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NITROGEN TRANSFORMATION DURING CO-COMPOSTING OF SPENT PIG MANURE, SAWDUST LITTER AND SLUDGE UNDER FORCED-AERATED SYSTEM

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ABSTRACT

Forced-aerated piles were built to investigate the transformation of nitrogen during co-composting of spent pig litter and sludge at 2:1 wet volume ratio. The total N concentration of spent litter-sludge increased during the early stage of composting due to the concentration effect as a consequence of strong degradation of carbon compounds in the spent litter-sludge piles. After day 21, the total N concentration decreased but there was no significant loss in nitrogen. The total mass of N was maintained at around 30 kg during the composting process. The ammonium- and nitrite-oxidizing bacteria increased in numbers during the active decomposition process (from day 0 to day 21), then dropped gradually as composting proceeded. At this early stage, the NH_4^+-N concentration declined and the $(NO_3^-+NO_2^-)$-N increased. However, the rapid decrease in NH_4^+-N concentration during the first 14 days of composting did not correspond to a rapid increase in $(NO_3^-+NO_2^-)$-N, indicating that some inorganic N was immobilized to organic N, some NH_4^+-N was volatilized, and some $(NO_3^-+NO_2^-)$-N was lost *via* denitrification. Denitrification only occurred during the early stage of composting and decreased significantly once the air was blown into the pile. Spatial variations in several physico-chemical and microbial parameters were found among four different locations of the forced-aerated piles, especially during the first 7 weeks of composting. The surface location had significantly lower temperature and population size of denitrifying bacteria than the top, middle and bottom locations, but higher concentration of total C and higher counts of ammonium-oxidizing bacteria were recorded in the surface location.

Keywords: Animal waste, nitrogen bacteria, nitrification, denitrification, heterotrophs

INTRODUCTION

Pig manure contains nutrients which can act as fertilizers, soil conditioners, or as a combination of both [1]. Composting of pig manure is often recommended prior to application to minimize phytotoxic substances [2-3], to control the spread of pathogens and improve handling and storage [4-5]. However, the composting process changes the nature of the waste which can affect its usefulness as a soil amendment. Previous studies have shown that the microbial activity, decomposition rate and concentrations of soluble carbon, total nitrogen, phosphorus and potassium in waste decreased; whereas CEC and humic acid contents increased during composting [6-9]. Since the composted product is often used as a soil amendment and any nutrients in the compost would be of beneficial value, it is important to know the fate of these nutrients during composting. The nutrient that has received the most attention in composting systems is nitrogen [10-12]. During composting, N could be lost due to ammonia volatilization [13-15], run-off and leaching [8, 14], and denitrification [16]. Such a loss will affect the agronomic quality of the composted product. Environmental factors such as aeration, moisture content and temperature, and C:N ratio of the initial composting material have been reported to affect losses of N during composting [10-11, 13, 17]. A very low C:N ratio would lead to loss of N through ammonia volatilization [18] especially when the compost piles were aerated mechanically or turned manually. Bertoldi *et al.* [11] reported that the N loss was greater with turning (18% N loss) than with the aeration method (5% N loss). Moreover, a pH > 7.0 would also enhance the loss of N through ammonia volatilization [17]. Such loss in N would decrease the fertility value of the mature composted product.

In the present study, the spent pig litter (a mixture of partially decomposed pig manure and sawdust) was co-composted with the pig sludge (the sludge that settled at the bottom of the primary sedimentation tanks in treating pig slurries). The spent litter had more organic matter but less nitrogen whereas pig sludge contained higher concentrations of nitrogen [9, 19-20]. Moreover, the pig sludge has a very low C:N ratio (<10) and it cannot be composted alone as significant loss of N could occur during composting.

Co-composting these two wastes together may not only increase the nutrient content of the spent litter but also supply an adequate amount of organic matter to the pig sludge, thus, improving the efficiency of composting and the quality of the composted product.

The forced-aerated composting method has been developed to reduce the labour cost and space requirement for conventional windrow composting (turning method) [12]. Under the forced-aerated composting system, the composting efficiency at different locations in the pile may be different due to variations in aeration levels. This might affect the efficiency of composting and the changes of N during composting. The present study therefore aims to (a) investigate the changes in N transformation during co-composting of spent litter and pig sludge and, (b) assess the variations in N transformation at different locations (top, middle, bottom and surface) of the spent litter-sludge piles under the forced-aerated system.

MATERIALS AND METHODS

The spent litter disposed from the pig-on-litter system was mixed homogeneously with pig sludge at a ratio of 2:1 (spent litter: sludge; wet volume). The pig sludge was collected from the primary sedimentation tank of the waste treatment plant at Ta Kwu Ling Pig Breeding Centre, New Territories of Hong Kong. The moisture content of the mixture (spent litter-sludge) was 65% (w/v) at the beginning of composting. The physico-chemical properties of the material used and the initial spent litter-sludge mixture are reported in Table 1. Three replicate spent litter-sludge piles were built on perforated pipes connected to an air pump. Each pile was triangular in shape, about 2 m in width at the base and 1.5 m in height. The total weight of each pile was 2000 kg. Twenty mm diameter polyvinyl chloride (PVC) pipes were laid on the base of the pile, with perforations (5 mm diameter) facing upwards. The distance between each perforation was 10 cm. The pipes were covered by wood chips to prevent blockages of the holes, and air was blown to the piles (from bottom to top of the pile) using a Cole Palmer air pump. The air pump with an average flow rate of 634 l min^{-1} and a maximum output of 764 l min^{-1} delivered air continuously during the entire period of composting. The spent litter-sludge piles were then topped off with a 5 cm layer of mature spent litter compost to insulate the piles, and act as a biofilter to minimize odours. Spent litter-sludge samples were taken at four different locations of the pile: top (130 cm from the base of the pile), middle (75 cm from the base of the pile), bottom (30 cm from the base of the pile), and surface (5 cm from the surface of the pile) at day 0, and then weekly until the end of the composting process (day 77).

The spent litter was analysed for the following parameters: moisture content (105°C for 24 hours); pH (1:10 w/v litter:water extract) using a pH probe; electrical conductivity (EC), total C (loss on ignition); Kjeldahl N; NH_4^+- and (NO_3^- + NO_2^-)-N; total P, total K, Cu and Zn, and water-extractable Cu and Zn using an atomic absorption spectrophotometer, nitrifying and denitrifying bacteria [21,22]. The theoretical total N concentration of the spent litter-sludge was calculated by adding the Kjeldahl N with

Table 1. Physico-chemical properties of the initial spent litter, pig sludge and spent litter-sludge mixture.

Parameters	Type of material		
	Spent litter*	Pig sludge**	Spent litter-sludge**
Moisture content (%)	30-45	89 ± 3.50	65 ± 1.77
pH	7.90-8.50	7.44 ± 0.30	8.60 ± 0.32
EC (dS m^{-1})	1.47-2.74	3.50 ± 0.11	2.85 ± 0.17
Ash content (g kg^{-1})	97-129	239 ± 2.28	148 ± 2.27
Total C (g kg-1)	505-525	441 ± 1.15	494 ± 1.45
Total N (g kg^{-1})	18.0-30.5	65.6 ± 0.22	28.1 ± 5.82
Organic N (g kg^{-1})	14-23	59.2 ± 1.15	25.0 ± 2.28
NH_4^+-N (g kg^{-1})	3.9-7.2	6.3 ± 0.15	3.0 ± 1.13
(NO_3^-+NO_2^-)-N (g kg^{-1})	0.08-0.27	0.13 ± 0.01	0.06 ± 0.02
C:N ratio	16.7-27.4	6.73 ± 1.20	17.58 ± 1.15
Total P (g kg^{-1})	13.2-18.0	12.2 ± 0.08	16.0 ± 0.12
Total K (g kg^{-1})	10.6-21.1	8.9 ± 0.02	11.0 ± 0.18
Total Cu (mg kg^{-1})	428-551	120 ± 29.38	276 ± 9.12
Water-extractable Cu (mg kg^{-1})	38.2-52.2	3.68 ± 0.58	42.6 ± 0.85
Total Zn (mg kg^{-1})	615-744	428 ± 174.96	556 ± 68.71
Water-extractable Zn (mg kg^{-1})	18.8-35.7	8.09 ± 1.29	7.87 ± 0.98

* data (range) were obtained from Tiquia [36]

** data (mean and standard deviation of 3 replicates) obtained from this study the $(NO_3^-+NO_2^-)$-N, whereas the organic N concentration was derived by subtracting the NH_4^+-N from the Kjeldahl N. The C:N ratio was then computed based on the concentration of total C and total N. The population sizes of ammonium-oxidizing, nitrite-oxidizing and total aerobic heterotrophic bacteria were assayed using the plate frequency technique [5, 23]. This technique involves inoculating 0.1 ml of the serially-diluted spent litter suspension on the 8 sections of the agar plate. A bacterial colony observed in any of the 8 sections was considered positive growth. The total number of positive growths was counted and the population size of microorganisms in the sample was estimated using a Most Probable Number (MPN) computing package [24].

The mean and standard deviation were reported for all parameters measured in the three replicate piles. To compare variations in the physical, chemical and microbial parameters at 4 locations (top, middle, bottom and surface) of the forced-aerated spent litter-sludge piles during the composting period, a two-way analysis of variance (ANOVA) statistical test (with sampling time and locations as the two factors) followed by the Bonferroni test were performed [25]. A stepwise multiple regression analysis was carried out to examine the most important physical and chemical factors affecting the transformation of N in the spent litter-sludge. All statistical analyses were computed using SigmaStat 1.0 for Windows statistical package.

RESULTS AND DISCUSSION

Changes in total and organic N concentration during composting

The changes in total N concentration at the top, middle, bottom and surface locations of the spent litter-sludge pile followed a similar pattern to that of the organic N concentration (Figure 1a and 1b). This is probably due to the low inorganic N concentration in all four locations which accounted for 4-10 % of total N (Figure 1c and 1d). Increases in organic N in the early stage of the composting process (first 3 weeks) could be due to immobilization of soluble inorganic nitrogen (mostly NH_4^+ and NO_3^-) by the microbes (such as bacteria, fungi and algae) into organic forms. Miller [26] and Vinten and Smith [27] reported that immobilization usually occurs during the decomposition process. Increases in total N during the early stages of composting have also been reported during composting of sludge and animal manure [6, 9]. This increase in concentration of total N during composting could also be attributed to a concentration effect as a consequence of strong degradation of labile organic-C compounds. A significant decrease in the concentration of total C in the spent litter-sludge was observed in the present study (Figure 1e). The concentration of total N decreased after day 21 and fluctuated at a level higher than the initial N value (Figure 1a). The total mass of N remained practically stable (~30 kg) during a 77-day composting period (Table 2).

Table 2. Mass of nitrogen (kg) during co-composting of spent litter and sludge.

Time (days)	Nitrogen (kg)
Day 0	25.61 ± 12.94
Day 7	32.75 ± 2.15
Day 14	31.78 ± 2.63
Day 35	29.24 ± 2.36
Day 63	27.47 ± 1.09
Day 77	28.21 ± 4.87

The mass of N was calculated by multiplying the mass of the pile with the concentration of N; means and standard deviations of 12 replicates (4 locations x 3 piles) are shown. Data were calculated based on 105°C dry weight basis.

Results of the stepwise multiple regression analyses revealed that the C:N ratio was the most critical factor affecting the changes in total and organic N concentration while pH was the most important chemical factor affecting the changes in inorganic forms of N (NH_4^+-N and $(NO_3^-+NO_2^-)$-N) (Table 3). Bertoldi *et al.* [18] reported that if the C:N ratio was greater than 35:1, the microorganism would go through many life cycles, oxidizing off the excess C until a more convenient C:N ratio (20:1) for the microorganism was reached. This would then slow down the composting process. On the other hand, low C:N values would lead to NH_3 volatilization. Bertoldi *et al.* [10] suggested that the general optimum C:N ratio in the material should be 25:1. In the present study, the initial C:N ratio of the spent litter-sludge was between 17:1 and 19:1, and declined to around 14:1 towards the end of the composting process (Figure 1f, Table 4). Although these C:N values were lower than the optimum value suggested by Bertoldi *et al.* [10], temperature data showed that active decomposition took place within the pile, and the overall nitrogen loss was insignificant despite some loss of NH_4^+-N *via* ammonia volatilization which was observed at the early stage of composting.

Changes in inorganic N and microbial numbers during composting

Golueke [29] pointed out that composting is mainly a decomposition/transformation process. For nitrogenous compounds, the conversion is from protein to organic N, then to NH_4^+ to NO_3^-/NO_2^- (with very little denitrification). It is generally believed that both ammonium- and nitrite-oxidizing bacteria are the major agents responsible for the conversion of ammonium to nitrite and nitrate [29-31]. There are also reports showing that heterotrophic organisms including bacteria, fungi and actinomycetes are able to oxidize NH_4^+ and to NO_2^- / NO_3^--N [32]. In the present study, the heterotrophic bacteria, ammonium- and nitrite-oxidizers were maintained at high population sizes during the active decomposition process (Figure 3a, 3b and 3d), suggesting a rapid oxidation of NH_4^+-N to NO_3^--N from day 0 to day 21. However, the dramatic decrease in NH_4^+-N concentration of

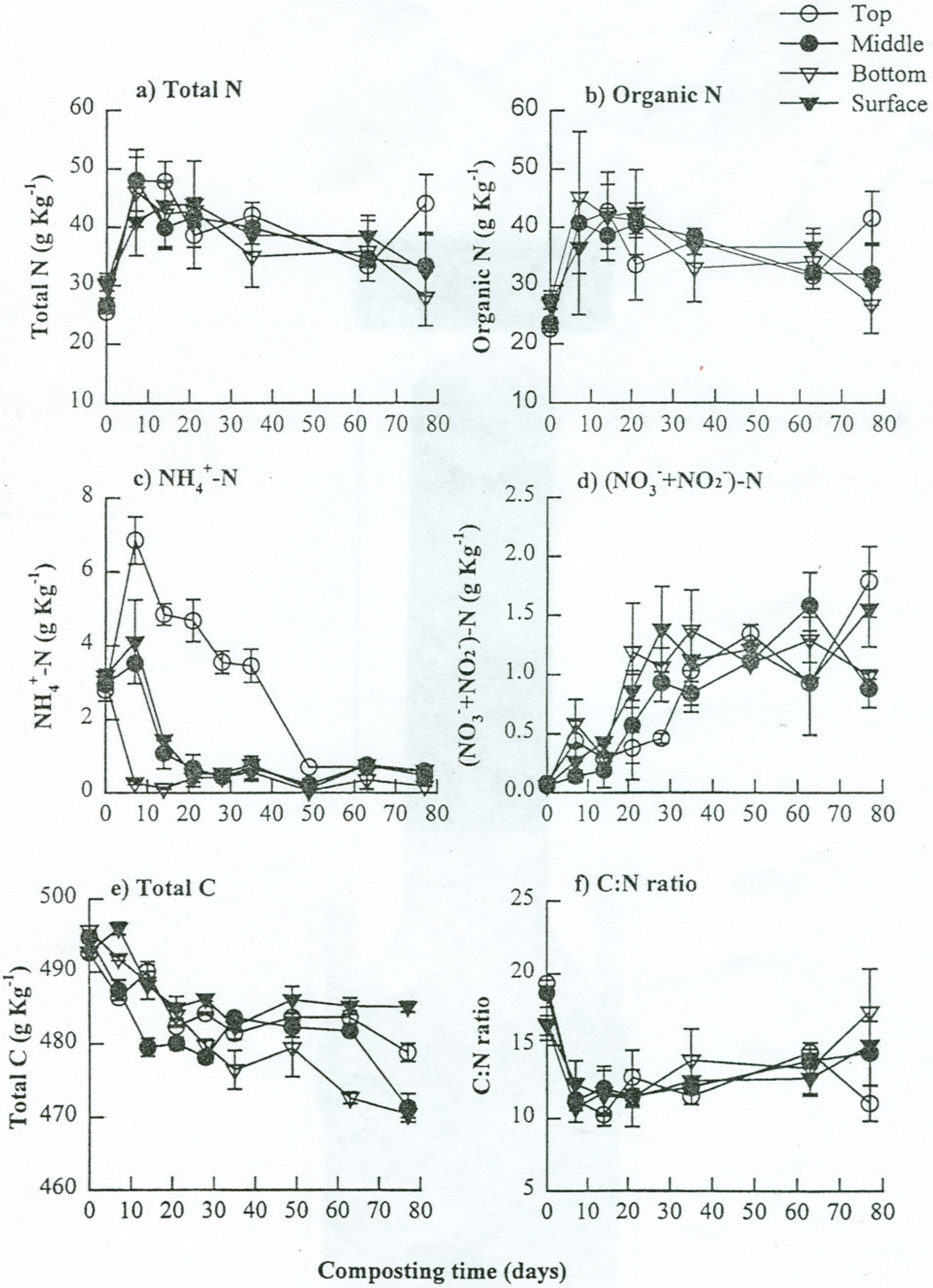

Figure 1. Changes in concentrations of different forms of nitrogen, total C, and C:N ratio of the spent litter-sludge piles at different locations during composting (mean and standard deviation of three replicates are shown).

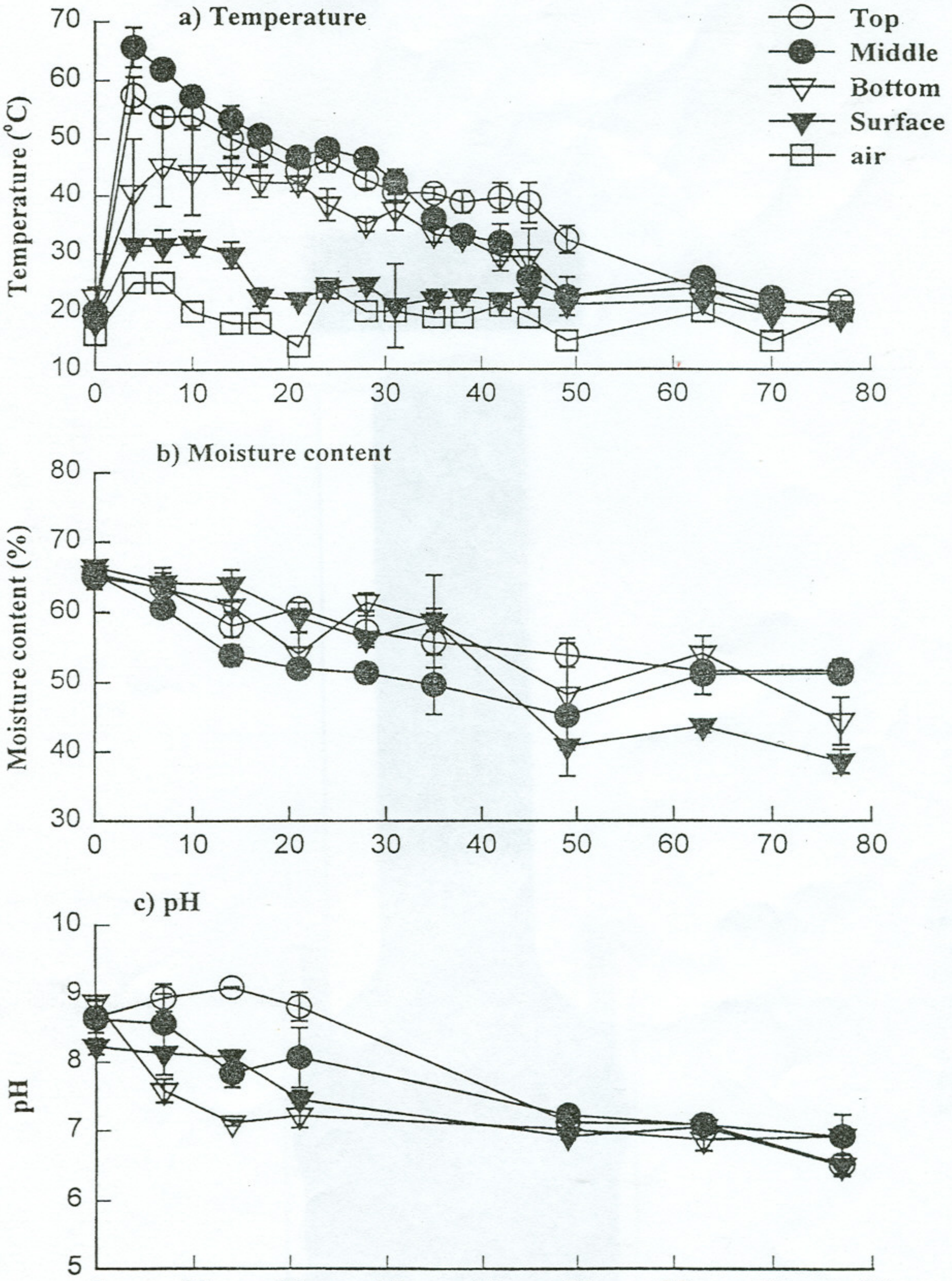

Figure 2. Changes in air and pile temperature, moisture content, and pH of the spent litter-sludge piles at different locations during composting (mean and standard deviation of three replicates are shown).

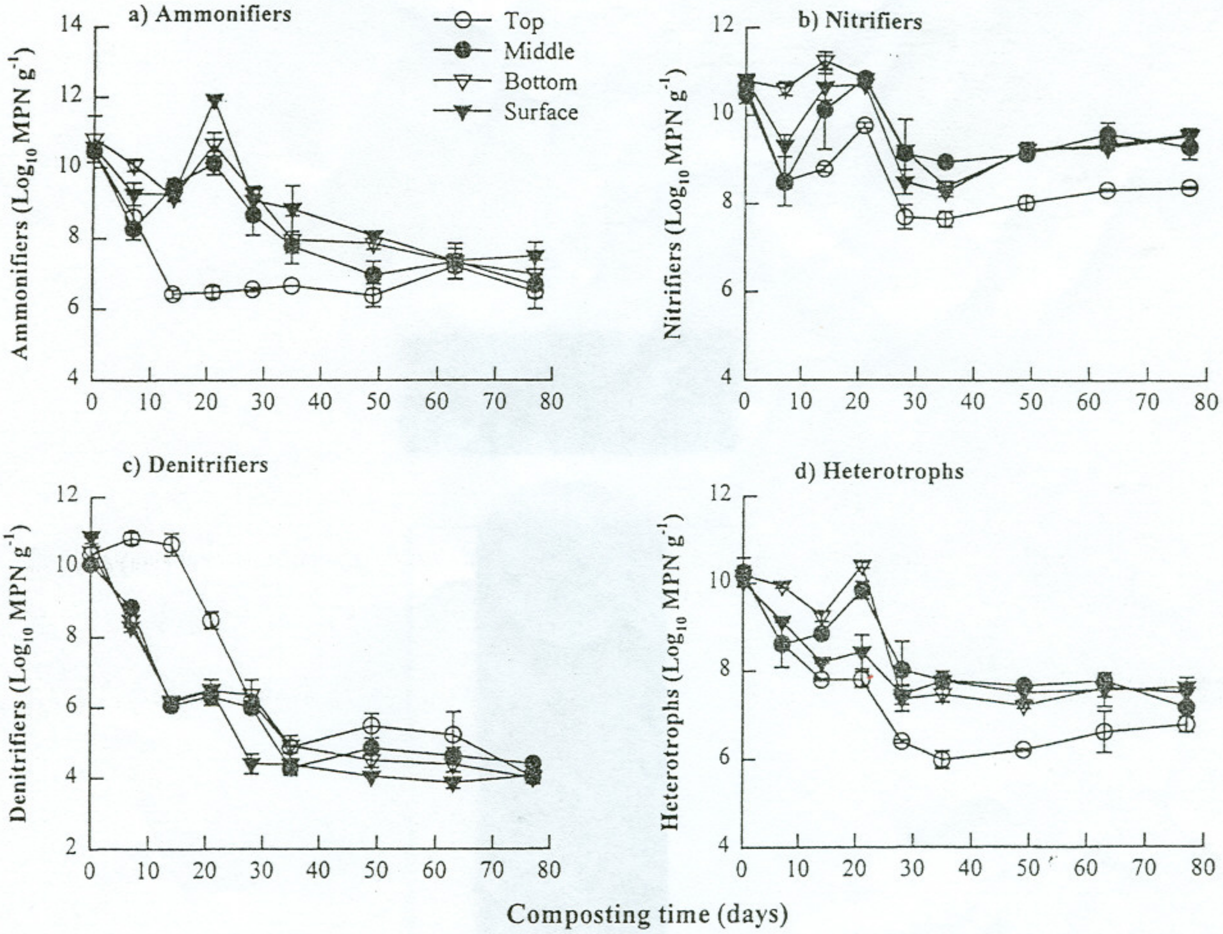

Figure 3. Changes in the population sizes of ammonium- and nitrite-oxidizing bacteria, denitrifiers, and total aerobic heterotrophs of the spent litter-sludge piles at different locations during composting (mean and standard deviation of three replicates are shown).

the spent litter-sludge during the first 14 days of composting did not correspond to a rapid increase in $(NO_3^-+NO_2^-)$-N (Figure 1c and 1d). The decrease in NH_4^+-N concentration was more than 2.5 g kg^{-1} in all 4 locations of the spent litter-sludge piles but the increase in $(NO_3^-+NO_2^-)$-N concentration was less than 0.6 g kg^{-1} during the first 14 days of composting. These results suggest that some NH_4^+-N was lost *via* ammonia volatilization at the beginning of composting when pH of the spent litter-sludge piles was above 8.0 (Figure 2c), which favours NH_3 volatilization. Some inorganic N (both NH_4^+-N and NO_3^--N) was immobilized to organic N since the concentration of organic N in the spent litter-sludge piles increased during the first 21 days of composting. Some of the $(NO_3^-+NO_2^-)$-N was lost *via* microbial denitrification as the population size of denitrifying bacteria was high during the early stage of composting (Figure 3c).

Denitrification normally occurs when poor aeration limits the amount of free oxygen in the soil or composting

Table 3. Multiple regression analysis of different forms of N (in concentration) and physical and chemical properties of the spent litter-sludge.

Multiple regression equation	Multiple R^2 value	*F* value	Significance of *F*
Total N = 64.38- (2.18 * C:N ratio) + (0.07 * temperature)	0.95	451.3	< 0.0001
Organic N = 68.22 - (2.41 * C:N ratio)	0.80	221.2	< 0.0001
NH_4^+-N = -12.2 + (1.8 * pH)	0.67	106.8	< 0.0001
$(NO_3^-+NO_2^-)$-N = 4.83 - (0.53 * pH)	0.64	98.8	< 0.0001

Regression analysis was calculated based on 2 physical (temperature and moisture content) and 3 chemical parameters (C:N ratio, concentration of total C, pH), with STEPWISE METHOD and PIN (probability of *f*-to-enter) = 0.050 limit.

Table 4. Results of two-way ANOVA showing the effects of sampling time and locations on changes in various physico-chemical and microbial parameters of the spent pig litter-sludge compost in the forced-aerated piles.

Parameters	Between time				Between locations			Interaction		Sample size
	F	P	Start	End	F	P	Locations	F	P	
Physico-chemical parameters.										
Temp (°C)	235.2	<0.0001	19.8	19.9	665.6	<0.0001	T=M>B>S	20.3	<0.0001	216
Total C	24.39	<0.0001	494.0	476.4	12.55	<0.0001	S>T>M=B	2.70	0.0006	108
C:N ratio	25.57	<0.0001	17.7	14.5	0.657	0.582	B=M=S=T	2.41	0.0063	84
Total N	14.71	<0.0001	28.1	34.4	0.974	0.412	T=S=M=B	1.39	0.1702	84
Organic N	12.88	<0.0001	25.0	32.7	0.075	0.974	S=T=B=M	1.43	0.1561	84
NH_4^+-N	177.6	<0.0001	2.99	0.46	360.3	<0.0001	T>S=M>B	40.7	<0.0001	108
$(NO_3^-+NO_2^-)$-N	58.21	<0.0001	0.066	1.300	5.09	0.003	B=S>T=M	5.33	<0.0001	108
pH	155.1	<0.0001	8.61	6.71	46.1	<0.0001	T>M>S=B	13.1	<0.0001	84
Population sizes of bacterial groups.										
Heterotrophs	257.9	<0.0001	10.2	7.26	131.9	<0.0001	B=M>S>T	11.7	<0.0001	108
Ammonifiers	152.4	<0.0001	10.6	6.94	155.9	<0.0001	S>B>M>T	16.6	<0.0001	108
Nitrifiers	129.7	<0.0001	10.7	9.22	122.3	<0.0001	B>S=M>T	8.35	<0.0001	108
Denitritrifiers	1073	<0.0001	10.4	4.12	205.6	<0.0001	T>B=M>S	36.0	<0.0001	108

Concentrations of total C and various forms of nitrogen are expressed in g kg^{-1} dry weight whilst population sizes of bacterial groups are in Log$_{10}$ MPN g^{-1} dry weight. Heterotrophs: total aerobic heterotrophic bacteria. Ammonifiers: ammonium-oxidizing bacteria. Nitrifiers: nitrite-oxidizing bacteria. Denitrifiers: denitrifying bacteria. Start and End represent mean values at the beginning and the end of the composting process, respectively. Locations were arranged in descending order, T= top, M=middle, B= bottom, S=surface.

system [13, 26]. In this study, the population sizes of denitrifying bacteria were highest at the beginning of composting (Figure 3c), indicating the presence of anaerobic or microaerophilic pockets in the spent litter-sludge pile. The high initial moisture content also hinders aeration and thereafter induces anaerobic condition [34]. Under this condition, the denitrifying bacteria use the NO_3^- as an electron acceptor instead of oxygen, leaving nitrogen and nitrous oxide (N_2O) gases to be released from the spent litter-sludge piles to the atmosphere. From week 3 onwards, the population size of denitrifying bacteria declined significantly (Figure 3c), indicating that very little denitrification took place once the air was blown into the pile. The $(NO_3^-+NO_2^-)$-N concentration actually started to increase from day 21 onwards.

Effect of composting at different locations of the forced-aerated piles

The peak temperatures occurred in the middle and top locations of the piles (66 and 58°C, respectively) and were significantly higher than those recorded in the bottom (41°C) and surface (32°C) locations (Figure 2a). The low surface temperature could be due to excess loss of heat as the surface location was close to the ambient air. The time required to return the temperatures to the ambient level also varied between locations. It took 63 days for the temperature in the top location to drop to the ambient level, 49 days for the middle and bottom locations, but only 28 days were needed for that in the surface location. Such variations in temperatures from one location to another have also been reported by previous researchers [33, 34]. These differences in temperatures indicate that the aeration condition and degree of decomposition varied at different locations in the pile. The air diffusion, oxygen availability and redox potential should be measured in future studies for a better understanding of the nitrogen transformation at different locations of the spent litter-sludge piles.

The temperature variations between the four locations were also reflected in differences in some physico-chemical and microbial parameters. For instance, the surface location, with the lowest pile temperatures, had the highest total carbon concentrations (Figure 1e, Table 4). This indicates that the degree of carbon degradation was the lowest, so less heat was released. The population size of ammonium-oxidizing bacteria in the surface location was the highest while its denitrifying bacteria count was the lowest when compared with the other three locations. On the contrary, the top location had the highest pH and NH_4^+-N concentration, but the lowest counts of the aerobic bacteria, including total heterotrophs, ammonium- and nitrite-oxidizing bacteria (Figure 3). The NH_4^+-N concentrations were very high in the top location of the spent litter-sludge pile by day 7 and remained at a relatively higher level than the other three locations (Figure 1c). These suggest that the conversion of NH_4^+-N to $(NO_3^-+NO_2^-)$-N was slower at the top compared to the other 3 locations of the forced-aerated pile during the first 28 days of composting. The denitrifying bacteria at the top location of the forced-aerated pile remained at a significantly higher level when compared to the other 3 locations during the first 14 days of composting. However, as composting progressed, their numbers decreased to a low level (4.5 Log_{10} MPN g^{-1}), and were similar to those of the middle, bottom and surface locations of the forced-aerated pile from day 35 onwards (Figure 3c). These suggest that spatial variations were most obvious in the first seven weeks, and differences between the four locations became less as composting proceeded. This also explained why some parameters commonly used as indicators of the composting process, such as C:N ratio, concentrations of total and organic nitrogen, did not show any significant difference between the four locations (Table 4). The present results suggest that it was not necessary to aerate the piles from week 7 onwards as there was little difference between the four locations thereafter. More detailed studies focusing on air distribution and oxygen availability in the piles, aiming to examine the homogeneity of the pile and reduce the cost of the forced aeration composting, will be conducted.

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