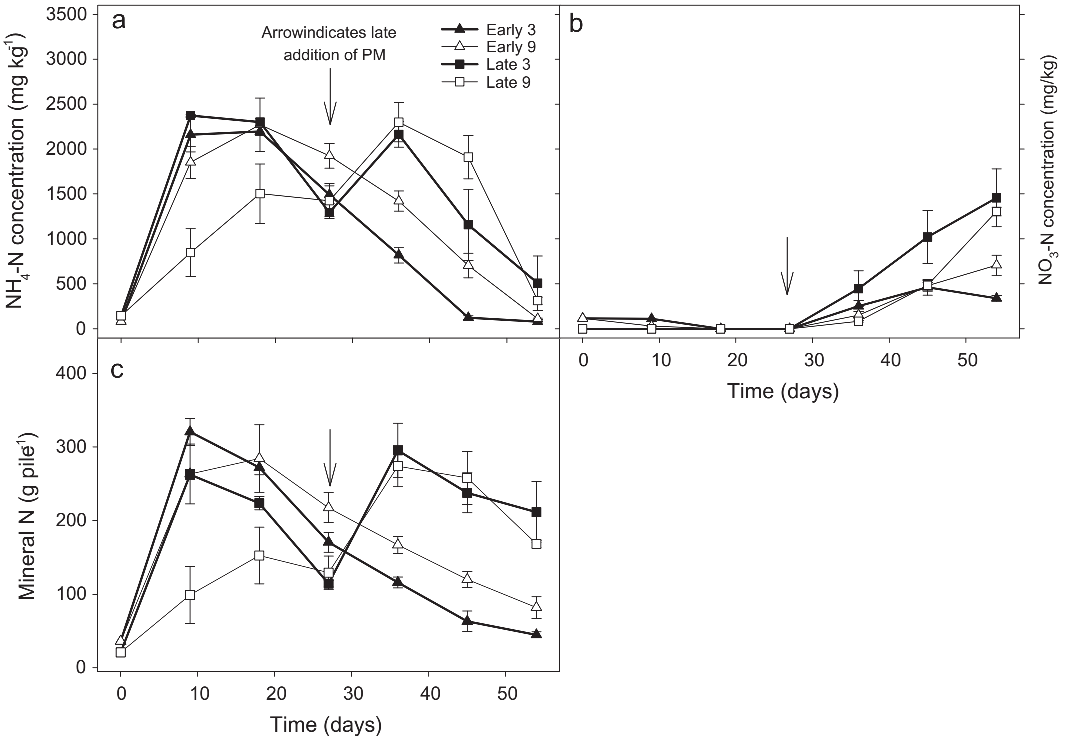

Fig. 3. a) Ammonia (NH_4-N) concentrations (μg g^{-1}), b) nitrate (NO_3-N) concentrations (μgg^{-1})and c) total mineral N (g pile^{-1}) (as the sum of NH_4-N and NO_3-N) during 54 days of composting. The arrow indicates the time when poultry manure (PM) was added to the two late addition treatments: Late 3 and Late 9. Bars indicate standard errors of means (n = 3).

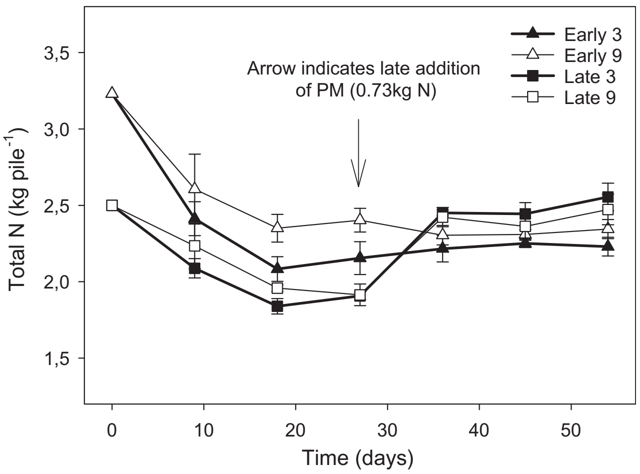

Fig. 4. Total N (kg pile^{-1}) during 54 days of composting. Arrow indicates time of PM addition to treatment: Late 3 and Late 9. Bars indicate standard error of means (n = 3).

NH_4^+ concentration (2300 mg N kg^{-1}) was observed within two weeks after early addition of poultry manure (Fig. 3a). The high N loss under early addition of poultry manure is explained by the co-existence of high NH_4^+ concentration (Fig. 3a), high temperature (>45 °C) (Fig. 1) and pH > 8 (data not shown) during the early stages of composting, all of which favor ammonia volatilization (Bernal et al., 2009; Eklind et al., 2007; Wang et al., 2013). The effectiveness of late addition on minimizing N loss is more pronounced in the present study as compared with earlier studies (Nigussie et al., 2017 and Dresboll and Thorup-Kristensen, 2005). This difference is explained by the presence of an extensive second thermophilic phase after delayed addition in the earlier studies (Nigussie et al., 2017 and Dresboll and Thorup-Kristensen, 2005) - which is a conducive environment for ammonia emissions. Since we used already matured poultry manure, we cannot account for the N losses in the previous processing of the manure, and furthermore, addition of fresh manure could possibly have led to higher N losses, also in the delayed addition. However, the manure used in this trial still had a much higher mineral N content than the plant materials, and in that sense still resembles typical collected, semi-stored manure from intensive livestock production.

The poultry manure addition did not alter the C:N ratio of the compost pile significantly (Table 4). However, delayed addition of poultry manure reduced N loss composting compared with early addition, suggesting that C:N ratio is a poor indicator of total N loss compared to the size of labile N pool. Even though turning frequency did not influence the total N loss (P = 0.84), less frequent turning reduced the total N loss by 12% in the early addition (Table 4), implying that frequent turning facilitates gaseous losses by liberating gases trapped in the compost air space (Parkinson et al., 2004) and exposing the dissolved NH_4^+ to the surrounding air. In agreement with our findings, Getahun et al. (2012) also found high N loss in frequently turned compost piles.

The total nitrogen content at day 54 was affected by turning frequency (P = 0.04) and time of poultry manure addition (P = 0.03). There was also a significant effect of interaction between the two factors (P = 0.02). Less frequent turning increased the total N content of the final compost by 13% during early addition of poultry manure. However, turning frequency did not influence the total N content during late addition of poultry manure. The total N content of the final compost ranged between 2.1% and 2.4%, and the lowest N content (2.1%) was observed during the combination of early addition of poultry manure and frequent turning of piles (Table 4). The mineral N (sum of NH_4^+-N and NO_3^--N) content of the final compost was significantly higher in late poultry manure addition (P = 0.001). However, there was no significant effect of

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Table 4
Development during composting of total C (%), total N (%), C:N ratio, dry-matter (DM) loss (% of total DM input), N loss (% of total N input) and mineral N (% of total N).

Treatment	Day	Total C (%)		Total N (%)		C:N ratio	DM loss (%)	TN loss (%)	Mineral N (%)
Early 3	9	31	(0.27)	1.6	(0.04)	19	17	25	13
	18	29	(0.76)	1.7	(0.08)	17	30	36	13
	27	26	(1.00)	1.9	(0.11)	14	36	33	8
	36	24	(0.62)	2.1	(0.11)	12	39	31	5
	45	24	(0.37)	2.1	(0.03)	11	40	30	3
	54	23	(0.60)	2.1	(0.10)	11	40	31	2
Early 9	9	32	(0.37)	1.9	(0.11)	17	21	19	10
	18	31	(0.24)	1.9	(0.03)	17	30	27	12
	27	28	(0.33)	2.1	(0.00)	13	36	26	9
	36	25	(0.50)	2.2	(0.10)	12	40	29	7
	45	25	(0.33)	2.3	(0.05)	11	43	29	5
	54	24	(0.36)	2.4	(0.07)	10	44	27	3
Late 3	9	34	(0.42)	1.9	(0.03)	18	23	16	13
	18	32	(0.33)	1.9	(0.02)	17	32	26	12
	27	28	(0.42)	2.2	(0.01)	13	38	24	6
	36	26	(0.49)	2.2	(0.00)	12	36	24	12
	45	25	(0.48)	2.2	(0.04)	11	39	24	10
	54	26	(0.25)	2.4	(0.09)	11	39	21	8
Late 9	9	36	(0.36)	1.9	(0.13)	18	19	11	4
	18	33	(0.33)	1.9	(0.02)	17	28	22	8
	27	29	(0.27)	2.1	(0.09)	14	37	23	7
	36	26	(0.40)	2.1	(0.09)	12	35	25	11
	45	25	(0.19)	2.2	(0.11)	12	39	27	11
	54	25	(0.08)	2.4	(0.06)	11	42	23	7
ANOVA									
Turning frequency		0.02		0.04		0.73	0.02	0.84	0.89
Timing of addition		<0.001		0.03		0.46	0.19	0.02	<0.001
Treatment interaction		0.25		0.02		0.10	0.35	0.26	0.11

Values are means of three replicates. Values in the parenthesis indicate standard error of means (SE); C = total C, N = total N; DM = dry-matter; Mineral N = Ammonium, nitrite and nitrate.

turning frequency (P = 0.89) and the interaction between the two factors (P = 0.11). Mineral N content of the final compost ranged between 420 and 2000 mg kg^{-1} dry matter. Late addition of poultry manure increased the mineral N content in the final compost by 200% compared with early addition (Fig. 3c). In consistence with our findings, Dresboll and Thorup-Kristensen (2005) showed almost twice as much increase in mineral N content during delayed addition of clover-grass as compared with early addition. Low N loss via ammonia is the obvious explanation for the higher mineral N content during delayed addition of poultry manure treatments.

Based on the results, we suggest that the poultry manure and other nitrogen-rich substrates should be added during the later stage of composting (mainly after the thermophilic phase) in order to reduce N losses and increase the agronomic-value of compost. This strategy might be implemented successfully among smallholder farmers who already add poultry manure to their compost. The effectiveness of the intervention may be affected by the labile C in the late addition substrate, and a subsequent second temperature peak. Hence mixing rates should be balanced to address this, as well as full-cycle N balances of various waste handling practices should be further investigated.

3.4. Nitrogen mineralization and soil microbial N during incubation

After 28 days of incubation, mineral N levels (mainly from NO_3-N) were significantly greater (P < 0.001) for compost age 54 days (1–3 µg N PE container^{-1}) compared to compost age 27 days (<0.6 µg PE container^{-1}) (Fig. 5). Compost age 27 days

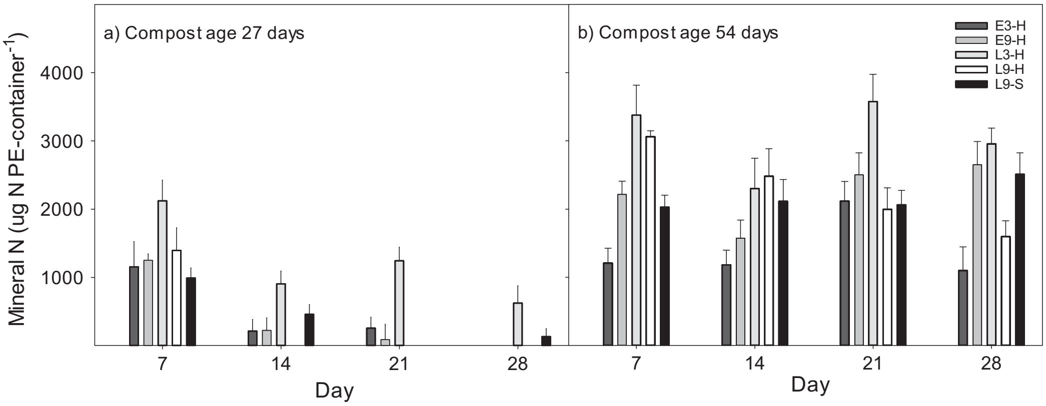

Fig. 5. Mineral N (µg container^{-1}) at days 7, 14, 21 and 28 during incubation. Control soil mineral N levels were subtracted from the presented values. Compost types E3, E9, L3, L9 were either stratified (S) or homogeneously mixed (H) in the soil. Stratified treatments are shown for the whole container (*i.e.* the sum of the detritusphere and bulk fraction). Bars indicate standard error of means (n = 3). a) Incubation of compost age 27 days before the L3 and L9 treatments received poultry manure input; b) Incubation of compost that had been aged for 54 days at the beginning of the incubation experiment.

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Table 5
Initial total N input, and mineral N and soil microbial biomass nitrogen (SMB-N) per PE container (% of initial mineral N input from compost). Final values are after 28 days of incubation.

Age	Type	Soil placement	Total N input (mg PE^{-1})	Mineral N input (μg PE^{-1})	Mineral N (% of mineral N input)	SMB-N (% of mineral N input)
27	E3	Homogenized	38	2984	0	NA
	E9	Homogenized	43	3849	0	3
	E9	Stratified	43	3849	3	5
	L3	Homogenized	43	2590	24	NA
	L9	Homogenized	42	2850	0	NA
54	E3	Homogenized	42	841	131	NA
	E9	Homogenized	47	1641	162	NA
	L3	Homogenized	47	3929	75	NA
	L9	Homogenized	48	3240	49	3
	L9	Stratified	48	3240	77	3

strongly immobilized N during the incubation, regardless of compost type and placement in soil, suggesting that this compost was still immature and the stabilization of organic matter was incomplete (Kabore et al., 2010). Furthermore for compost age 27 days, no difference was found in N mineralization between the compost types with early PM addition and late PM addition, even though at that age the late addition treatments had yet not received their PM input (Fig. 5a).

Incubation of composts age 54 days did not result in high immobilization of N, suggesting that the compost was more mature (Fig. 5b). When comparing the mineral N level relative to the initial compost mineral N content, the early PM addition compost age 54 days (E3-54 and E9-54) were the only composts showing a net N mineralization after 28 days of incubation (Table 5). In contrast, these composts (*i.e.* E3-54 and E9-54) contributed the lowest initial mineral N inputs, suggesting that both maturity and initial mineral N input are determinants in the N release pattern.

Treatments subjected to SMB-N analysis did not show a net increase in SMB-N compared to the control soil until day 21 (Fig. 6). The measured SMB-N levels after 28 days of incubation were only 3–5% of the initial mineral N inputs, and compost age, type or placement did not influence final SMB-N (Table 5). The results indicate that the stratified placement of compost did not accelerate the rate of decomposition of the compost or the soil microbial biomass, but that the degree of compost maturity was the main driver for N mineralization from composts. In order to explore fully the effects of placement of compost rather than homogeneous mixing, similar studies should be conducted that include a growing plant, to investigate the N dynamics when a plant root is involved.

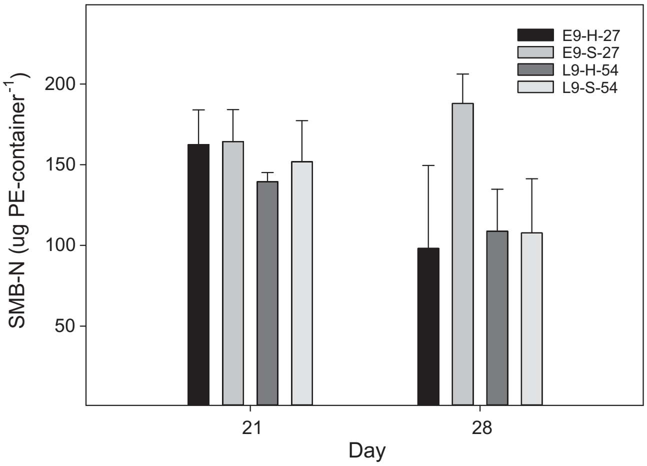

Fig. 6. Soil microbial biomass N (SMB-N) (μg $container^{-1}$) at days 21 and 28 of incubation. Control soil SMB-N levels were subtracted from the presented values. Compost types E9 and L9 were either stratified (S) or homogeneously mixed (H) in the soil for each of the compost ages 27 days (27) or 54 days (54). Stratified treatments are shown for the whole container (*i.e.* the sum of the detritusphere and bulk fraction). Bars indicate standard error of means (n = 3).

4. Conclusions

Late addition of PM resulted in mineral N in maturing compost being twice that found with the early addition, suggesting that late addition is an effective strategy for increasing N availability, while reducing N losses by up to 33%. Less frequent turning reduced N losses when PM was added early and resulted in better conservation of heat and moisture, which had a positive effect on decomposition. Nitrogen mineralization from compost occurred primarily when compost was more mature after 54 days (vs 27 days). Stratified placement of compost in soil did not enhance N mineralization in comparison to a homogeneous mix of the compost materials.

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