Microbial Transformation of Nitrogen During Composting

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Abstract. Microorganisms are fundamentally involved in important changes to the N compounds during composting. However, their role in composting systems is not well understood. Hence, this study was conducted to evaluate the microbial transformation of nitrogen during composting of spent pig litter-sludge and poultry litter in forced-aeration piles. Most N in spent pig litter sludge and poultry litter is in organic forms, which serve as a reservoir of N. During composting, some of the organic N was slowly converted to the much smaller inorganic N pools. The mineralization process was continued further by conversion of ammonium to nitrite/nitrate by nitrifiers. The ammonium- and nitrite-oxidizing bacteria are the microorganisms that gain their energy from these inorganic oxidations. Denitrification occurred during the early stage of composting, as indicated by a higher population of denitrifying bacteria. However, as composting proceeded, the population of denitrifying bacteria declined significantly, indicating that very little denitrification took place once the air was forced into the pile. The multiple regression analysis showed that the physico-chemical properties of the spent pig litter-sludge and poultry litter are the most critical factors affecting the changes in N and its different forms during composting. The equilibria and rates of N were affected by interactions between microbial biomass community structure and the physico-chemical properties of the manure such as temperature, water content, pH, and C:N ratio during composting.

Introduction

Nitrogen is an essential nutrient for all life on earth (Galloway 1998). Thus, its fixation into usable forms by microorganisms and subsequent transformations and recycling through organic and inorganic forms are of great interest. Indeed, N is the nutrient most limiting plant growth in terrestrial ecosystems (Wild 1988). Current concerns include high concentrations of nitrate in ground and surface waters and the contribution of gaseous nitrogen oxides, such as NO and N_2O, to large-scale environmental problems of acid rain, ozone depletion, and greenhouse warming (Galloway 1998). The large diversity of N-containing compounds, which exist in numerous oxidation states, and the wide array of microbial transformations, make the N cycle an extremely interesting intellectual knowledge.

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Microbiology of Composting

Nitrogen is also the nutrient that has received the most attention in composting, as it could be lost significantly during the composting process. Recent studies have showed that about 20–70% of the initial N of the initial feedstock could be lost due to ammonia volatilization, leaching, and runoff during composting (Martins and Dewes 1992; Rao Bhamidimarri and Pandey 1996; Tiquia and Tam 2000a). These losses not only reduce the value of the composted product as an N fertilizer, but they could also lead to serious environmental pollution (Kirchmann and Lundvall 1998). Apart from N losses through ammonia volatilization, leaching, and run-off, there are a number of microbially mediated processes (ammonification, immobilization, nitrification, and denitrification) that are involved in other changes to the N compounds during composting. These processes are responsible for moving the fixed nitrogen from one form to another in the compost, and are vital in understanding the composting process.

Despite the unique role played by microorganisms in determining the characteristics of composts added to soil, very few studies have been published on the microbial communities of such residues, particular those populations that are involved in nitrogen transformation during composting (Finstein and Morris 1975; Diaz-Ravina et al. 1989; Nodar et al. 1990). Hence, this study was carried out to describe the evolution of microbial populations involve in N transformation during composting. Since the key processes in N dynamics are also affected by other controlling factors such as environmental conditions and physico-chemical properties of the compost, this study will also examine the most important physical and chemical factors affecting the transformation of N in the spent litter-sludge and poultry litter.

Materials and Methods

Composting Setup and Sampling

Spent litter-sludge (a mixture of spent pig litter and sludge; 2:1 litter: sludge, wet volume) and poultry litter (a mixture of poultry manure, wood shavings, wastes feed, and feathers) were composted using forced-aeration method (Tiquia and Tam 2000b). Three forced-aeration piles were setup for the spent pig litter-sludge and also for the poutry litter. Triplicate composite samples (approximately 1 kg each) were taken from each pile at day 0 and then weekly until the termination of the composting trial. Average temperature readings were three locations of the forced-aeration piles every 4 days.

Chemical and Microbial Analysis

The manure composts were characterized for the following parameters: water content, pH; electrical conductivity, organic matter (OM), ash; different forms of

N (Sparks 1996). The theoretical total N concentration of the compost samples was calculated by adding the Kjeldahl N with the NO_x^--N, whereas the organic N concentration was derived from subtracting the NH_4^+-N from the Kjeldahl N. Denitrifying bacterial population was quantified by inoculation of tubed liquid media (Tiedje 1994) using the most probable number (MPN) method (Woomer et al.1990). Total aerobic heterotrophs, and ammonium- and nitrite-oxidizing bacteria were quantified on appropriate media using the plate frequency technique (Tiquia et al. 1998).

Statistical Analysis

Pearson product-moment correlation coefficients were calculated to show the relationship between different forms of N and physico-chemical and microbial properties of the manure compost. To determine the most important factors (physical, chemical, or microbial) affecting the transformation of N in the spent pig litter-sludge and poultry litter, a stepwise multiple regression analysis was performed. Statistical analyses were computed using SigmaStat 1.0 for Windows statistical package.

Results and Discussion

The Composting Process

The temperature within the composting mass determines the rate at which many biological processes take place, and the material is considered mature if the declining temperature reaches ambient level (Golueke 1972). The temperature of the spent pig litter-sludge piles mass increased rapidly to between 55 and 65 °C, and persisted until the active decomposition was over, thereafter slowly decreasing (Fig. 1A).

The declining temperature of spent litter-sludge reached ambient level by day 77, which was 111 days earlier than the poultry litter piles (Fig. 1A and B). The temperature of the poultry litter took 168 days to reach ambient temperature (Fig. 1B). Higher peak temperatures (70–75 °C) also persisted during the first 21 days of composting (Fig. 1B). The poultry litter piles had higher peak temperatures and conserved heat longer than spent pig litter-sludge piles due to heat generated by on-going microbial activities (Fig. 1D), and possibly due to the higher insulating quality of the poultry litter (Mathur 1998).

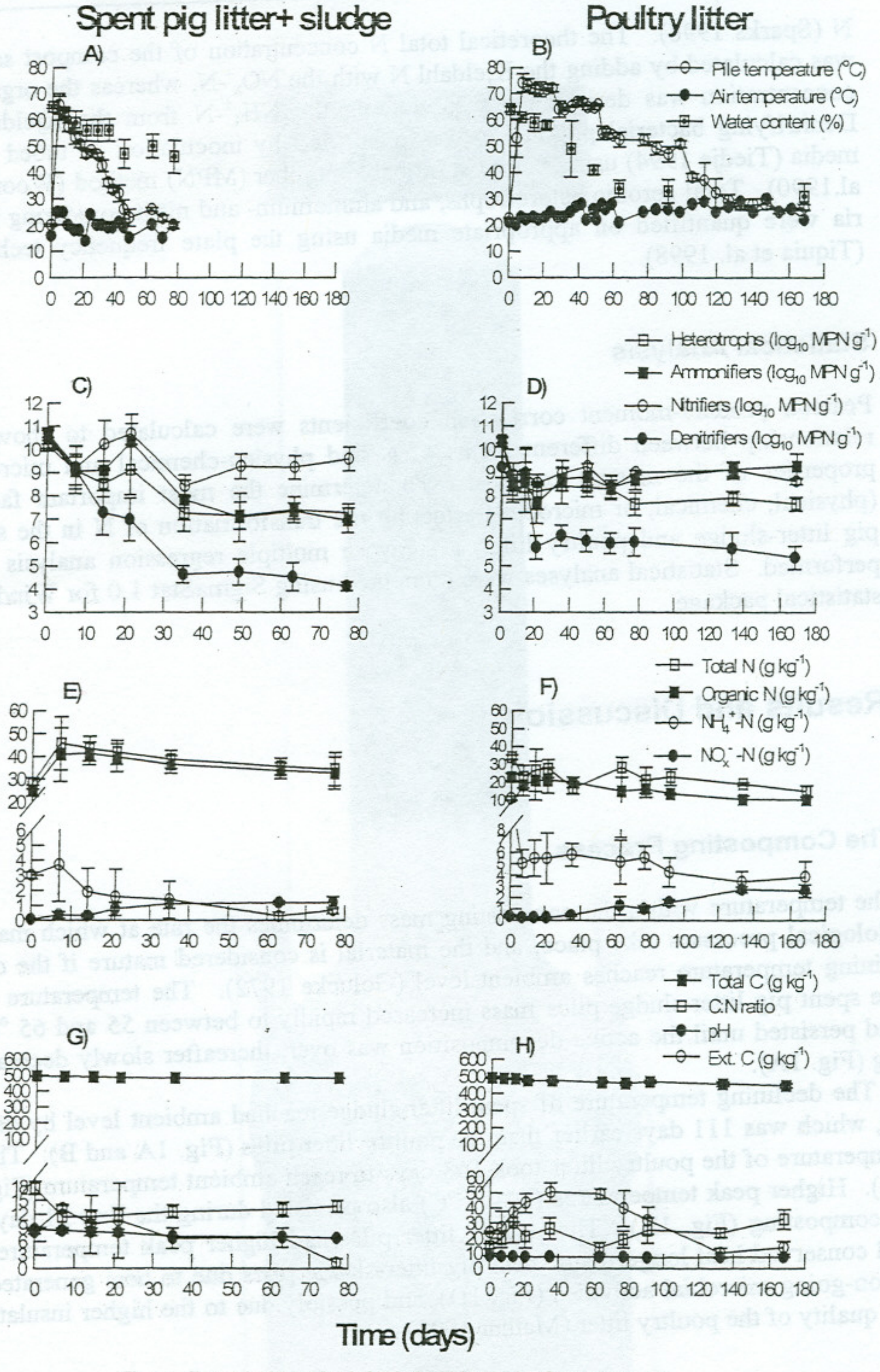

Figure 1. Changes in physico-chemical and microbial properties of manure composts.

The poultry litter also had higher concentrations of available nutrients and water-extractable C than the spent pig litter-sludge (Tiquia and Tam 2000a,b).

Nitrogen Dynamics During Composting

The changes in total N concentration of the manure composts were very similar to the organic N (Fig. 1E–F). This result relates to the facts that the inorganic fractions (NH_4^+-N and NO_x^--N) were low, and that organic N was the major nitrogenous constituent in both feedstocks. After the slight increase in organic N by day 14 (for spent pig litter-sludge) and day 21 (for poultry litter), the organic N gradually decreased (Fig. 1E,F). The initial increase can be attributed to a concentration effect as a consequence of degradation of organic C compounds (Fig. 1G,H), which reduced the dry mass. On the other hand, the subsequent decrease was a result of the ammonification process, which converted a fraction of the organic N to NH_3 and NH_4^+ ions.

The NH_4^+-N concentration decreased dramatically during composting. The dramatic decline in NH_4^+-N concentrations of the spent pig litter-sludge and poultry litter did not, however, correspond to a rapid increase in NO_x^--N, which indicated that some of the NH_4^+-N in these piles had been lost through NH_3 volatilization and/or microbial denitrification. The increase in NO_x^--N concentration during composting was only 1.3 g kg^{-1} and 2.6 g kg^{-1} for spent pig litter-sludge and poultry litter, respectively. This means that about 1.24 g kg^{-1} and 4.60 g kg^{-1} of NH_4^+-N in the spent pig litter-sludge and poultry litter, respectively were lost during composting. Some of the NH_4^+ ions were lost through NH_3 volatilization as the pH of both piles were > 7.0 (Fig. 1G,H), giving conditions that favored NH_3 volatilization. Some of the NO_3^-/NO_2^- ions were lost through microbial denitrification as the population of denitrifying bacteria was high during the early stage of composting (Fig. 1C,D).

Evolution of Microbial Populations Related to N Transformation

It is generally believed that microorganims are fundamentally involved in biochemical transformations during composting (Mathur 1998). The ammonium-oxidizing bacteria are the microorganisms involved in the oxidation of NH_4^+ to NO_2^-, while nitrite-oxidizing bacteria are involved in the subsequent oxidation of NO_2^- to NO_3^-. There are also reports that heterotrophic organisms including bacteria, fungi, and actinomycetes are able to oxidize NH_4^+ to NO_2^-/NO_3^- (Wild 1988). Numbers of total aerobic heterotrophs, and ammonium- and nitrite-oxidizing bacteria were maintained at high population sizes during the active decomposition process (Fig. 1C,D), which suggests a rapid nitrification rate. Myrold (1999) estimated that about 3 x 10^5 nitrifiers g^{-1} soil is required to produce a nitrification rate of 1 mg N kg^{-1} day^{-1}. This population is about half the initial population of total aerobic heterotrophs (10.16 and 10.40 $\log_{10}$ MPN g^{-1}), ammonium-oxidizing bacteria (10.50 and 10.31 $\log_{10}$ MPN g^{-1}), and nitrite-oxidizing bacteria (10.70 and

10.29 $\log_{10}$ MPN g^{-1}) of the spent pig litter-sludge and poultry litter, respectively. Despite this, there was no dramatic increase in NO_x^--N concentration (Fig. 1E,F). This result relates to the facts that a fraction of the NH_4^+-N had been lost through ammonia volatilization and denitrification caused by other interacting factors such as aeration, pH, temperature, water content, and initial C:N ratio (Mathur 1998; Tiquia and Tam 2000a). If these factors are not optimized during composting, they could stimulate volatilization and denitrification of N in the compost piles.

Denitrification is the dissimilatory reduction of NO_3^- to N gases NO, N_2O, and N_2. This process is carried out by a wide variety of mainly heterotrophic bacteria that use NO_3^- as a terminal electron acceptor when O_2 is unavailable. Hence, denitrification occurs in composting where C and NO_3^- are available during periods of restricted O_2 availability (Bishop and Godfrey 1983). In this study, the numbers of denitrifying bacteria were highest at the beginning of composting (Fig. 1C,D) due to higher initial water content (65%) of the piles, which could hinder aeration and subsequently produce anaerobic or microaerophilic pockets in the compost piles. Under these conditions, the denitrifying bacteria use NO_3^- as an electron acceptor instead of O_2, leaving N and N_2O gases to be released from the compost piles to the atmosphere. As composting proceeded, the population of denitrifying bacteria declined significantly (Fig. 1C,D), indicating that very little denitrification took place once the air was forced into the pile. The NO_x^--N concentration also started to increase at a faster rate as composting progressed (Fig. 1C,D).

Relationship Between Different Forms of N and Other Compost Properties

Significant negative correlations were found between C:N ratio and total and organic N of the manure composts (Table 1). This result means that as the total and organic N decreased, the C:N ratio increased. The C:N ratio of the compost pile normally decreases during composting (Golueke 1972); however, due to vigorous losses of N, the C:N ratio increased (Fig. 1E,F). Morisaki et al. (1989), Tiquia and Tam (2000a), and Tiquia et al. (2000) also reported increases in C:N ratio during composting. These losses were attributed mostly through ammonia volatilization. Here, significant correlations were found between NH_4^+-N and temperature, water content, pH, and carbon, while significant negative correlations were found between NO_x^--N and these four parameters (Table 1). The NH_4^+-N was positively correlated with the microbial properties, with the exception of nitrite-oxidizing populations of spent pig litter-sludge where no correlation was found. On the other hand, significant negative correlations were found between NO_x^--N and microbial properties of these two manures (Table 1). The multiple regression analysis demonstrated that C:N ratio was the most important factor affecting the changes in total N during composting of spent pig litter-sludge and poultry litter.

Table 1. Pearson product-moment correlation coefficient (*r*) values between different forms of N and physico-chemical and microbial properties of spent pig litter-sludge and poultry litter

Different forms of N	Parameters									
	temperature	watercontent	pH	Carbon	ext. C	C:N ratio	hetero	ammoni	nitri	denitri
Spent pig litter-sludge										
Total N=	0.94***	0.08	0.12	-0.003	0.62*	-0.97***	-0.07	-0.02	-0.21	-0.01
Organic N=	0.88**	-0.05	-0.01	-0.14	0.52	-0.99***	-0.19	-0.06	-0.21	-0.15
NH_4^+-N	0.60*	0.94***	0.90***	0.87**	0.76*	0.02	0.83*	0.74*	0.29	0.91***
$(NO_3^-+NO_2^-)$-N	-0.59*	-0.93***	-0.97***	-0.93***	-0.68*	-0.13	-0.91***	-0.85**	-0.61*	-0.96***
Poultry litter										
Total N=	0.04	0.64*	0.56	0.70*	0.24	-0.66*	0.71*	0.19	0.63*	0.61*
Organic N=	0.35	0.91***	0.76*	0.89**	0.53	-0.60*	0.68*	-0.06	0.33	0.69*
NH_4^+-N	0.59*	0.66*	0.78**	0.68*	0.13	-0.51	0.98***	0.64*	0.74*	0.66*
$(NO_3^-+NO_2^-)$-N	-0.61*	-0.89***	-0.67*	-0.87**	-0.63*	0.31	-0.64*	0.60*	-0.61*	-0.64*

Ext. C= water extractable carbon; ammoni=ammonium oxidizing bacteria; nitri= nitrite oxidizing bacteria; denitri=denitrifying bacteria.
Correlations were based on 10 average data of the spent pig litter-sludge and poultry litter piles; *, ** and *** indicate significance at 0.05, 0.01 and 0.001 probability levels, respectively.

For the spent pig litter-sludge, the C:N ratio, water content, and pH are the most critical factors affecting the changes of organic N, NH_4^+-N, and NO_x^--N, respectively. For poultry litter, the changes in organic N and NO_3^--N were affected by water content, while change in NH_4^+-N was affected by population of total aerobic heterotrophs (Table 2).

Results of this study revealed that the microbial transformation of N during composting varies with the properties of the initial composting materials. The microbial biomass community structure as well as the physico-chemical properties of the manure during composting such as temperature, water content, pH, and C:N ratio would affect the transformation of N. The equilibria and rates of N dymanics were affected by interactions between microbial biomass community structure and populations, and the physico-chemical properties of the manure.

Table 2. Multiple regression analysis between different forms of N and microbial and physico-chemical properties of spent pig litter-sludge and poultry litter

Regression equation	Multiple R^2 value	F value	Significance of F
Spent pig litter-sludge			
Total N= 72.3 - (2.57 * C:N ratio)	0.92	70.30	0.0004
Organic N= 68.7 - (2.5 * C:N ratio)	0.98	285.00	<0.0001
NH_4^+-N= -7.82 + (0.12 * water content) + (0.35 * pH)	0.79	12.40	0.0192
$(NO_3^-+NO_2^-)$-N= 5.71 - (0.65 * pH)	0.93	81.50	0.0003
Poultry litter			
Total N= -37.6 - (0.62 * C:N ratio)	0.57	6.33	0.0360
Organic N= 3.33 + (0.30 * water content)	0.82	40.90	0.0002
NH_4^+-N= -16.2 + (2.6 * heterotrophs)	0.96	183.50	<0.0001
$(NO_3^-+NO_2^-)$-N= 3.86 - (0.06 * water content)	0.76	29.60	0.0006

Regression analysis was calculated based on four microbial (total aerobic heterotrophs, ammonium- and nitrite-oxidizing bacteria, and denitrifying bacteria) and six physico-chemical (temperature, water content, pH, total C, water-extractable C, and C:N ratio) parameters with stepwise method and PIN (probability of f-to-enter) = 0.050 limit. Heterotrophs=total aerobic heterotrophs.

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