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Nitrogen mineralization dynamics of different valuable organic amendments commonly used in agriculture

Raphael Habai Masunga[a,e], Veronica Nwakaego Uzokwe[b,*], Peter Deusdedit Mlay[a], Inakwu Odeh[c], Ajay Singh[d], David Buchan[e], Stefaan De Neve[e]

[a] Lake Zone Agricultural Research and Development Institute, Ukiriguru, P.O.Box 1433, Misungwi District, Mwanza, Tanzania
[b] International Institute of Tropical Agriculture, Eastern African Hub, Plot No. 25, Mikocheni Light Industrial Area, Mwenge-Coca Cola Road, Mikocheni B, P.O. Box 34441, Dar es Salaam, Tanzania
[c] Department of Environmental Sciences, The University of Sydney, Rm 409, Biomedical Building C8, 1, Central Avenue, Australian Technology Park, Eveleigh, Sydney, NSW 2006, Australia
[d] Agricultural and Food Engineering Department, Indian Institute of Technology Kharagpur, West Bengal, 721 302, India
[e] Faculty of Bioscience Engineering, Ghent University, Coupure Links, 653, 9000 Ghent, Belgium

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ABSTRACT

Sustainable agriculture requires the careful optimization of the use of organic amendments to improve soil fertility while minimizing any harmful environmental effects. To understand the events that occur in soil after the addition of different organic amendments, we evaluated the nitrogen (N) mineralization dynamics in soil after adding organic amendments, and evaluated changes in the microbial population. The four organic amendments were fresh dairy cattle manure, fresh white clover, vegetable, fruit, and yard waste compost, and poplar tree compost. The N mineralization potential of each organic amendment was determined by analyzing total mineral nitrogen during a 97-day laboratory incubation experiment. Soils amended with clover released 240 $\mu g\,N\,g^{-1}$ soil during the 97-day incubation, more than twice as much as that released from soils amended with manure or composts (76–100 $\mu g\,N\,g^{-1}$ soil). At the end of the incubation, the net N mineralization in clover-amended soils was 54%, more than five times higher than that in soils amended with composts or manure (4%–9%). Nitrogen was mineralized faster in clover-amended soil (1.056 $\mu g\,N\,g^{-1}$ soil day^{-1}) than in soil amended with composts (0.361–0.417 $\mu g\,N\,g^{-1}$ soil day^{-1}). The microbial biomass carbon content was higher in clover-amended soil than in the soils amended with manure or composts. We monitored changes in the microbial population in amended soils by a phospholipid fatty acid (PLFA) analysis. On day 97, there were higher concentrations of total PLFAs in soils with organic amendments (e.g., 14.41 nmol g^{-1} in clover-amended soil) than in control soil without amendments (9.84 nmol g^{-1}). Bacteria (Gram-positive and Gram-negative), actinomycetes, and fungi were more abundant in clover-amended soils than soils amended with manure or composts. The N mineralization potential varied among the four organic amendments. Therefore, the timing of application and the type of organic amendment should be matched to the nutrient needs of the crop.

1. Introduction

To increase crop yields, reduce environmental pollution, and achieve sustainable agriculture, soil fertility needs to be maintained at an appropriate level, or restored if it has decreased (Diacono and Montemurro, 2010; Fageria, 2007). In farming systems with low inputs of chemical fertilizers and pesticides, this can be achieved by rotating leguminous and non-leguminous crops, and by addition of organic amendments (OA). These OAs can be composted or non-composted organic wastes from agriculture, industry, municipal operations, seaweed, or blood and bone meal (Quilty and Cattle, 2011).

Abbreviations: AMF, arbuscular mycorrhizal fungi; CLO, fresh white clover; COI, poplar tree compost; COV, vegetable, fruit, and yard waste compost; ILVO, Institute for Agriculture and Fisheries Research; MAN, cattle manure; OA, organic amendments; PLFA, phospholipid fatty acid; SOM, soil organic matter; TOC, total organic carbon.

* Corresponding author. Fax: +255 22 2775021.
E-mail addresses: v.uzokwe@cgiar.org, uzokwene2008@googlemail.com (V.N. Uzokwe).

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The application of OAs is common in organic farming systems. These OAs enhance plant growth and may reduce the need for mineral fertilizers (Mohanty et al., 2011), which reduces costs for farmers. Organic amendments restore and reclaim degraded soils by maintaining organic matter and sustaining soil fertility for agricultural production, particularly in the long-term, by slowly releasing nutrients (Tejada et al., 2009). Thus, OAs recycle nutrients and organic matter to support crop productivity and maintain soil quality (Whalen et al., 2001).

Soil organic matter (SOM) is a storehouse and supplier of nutrients such as nitrogen (N), phosphorus, and sulfur to crops (Schulten and Schnitzer, 1998), and it improves the physical, chemical, and biological properties of soils (Diacono and Montemurro, 2010). The growth and activity of soil microbes are stimulated by SOM, leading to efficient mineralization of crop nutrients (Tejada et al., 2009). The SOM is derived from plants, animals, and microbes. These organic materials, either added to the field or already on-site, decompose via mineralization to release the nutrients required for crop growth and development (Diacono and Montemurro, 2010). The recent popularity of OAs in agriculture represents an alternative strategy to manage wastes and improve the SOM content in low-fertility soils (Flavel and Murphy, 2006).

Nitrogen mineralization is a biological process. The amount of N released to crops depends on the chemical composition of organic matter (e.g., N content, carbon:N ratio, and contents of cellulose and hemicelluloses, lignin, and polyphenols) (Calderón et al., 2005; Mohanty et al., 2011) and on the physical, chemical, and biological properties of soil microbes (Manojlović et al., 2010). Organic amendments with high N contents and low C:N ratios mineralize sufficient N to satisfy plant growth (Cordovil et al., 2005; Seneviratne, 2000). Conversely, N can be immobilized in OAs with lower N contents and higher C:N ratios (Manojlović et al., 2010).

It is important to manage OAs appropriately to avoid contaminating the environment (Manojlović et al., 2010). Groundwater and atmospheric contamination are the main impacts of excessive use of organic fertilizers in agriculture (Calderón et al., 2005). There are two main reasons for exploring the N mineralization dynamics of OAs used in agriculture; first, to avoid excess fertilizer application and reduce N losses to the environment; and second, to optimize residue management to maximize crop production, especially in low-input agriculture based on nutrient recycling (Bruun et al., 2006).

There have been some studies on the dynamics of N mineralization from OAs in agro-ecosystems; for example, animal manures, crop residues, and composts (Abbasi et al., 2007; Amanullah, 2007; Azeez and Van Averbeke, 2010; De Neve and Hofman, 1996; Van Kessel and Reeves, 2002). However, few studies have specifically compared the effects of various kinds of OAs (e.g., cattle manure, compost, and green manure) on N mineralization and on the microbial population as the main decomposers. In this study, we focused on the effects of various OAs on both N mineralization and the microbial population, which plays an important role in nutrient recycling, especially in organic farming systems. Two hypotheses were tested. First, we hypothesized that the N mineralization dynamics in soil will differ depending on the type of OA added. Second, we hypothesized that the addition of different OAs will have different consequences in terms of the size and composition of the microbial population.

2. Materials and methods

2.1. Soil collection and analysis

Sandy loam soil was collected from the surface layer (0–20 cm) at the Research Farm of the Institute for Agriculture and Fisheries Research (ILVO), Merelbeke, Belgium. White clover had been cultivated in this soil continuously for several years. The soil was obtained in September 2011 at 17% (w/w) field moisture content, and a subsample was taken for chemical analysis. Field-moist soil was passed gently through a 4.75-mm sieve to remove root materials, surface litter, and stones. The soil pH was measured with a pH meter in a potassium chloride suspension (1.0 g soil: 2.5 ml KCl). Total N and C contents were determined using a CNS Analyzer (Variomax CNS Elementar, Hanau, Germany). The soil had a pH_{KCl} of 5.5 (1:2.5 w/v), a bulk density of 1.3 g cm^{-3}, 0.09% total N content, and 1.12% total C content.

2.2. Organic amendments

We used four different OAs in the 97-day laboratory incubation experiment. Fresh dairy cattle manure (MAN) and fresh white clover (CLO) were obtained from the ILVO. The other two OAs were composts: vegetable, fruit, and yard waste compost (COV), produced from household wastes and obtained from Vlaamse Compostorganisatie (VLACO; Mechelen, Belgium); and poplar tree and grass compost (COI), which was obtained from the ILVO.

2.3. Laboratory incubation procedure

Before the incubation experiment, the fresh white clover was chopped into small pieces (with dimensions of approximately 2–10 mm) using a kitchen knife. Each OA was mixed with 200 g of moist soil at a rate indicated in Table 6 then placed in plastic tubes (7.2-cm length, 6.8-cm diameter). Then, the soil was compacted to give a bulk density of 1.3 g cm^{-3} (identical to that measured in the field). The tubes were covered with pin-holed parafilm to allow air circulation and minimize water evaporation, then incubated in the dark at 20 °C for 97 days. For the control, no OA was added to the soil, but the soil samples were mixed, compacted, covered, and incubated in exactly the same way as the soils in the OA treatments. During the incubation period, all soils were maintained at 55% water-filled pore space (WFPS), which was calculated from the bulk density and the gravimetric moisture content. The weight loss of each tube was checked daily, and distilled water was added to each tube to maintain a constant soil moisture content as required.

Four separate replicates of each of the four treatments and the control were analyzed at each sampling time (days 7, 21, 40, 68, and 97 days of incubation). All parameters (mineral N, microbial biomass C (C_{mic}), PLFA concentration, and moisture content) were measured for each replicate.

2.4. Measurements of mineral nitrogen and microbial biomass carbon

To measure mineral N, 30 g of soil was extracted in 60 ml 1 M KCl with shaking for 1 h. The mixture was filtered through mineral N filter paper (MN 616), then the extract was stored at −18 °C until analysis. Mineral N (NO_3^--N and NH_4^+-N) was determined with a continuous flow auto-analyzer (Chemlab System 4, Skalar, The Netherlands).

The net N mineralization of each OA was calculated as the difference in the amount of mineral N released between amended and control soil (Mohanty et al., 2011). The percentage of total N mineralized from each OA at each sampling time was calculated as described by Azeez and Van Averbeke (2010) and Abbasi et al.

(2007), as follows:

$$\%\,\text{Mineralization} = \frac{\text{MN(amended)} - \text{MN(control)}}{\text{N Total (applied)}} \times 100$$

where MN is total mineral N, and N total is total N in the applied OA.

The chloroform fumigation–extraction method (Vance et al., 1987) was used to determine C_{mic}. Each 30-g soil sample was fumigated with chloroform for 24 h, then 60 ml 0.5 M K_2SO_4 was added and the mixture was shaken for 1 h. The samples were filtered through Whatman No. 5 filter paper and the filtrates were stored at −18 °C until analysis. Non-fumigated samples were simultaneously extracted using the same procedure. The organic C content of the extracts was determined with a total organic carbon (TOC) analyzer. The C_{mic} value was calculated as the difference in organic carbon content between fumigated and non-fumigated samples (Wang et al., 2007; Moore et al., 2000). The K_2SO_4 extraction efficiency factor of 0.45 stated by Joergensen (1996) was used to estimate C_{mic} with the following equation:

$$C_{mic} = \frac{C_f - C_{nf}}{kEC},$$

where C_f and C_{nf} represent the amount of carbon extracted from fumigated and non-fumigated samples, respectively, and kEC is the extraction factor.

2.5. Phospholipid fatty acids (PLFAs) analysis

Soils sampled at each time were freeze-dried and stored at −18 °C until analysis. The PLFAs were extracted from 4 g of soil using a modified technique. The extracted samples were analyzed by gas chromatography mass spectrometry (GC–MS) with a Thermo Focus GC coupled to a Thermo DSQ MS (Thermo Fisher Scientific Inc., Waltham, USA) in electron ionization mode.

Fatty acids were designated as *X*: *YωZ*, where '*X*' is the number of carbon atoms in the chain, '*Y*' is the number of double bonds (unsaturations), and '*Z*' is the number of carbon atoms from the methyl end of the molecule to the first unsaturated bond (Bossio et al., 1998; Fraterrigo et al., 2006; Peacock et al., 2001; Zelles, 1999). The prefixes *a*,*i*, *cy*, and *d* refer to *anteiso*, *iso*, cyclopropyl branching, and dicarboxylic fatty acids, respectively; *br* indicates unknown branching type, and ME indicates the position of a methyl group. The prefixes α and β indicate that the OH groups of an OH fatty acid are located at positions 2 and 3, respectively. Numbers preceded by *ω* indicate the position of OH groups from the aliphatic end of the fatty acid. The suffixes *c* and *t* indicate *cis* and *trans* geometry, respectively.

Different fatty acids indicate different microbial groups: cyclopropyl fatty acids are indicative of Gram-negative bacteria; branched fatty acids (e.g., *iso* and *anteiso*) indicate Gram-positive bacteria (Balser and Firestone, 2005 Zelles, 1999); monounsaturated fatty acids are indicative of fungi and bacteria (Peacock et al., 2001 Zelles, 1999); and polyunsaturated fatty acids are indicative of eukaryotes, excluding cyanobacteria. The fatty acids with methyl branching on the tenth carbon atom are indicative of actinomycetes (Zelles, 1999).

We calculated the total PLFA and PLFA concentrations for different microbial groups for each sample. The sum of iC15:0, aC15:0, iC16:0, aC16:0, iC17:0 and aC17:0 represented Gram-positive bacteria. The sum of cyC17:0 and cyC19:0 fatty acids represented Gram-negative bacteria. The total bacterial community was the sum of marker PLFAs for Gram-positive bacteria, Gram-negative bacteria, C15:0, and C17:0. The sum of C18:2ω9, 12c, C18:1ω9c, and C18: 3ω9, 12, and 15c represented fungi, and C16:1ω11 represented arbuscular mycorrhizal fungi (AMF). Actinomycetes were calculated as the sum of 10Me16:0 and 10Me18:0. We also calculated the ratio of bacteria to fungi (B:F).

2.6. Statistical analyses

All data (MN, C_{mic}, and PLFA concentrations) were statistically analyzed by two-way analysis of variance (ANOVA) using SPSS version 16.0. Tukey's (honestly significant difference) post-hoc test was used to compare means when significant differences were found. The main effects (time and treatment) and their interaction were considered significant at a probability level (p) of 0.05. Pearson's correlation analysis was performed to test relationships between variables.

3. Results

3.1. Initial properties of organic amendments

Table 1 shows the chemical characteristics of the OAs used in the experiment. Total N content was significantly higher in CLO than in the other OA materials. The carbon content was significantly higher in CLO and MAN than in COI and COV. The carbon:N ratio was significantly lower in CLO than in the other OAs. From the lowest carbon:N ratio to the highest, the four amendments were ranked as follows: CLO < COV < COI < MAN.

3.2. Nitrogen mineralization

The total mineral N (MN) content increased in all OA treatments and the control during the incubation experiment. The range was 6–71.7 μg N g^{-1} soil in the control, and 37.4–240.9 μg N g^{-1} soil in the OA treatments (Fig. 1, Table 7). In CLO-amended soil, the total MN increased from 148.7 μg N g^{-1} soil (day 7) to 240.9 μg N g^{-1} soil (day 97). The total MN released during the entire incubation period was lowest in the control (65.7 μg N g^{-1} soil) and highest in CLO-amended soil (240.9 μg N g^{-1}). Intergrated over the whole incubation period, the highest percentage of N mineralization was in CLO-amended soil and the lowest was in COI-amended soil. Net N mineralization increased at the beginning of the incubation period. The maximum amount of mineralized N was recorded at 40 days of incubation in COI- and MAN-amended soils, and at 68 days in CLO- and COV-amended soils. Afterwards, net mineralization decreased in CLO-, COV-, and COI-amended soils, but leveled off in MAN-amended soil after 40 days of incubation.

Net mineralization (Table 2) represents the difference in the amount of mineral N released between OA-amended soil and control soil. The net N mineralization was markedly higher in CLO-amended soil than in the other amended soils. Consequently, net N mineralization showed an increasing trend in all treatments from day 7 until day 68, then decreased. The relative decrease was larger in the COI-amended soil and COV-amended soil (from 18.5 to 4.6 μg N g^{-1} in COI-amended soil, and from 28.6 to 6.5 μg N g^{-1} in COV-amended soil).

Table 1
Chemical properties of organic amendments used in 97-day incubation experiment.

Material	C% (DM)	N% (DM)	C:N ratio	pH (KCl)
CLO	46.2 (0.05) c	6.3 (0.03) c	7.4 (0.02) c	n.d.
COI	25.3 (0.7) b	2.2 (0.06) ab	11.4 (0.18) a	7.6 (0.04) b
COV	26.0 (6.58) b	2.9 (0.89) b	9.1 (0.52) b	6.9 (0.01) ab
MAN	40.6 (0.54) bc	3.3 (0.03) b	12.3 (0.64) a	7.3 (0.50) b

Notes: n.d. = not determined, C = carbon, N = nitrogen, DM = dry matter, CLO = fresh white clover, COI = poplar tree compost, COV = vegetable, fruit, and yard waste compost, MAN = fresh dairy cattle manure. Different letters in the same column indicate significant differences ($p < 0.05$). Values shown are means ($n = 4$; standard errors in parentheses).

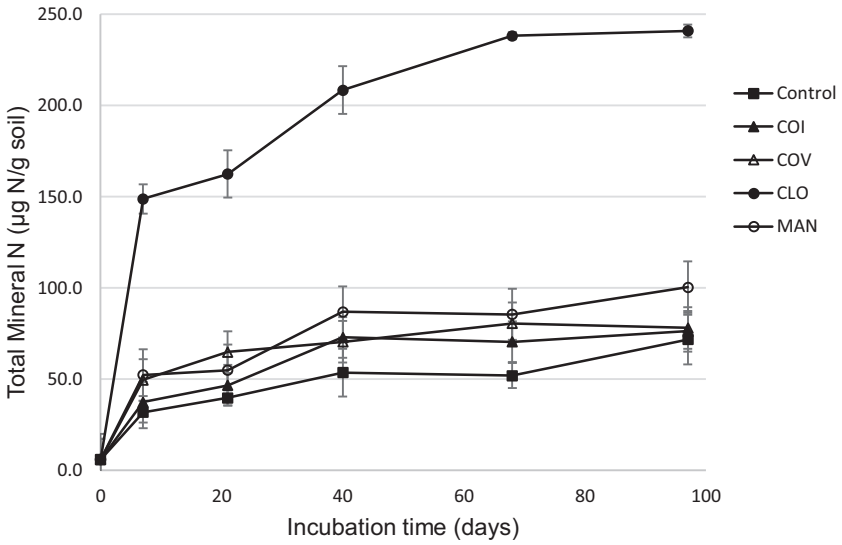

Fig. 1. Nitrogen mineralization in soils with organic amendments. A. Total mineral nitrogen released in soils with or without organic amendments during a 97-day laboratory incubation experiment. Values are means. Error bars show standard deviation.

Table 2
Net total mineral nitrogen released ($\mu g\,N\,g^{-1}$ soil) from amendments during a 97-day laboratory incubation experiment.

Time (days)	Treatment			
	COI	COV	CLO	MAN
	($\mu g\,N\,g^{-1}$ soil)			
7	5.6	17.71	125.43	20.44
21	6.92	25.21	122.64	25.94
40	19.33	16.83	163.02	33.33
68	18.51	28.6	192.49	33.47
97	4.61	6.46	169.21	28.73

Notes: CLO = fresh white clover, COI = poplar tree compost, COV = vegetable, fruit, and yard waste compost, MAN = fresh dairy cattle manure.

Table 3
Total mineral nitrogen (MN) released ($\mu g\,N\,g^{-1}$ soil) at different sampling times during incubation, and in different treatments.

Time	MN ($\mu g\,N\,g^{-1}$)	Treatment	MN ($\mu g\,N\,g^{-1}$)
0	5.96 (7.9) a	CTR	44.20 (3.2) a
7	65.65 (3.5) b	COI	60.73 (3.5) b
21	75.87 (3.5) b	COV	70.0 (3.5) bc
40	100.06 (3.5) c	CLO	204.30 (3.5) d
68	106.57 (3.5) c	MAN	78.12 (3.5) c
97	116.85 (3.5) c		

Notes: different letters in the same column indicates significant differences between means (Tukey's test: $p < 0.05$). Values shown are means (standard errors in parentheses); CTR = Control, CLO = fresh white clover, COI = poplar tree compost, COV = vegetable, fruit, and yard waste compost, MAN = fresh dairy cattle manure.

In the two-way ANOVA, the effects of time, treatment, and their interaction on total MN were significant ($p < 0.05$; Table 3). That is, time and the type of OA affected total MN, and the effects of the OA depended on the incubation time. The amount of total MN released was lower on day 7 than on other days. Comparing OA treatments, the amount of total MN released was always higher in CLO-amended soil than in the other treatments. The amount of total MN released did not differ significantly between the COI- and COV-amended soil, or between MAN- and COV-amended soil.

A zero-order mineralization kinetics equation was fitted to the total mineral N data, as follows

$$N_{min}(t) = k \times t \times C,$$

where $N_{min}(t)$ is the cumulative amount of N mineralized at time t (days), k is the zero-order mineralization rate (day^{-1}) and C is the intercept parameter (amount of N mineralized at $t = 0$). The results of these calculations are shown in Table 4. The N mineralization rate was higher in CLO-amended soil (1.056 $\mu g\,N\,g^{-1}\,day^{-1}$) than in COV-amended soil and COI-amended soil (0.361 and 0.417 $\mu g\,N\,g^{-1}\,day^{-1}$, respectively).

3.3. Microbial biomass carbon

The C_{mic} values showed similar trends in the control and OA-containing soils over the 97-day incubation period (Fig. 2). The C_{mic} peaked on day 21 in all treatments. The highest C_{mic} values were in CLO-amended soil (280 $\mu g\,C\,g^{-1}$ soil) and the lowest in the control (180 $\mu g\,C\,g^{-1}$ soil). The C_{mic} then decreased until day 68 in all treatments. After that, it peaked again in MAN- and CLO-amended soils on day 97, but did not increase again in COI- or

Table 4
Nitrogen mineralization, nitrogen mineralization rate, and field nitrogen mineralization rate in soils with or without organic amendments during a 97-day incubation experiment.

Treatment	Mineralization at $t = 0$ (intercept) ($\mu g\,N\,g^{-1}$ soil)	Mineralization rate (k) (at 20 °C) ($\mu g\,N\,g^{-1}$ soil day^{-1})	R^2	Mineralization rate ($\mu g\,N\,g^{-1}$ soil yr^{-1})	Mineralization rate (0.2 m depth) ($kg\,N\,ha^{-1}\,yr^{-1}$)
CTR	28.63 a	0.498 a	0.89	182.5	70.19
COI	41.29 b	0.417 b	0.75	153.3	58.96
COV	53.19 c	0.361 c	0.89	146	56.15
CLO	155.10 d	1.056 d	0.83	386.9	148.81
MAN	55.42 e	0.487 e	0.86	175.2	67.39

Notes: different letters in the same column indicate significant differences ($p < 0.05$). Values shown are means.

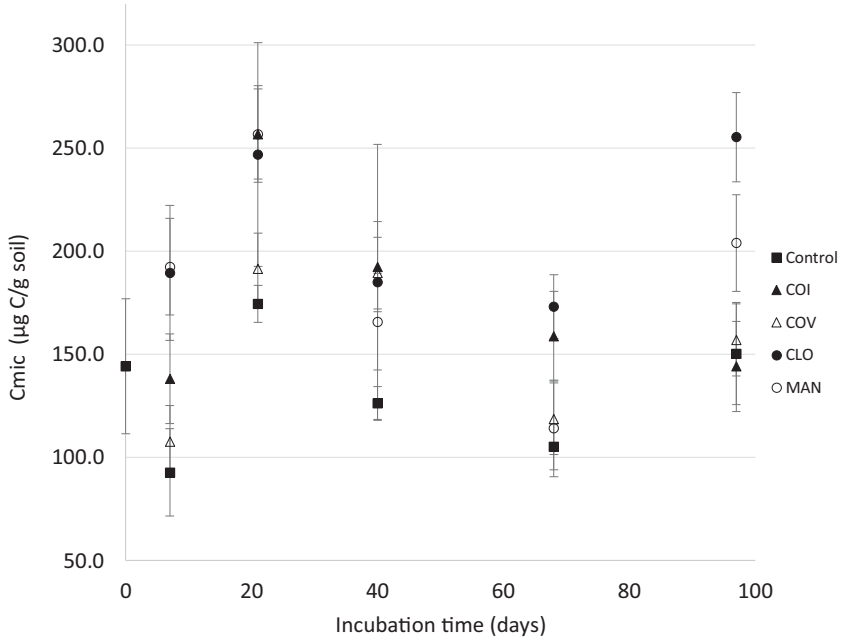

Fig. 2. Microbial biomass carbon ($\mu g\,C\,g^{-1}$ soil) over time in soil with or without organic amendments during a 97-day laboratory incubation. Value shown are means. Error bars show standard errors. Organic amendments: fresh dairy cattle manure (MAN), fresh white clover (CLO), vegetable, fruit, and yard waste compost (COV), and poplar tree compost (COI). Control (CTR).

COV-amended soils. There was a weak but highly significant positive correlation ($R^2 = 0.473$, $p < 0.01$) between total MN and C_{mic}.

There were significant effects of time, type of OA, and their interaction on C_{mic}. Averaged over all of the sampling times, C_{mic} was significantly higher in CLO-amended soil (mean ± S.E., $219.6 \pm 7.8\,\mu g\,g^{-1}$) than in other treatments (ranging from 161.1 ± 7.8 in COV-amended soil to $189.6 \pm 8.0\,\mu g\,g^{-1}$ in MAN-amended soil) and the control ($132.2 \pm 7.5\,\mu g\,g^{-1}$).

3.4. Phospholipid analysis

We analyzed the changes in PLFAs concentrations over the 97-day incubation period (Fig. 3A; Table 5). In all treatments and the control, the total PLFAs concentration decreased over time. On day 7, the highest PLFA concentrations were in CLO- and MAN-amended soil (25 nmol g^{-1} soil) and the lowest was in the control (14 nmol g^{-1} soil). The total PLFAs concentration did not change significantly from day 40 to day 97 in CLO-amended soil, MAN-amended soil, and the control. Based on the fatty acid biomarkers (Table 5; Fig. 3B), Gram-positive bacteria and fungi were the dominant microbes in all treatments, and other groups made up a smaller proportion of the total. The two-way ANOVA revealed that time, type of OA, and their interaction affected the total PLFAs concentration (Table 5). That is, the mean total PLFAs concentration differed among treatments and among sampling times, and the effect of OAs depended on the incubation time. Overall, the total PLFAs concentration was significantly higher in CLO-and MAN-amended soils than in the control, COV-amended soil, and COI-amended soil. The total PLFAs concentration did not differ significantly between COI- and COV-amended soils, nor between CLO- and MAN-amended soils. The total PLFAs concentration was significantly higher on day 7 than on any other sampling day.

The population of Gram-positive bacteria was significantly larger in COI-amended soil than in the other treatments (Table 5). There were more fungi, protozoa, and actinomycetes in CLO-amended soil than in the other treatments. There were more Gram-positive bacteria and AMF in COI-amended soil than in the other treatments and the control. The effect of time on the concentration of fatty acid biomarkers was significantly higher ($p < 0.05$) on the first days of incubation than on the other days. The B:F ratio was significantly higher in CLO-amended soil (1:4.5) than in the other treatments (1:4.0; 1:8, and 1:3.9 for COI, COV, and MAN, respectively) and the control (1:3).

4. Discussion

4.1. Chemical properties of organic amendments

The OAs showed significant differences in their chemical composition (Table 1). The highest total N content was in CLO, because clover fixes atmospheric N as well as taking up N from soil (Seneviratne, 2000). The high N content in CLO resulted in a low C:N ratio (approx. 7). The N and C contents did not differ significantly between the two composts, but both were significantly lower in the composts than in CLO. The total N content was lower in MAN than in CLO, giving a higher C:N ratio for MA Bernal et al. (1998) reported that nutrient contents were lower in composted organic materials than in other materials, because the decomposition during composting degraded organic matter. The range of C:N ratios of composts in this study (9.1–11.4) was similar to the range (8–10) reported by Gale et al. (2006).

4.2. Nitrogen mineralization

Nitrogen availability is often limited in agro-ecosystems. The main source of N for crops and microbes is SOM, via mineralization. High-quality organic matter is that with a low C:N ratio and with sufficient N to sustain microbe and crop growth. In most cases, soil is N deficient because it contains little or poor-quality organic matter. Addition of OAs, which are usually N-rich, to soil usually improves the quality of soil organic matter.

Nitrogen mineralization differed among the OAs, with the highest N mineralization in CLO-amended soil (Table 4). Ross et al. (2009) compared seven clover species as green manure, and white clover showed the highest N mineralization. In the present study,

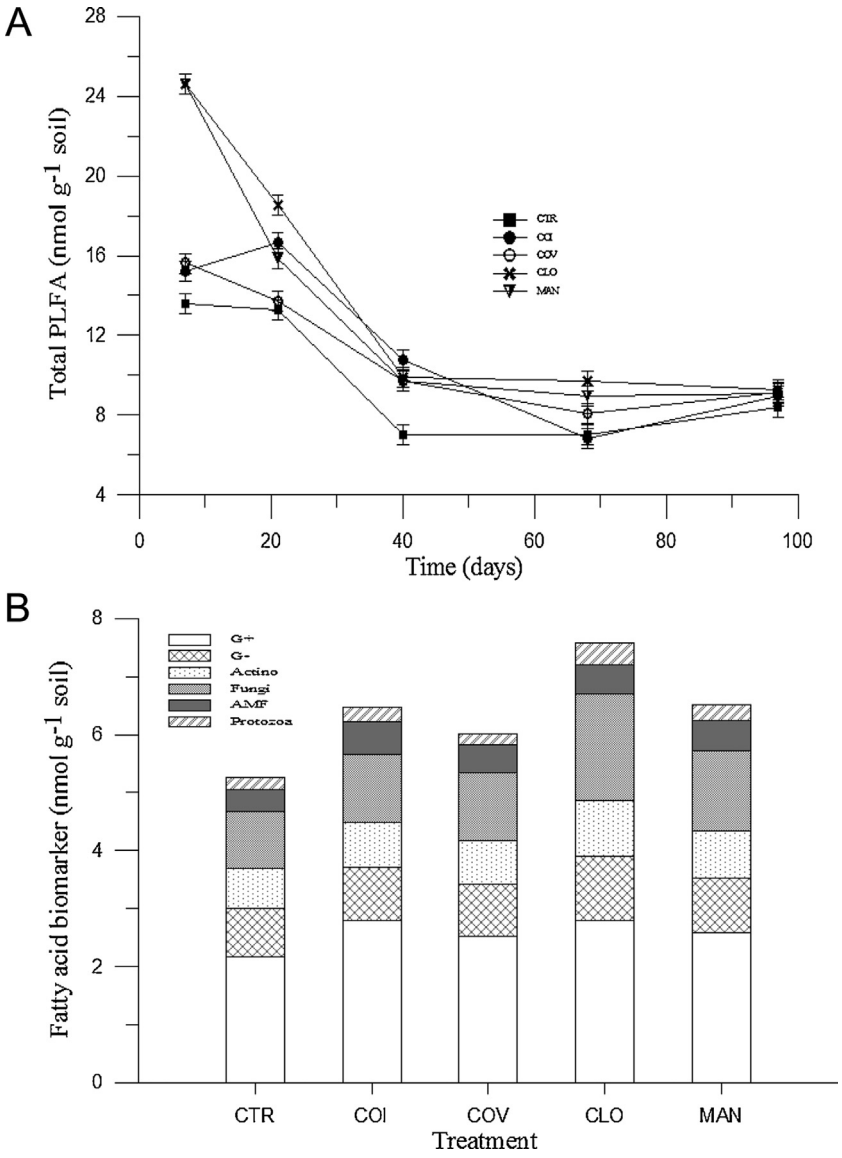

Fig. 3. Phospholipid fatty acids (PLFAs) and fatty acid biomarkers in soils with or without organic amendments over a 97-day incubation period. A. Total PLFAs concentration (nmol g^{-1} soil) in soils with or without organic amendments during a 97-day laboratory incubation. Values shown are means. Bars show standard error. B. Concentrations (nmol g^{-1}) of fatty acid biomarkers (Gram-positive (G+) bacteria, Gram-negative (G-) bacteria, actinomycetes (Actino), fungi, arbuscular mycorrhizal fungi (AMF), and protozoa) in different treatments after a 97-day laboratory incubation. Organic amendments: fresh dairy cattle manure (MAN), fresh white clover (CLO), vegetable, fruit, and yard waste compost (COV), and poplar tree compost (COI). Control (CTR).

Table 5
Effects of treatments and time on fatty acid biomarker concentrations (nmol g^{-1}) in soils with or without organic amendments incubated for 97 days.

	Fatty acid biomarkers (nmol g^{-1} soil)						
Treatment	Gram+	Gram-	Actino	Fungi	AMF	Protozoa	Total PLFA
CTR	2.16 (0.8) a	0.83 (0.2) a	0.70 (0.2) a	0.99 (0.3) a	0.38 (0.1) a	0.21 (0.1) b	9.84 (3.1) a
COI	2.80 (1.1) c	0.91 (0.2) ab	0.78 (0.3) ab	1.18 (0.4) b	0.56 (0.2) c	0.23 (0.1) b	11.69 (4.1) b
COV	2.52 (0.8) b	0.90 (0.2) ab	0.76 (0.2) ab	1.15 (0.3) b	0.48 (0.1) b	0.20 (0) b	11.25 (3.1) b
CLO	2.79 (1.3) bc	1.11 (0.3) c	0.97 (0.3) c	1.82 (0.8) c	0.50 (0.3) bc	0.38 (0.2) a	14.41 (6.5) c
MAN	2.58 (1.0) bc	0.95 (0.2) b	0.80 (0.2) b	1.38 (0.5) d	0.52 (0.2) bc	0.27 (0.1) b	13.6 (6.4) c
Time							
7	3.77 (0.6) a	1.24 (0.2) a	1.06 (0.2) a	1.96 (0.7) a	0.75 (0.2) a	0.25 (0.1) a	18.73 (5.2) a
21	3.57 (0.4) a	1.12 (0.1) b	1.05 0.1) a	1.58 (0.3) b	0.67 (0.1) b	0.39 (0.2) b	15.60 (2.1) b
40	1.94 (0.4) b	0.75 (0.1) d	0.64 (0.1) bc	1.14 (0.2) c	0.37 (0.1) c	0.23 (0) b	9.42 (1.5) c
68	1.82 (0.2) b	0.66 (0.1) d	0.56 (0.1) c	0.92 (0.2) d	0.30 (0) d	0.18 (0) b	8.12 (1.2) d
97	1.76 (0.2) b	0.92 (0.1) c	0.70 (0.1) b	0.90 (0.1) d	0.35 (0.1) cd	0.23 (0) b	8.96 (0.5) cd

Notes: actino = actinomycetes, AMF = arbuscular mycorrhizal fungi. Different letters in the same column indicate significant differences (Tukey's test, $p < 0.05$). Values shown are means (standard errors in parentheses).

Table 6
Application rates of amendments used in 97-day laboratory incubation experiment.

Amendment	Moist soil	Water	Application rates of organic amendments			
			Moist	Dry basis		
	(g tube^{-1})	(ml)	(g tube^{-1})	(g tube^{-1})	(kg N ha^{-1})	(t ha^{-1})
COI	200	2.2	4.28	2.09	126.5	5.8
COV	200	1.0	2.57	1.59	127.6	4.4
CLO	200	3.9	5.59	0.73	126.0	2.0
MAN	200	4.3	4.82	1.39	125.4	3.8

the high net N mineralization from CLO (154 μg N g^{-1} soil, or 54% of the total N applied) may have been because of the low C:N ratio and high N content in CLO. In other reports, N mineralization in soil containing clover residues ranged from 30% (Marstorp and Kirchmann, 1991) to 60% (Cookson et al., 2002) of the total N applied. Differences in N mineralization may be because of differences in incubation conditions (e.g., temperature, duration), and/or the characteristics and composition of the clover materials (plant age, plant part, crop management, and proportions of cellulose, hemicelluloses, and lignin).

Nitrogen mineralization did not differ significantly between COV- and COI-amended soils (19 and 11 μg N g^{-1} soil, respectively, or 7% and 4% of the total N applied, respectively). These low rates of N mineralization may be because these composts contained a high proportion of stable, recalcitrant materials, and most N was complexed in organic forms. The composting process stabilizes organic compounds, reducing the proportion of soluble forms of C and N (Flavel and Murphy, 2006). In another study, only a small proportion of organic N in composts was mineralized over time (Zaman et al., 2004). Therefore, even if compost is N-rich, the N is mineralized slowly. Nevens and Reheul (2003) observed that N release was limited in compost made from vegetable, fruit and garden waste, despite its high N content (1.5% of fresh weight). Hartz et al. (2000) also observed that N mineralization was higher in manure than in composts (7% and 1% of organic N applied, respectively), despite their similar C:N ratios and N contents (10.1 and 9.3, respectively, and 1.2% and 2.2%, respectively).

Nitrogen mineralization was lower in MAN-amended soil (28 μg N g^{-1}, or 9% of total N applied) than in CLO-amended soil. The same N mineralization value was reported for cattle manure by Abbasi et al. (2007) (9% of total N applied after 120 days), but a lower value (2%) was reported by Chadwick et al. (2000). Hartz et al. (2000) reported slightly higher N mineralization from manure (16% of organic N) than from composts (1%–7%). In this study, the MAN was obtained from intensively managed dairy cattle whose nutrients requirements were met. Possible reasons for the low N mineralization from MAN include the age of the animals and the type of diet (Chadwick et al., 2000).

In this study, N mineralization continuously increased over time in MAN- and CLO-amended soils, but leveled off in the compost-amended soils after day 68 (Fig. 1). The largest amounts of mineral N released by the end of the incubation were in the control, CLO-amended soil, and MAN-amended soil. In these treatments, N was mineralized steadily over time.

In COI- and COV-amended soils, N was immobilized from day 40 to day 97. The N immobilization from day 40 corresponded to the increase in the microbial population. In other incubation studies, N was first immobilized but then re-mineralized (Azeez and Van Averbeke, 2010) because OAs stimulated microbial growth and reproduction, and hence, increased competition for available nutrients. Cordovil et al. (2005) reported that N mineralization from composted pig manure increased up to 35 days of incubation, but then slowed and stopped after 57 days. This was because most of the organic N was complexed in the stable recalcitrant compounds that remained after the initial rapid mineralization.

The N mineralization rate (k) in CLO-amended soil was at least twice that in the other treatments (Table 4), possibly because of the large pool of labile N in CLO. The two composts showed the slowest N mineralization rates, implying that N was bound more strongly in composts than in the other OAs. In a laboratory incubation experiment, the N mineralization rate of winter wheat was 0.952 mg N kg^{-1} day^{-1} (Watkins and Barraclough, 1996), comparable to that in CLO-amended soil.

4.3. Soil microbial biomass carbon

Addition of OAs to soil stimulates soil microbial activity, and changes the size of the soil microbial biomass (Wang et al., 2007). Also, the quality and quantities of OAs applied can affect the microbial biomass in soil (Černý et al., 2008). In this study, C_{mic} was higher in OA-amended soils than in the control soil (Fig. 2), possibly because the high C content in OA-amended soils created suitable conditions for microbial growth. The increase in C_{mic} was consistent with the size of the C input. Flavel and Murphy (2006) found that the increase in C_{mic} in soils with amendments could be attributed to increased available carbon, which served as an energy source for rapid microbial growth. In this study, C_{mic} was higher in CLO- and MAN-amended soils than in compost-amended soils, because CLO and MAN had higher C contents than did the composts (Table 1). The C_{mic} did not differ significantly between COI- and COV-amended soils because the size of the C inputs was similar (Table 1). A previous study showed that C in composts is complexed in stable forms, and thus, is not readily available to soil microbes (Zaman et al., 1999). Tu et al. (2006) reported higher C_{mic} in plots treated with composts than in those treated with animal manure and rye/vetch green manures. Bhattacharyya et al. (2005) observed higher C_{mic} in soils treated with cow manure composts than in those treated with municipal solid waste. The quality and quantity of C inputs, as well as environmental factors, may explain such variations.

Incubation time significantly affected C_{mic}, with C_{mic} values peaking on day 21 in soils containing OAs (Fig. 2). After day 21, the

Table 7
Changes in mineral nitrogen release (μg N g^{-1} soil) over time from amended and non-amended soil incubated for 97 days in controlled laboratory conditions.

Days	Treatment														
	CTR			COI			COV			CLO			MAN		
	NH_4	NO_3	TMN	NH_4	NO_3	TMN	NH_4	NO_3	TMN	NH_4	NO_3	TMN	NH_4	NO_3	TMN
0	0.7	5.3	6.0	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
7	0.7	31.1	31.8	1.3	36.1	37.4	0.8	48.7	49.5	1.2	147.6	148.2	1.1	51.2	52.2
21	0.0	39.7	39.7	0.1	46.6	46.6	0.2	64.8	64.9	0.6	161.7	162.3	0.6	54.3	54.8
40	0.5	53.0	53.6	0.6	72.3	72.9	0.5	69.9	70.4	0.7	207.7	208.4	0.6	86.3	86.9
68	4.7	48.0	52.0	2.2	68.3	70.5	2.6	75.8	80.6	2.1	236.4	238.2	2.6	82.8	85.4
97	0.4	71.3	71.7	0.6	75.7	76.3	0.4	77.7	84.6	0.5	240.3	240.9	0.7	99.7	100.4

n. a. indicates not applicable, because mineral nitrogen was included in the control on day 0.

C_{mic} decreased, then increased in CLO— and MAN-amended soils, but remained low in compost-amended soils. The C_{mic} in control soil did not change during the incubation experiment. The higher C_{mic} values at day 21 probably represented the C inputs from the OAs, and the resulting stimulation of microbial growth. The decreases in C_{mic} after day 21 probably represented C depletion. Zaman et al. (1999) observed that the C_{mic} in soil containing dairy shed effluent peaked at 16 days, then decreased. Flavel and Murphy (2006) also observed a peak of C_{mic} after 16 days of incubation in soils containing manure and composts.

The variability in C_{mic} and microbial activity among different OAs has implications for nutrient availability to crops. High microbial biomass and activity can increase nutrient availability to crops as a result of greater microbial biomass turnover and degradation of non-microbial organic materials (Zaman et al., 1999). Therefore, CLO, which resulted in high microbial biomass in soil, is likely to be a good source of nutrients to crops.

In this study, C_{mic} was weakly positively correlated ($R^2 = 0.473$) with total MN in OA-amended soils, implying that OAs increased the activities of enzymes that accelerate N mineralization. Wang et al. (2007) found a positive relationship ($R^2 = 0.57$) between mineral N concentrations and C_{mic} in different cover crops. Zaman et al. (1999) reported a positive correlation ($R^2 = 0.61$, $p < 0.001$) between N mineralization and C_{mic} in dairy shed effluent. Variations in the relationship between C_{mic} and N mineralization may be explained by differences in the quality of materials used. The weak correlation between N mineralization and C_{mic} in this study indicated that C_{mic} was not the only factor affecting mineralization. Other important factors include the quality of materials, N content, and the C:N ratio.

4.4. Phospholipid fatty acids analysis

We evaluated the microbial community after OA addition by monitoring PLFAs, which are indicators of microbial biomass (Zelles, 1999). PLFAs particularly reflect the active microbial population "flushed" in response to substract. The total PLFAs decreased over the 97-day incubation period in all treatments and in the control. This trend was consistent with the carbon substrate depletion reflected by the increase in C_{mic} over time. At day 97, the total PLFA concentration was significantly higher in OA-amended soils than in control soil (Table 5). Carbon is a substrate for microbial growth. In another study, the amount of carbon input into soils by the OAs affected the microbial population (Peacock et al., 2001). In the present study, the total PLFA concentration was significantly higher in the CLO- and MAN-amended soils than in the compost-amended soils, possibly because of the higher carbon inputs from CLO and MAN (Table 1).

The microbial communities differed significantly among the OA treatments (Table 5). There were more bacteria, actinomycetes, and fungi in the CLO-amended soil than in the other treatments. The B:F ratio was higher in CLO-amended soil than in soils containing composts. Fungi made up a larger proportion of total microbes in soils containing compost. The differences in microbial community composition among treatments were likely due to differences in composition and substrate availability among the OAs (Marschner et al., 2003). The dominance of fungi in soil containing composts and of bacteria in soils containing CLO and MAN implies that bacteria preferentially degrade decomposable materials over recalcitrant ones, whereas fungi degrade the recalcitrant and insoluble compounds (Marschner et al., 2003). Previous studies found that bacteria were dominant in soils containing dairy cattle manure (Peacock et al., 2001).

The significant positive linear relationship between total mineral N and C_{mic} in OA-amended soils indicated that OAs stimulated microbial carbon, ultimately benefiting soil microbes. However, the total PLFAs decreased over time in all soils because of depletion of the carbon substrate (represented by the increase in C_{mic} over time). The higher total PLFAs concentration in soil with CLO was indicative of its large and varied microbial population, which could be related to efficient CLO decomposition.

5. Conclusions

Nitrogen mineralization from OAs is very important for efficient nutrient use in agriculture. Our results showed that the N mineralization potential differed among various OAs, suggesting that the type of OA should be matched to the needs of the crop. Net N release peaked more than 2 months after OA application. This suggests that OAs should be applied approximately 2 months in advance to synchronize N release with crop uptake, depending on the crop and time of year. Also, farmers should try to avoid losses of mineral N via leaching. Of the four OAs evaluated in this study, CLO showed the highest N release (54%). Therefore, CLO may be the best organic N source for crops, because it could meet the needs of the crop without an additional mineral N source. However, the high N mineralization from CLO may pose a risk to the environment if its application is not timed to match plant uptake. The other OAs showed low N mineralization rates. Therefore, the application rates of these OAs should be increased to satisfy the needs of the microbial community and the crop. Addition of composts can improve other soil qualities, e.g., reduce the leaching risk when the N demands of crops are low.

In further research, these OAs should be tested in a field experiment to confirm the results of the laboratory incubation experiment.

The results of this study highlight that the N content of an OA does not always correspond to the amount of N released. After OAs are added to soil, the N dynamics are complex and depend on multiple factors including temperature, time, moisture content, and the microbial community. Consequently, the use of OAs for N nutrition in cropping systems is much more sophisticated than the use of mineral compounds, which have known compositions and release nutrients in a predictable manner. The implications of these findings are that farmers need to understand how to use OAs appropriately, and to understand the advantages and disadvantages of using such materials to supply the nutrient needs of crops. More research on the synchronization of nutrient mineralization and plant up-take is required.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2016.01.006.

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