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Soil microbial community responses to dairy manure or ammonium nitrate applications

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Abstract

Soil management practices that result in increased soil C also impact soil microbial biomass and community structure. In this study, the effects of dairy manure applications and inorganic N fertilizer on microbial biomass and microbial community composition were determined. Treatments examined were a control with no nutrient additions (*CT*), ammonium nitrate at 218 kg N ha^{-1} (*AN*), and manure N rates of 252 kg manure-N ha^{-1} (*LM*) and 504 kg manure-N ha^{-1} (*HM*). All plots were no-till cropped to silage corn (*Zea mays*, L. Merr) followed by a Crimson clover (*Trifolium incarnatum*, L.)/annual ryegrass (*Lolium multiflorum*, Lam.) winter cover crop. Treatments were applied yearly, with two-thirds of the N applied in late April or early May, and the remainder applied in September. Soil samples (0–5, 5–10, and 10–15 cm) were taken in March 1996, prior to the spring nutrient application. Polar lipid fatty acid (PLFA) analysis was used to assess changes in microbial biomass and community structure. Significantly greater soil C, N and microbial biomass in the 0–5 cm depth were observed under both manure treatments than in the *CT* and *AN* treatments. There was also a definable shift in the microbial community composition of the surface soils (0–5cm). Typical Gram-negative bacteria PLFA biomarkers were 15 and 27% higher in the *LM* and *HM* treatments than in the control. The *AN* treatment resulted in a 15% decrease in these PLFA compared with the control. Factor analysis of the polar lipid fatty acid profiles from all treatments revealed that the two manure amendments were correlated and could be described by a single factor comprised of typical Gram-negative bacterial biomarkers. The *AN* treatments from all three depths were also correlated and were described by a second factor comprised of typical Gram-positive bacterial biomarkers. These results demonstrate that soil management practices, such as manuring, that result in accumulations of organic carbon will result in increased microbial biomass and changes in community structure.

Keywords: Microbial biomass; Community structure; Manure; Fatty acids; PLFA

1. Introduction

It has been well documented that soil management can significantly impact soil biological and biochemical properties. Doran (1980) observed that soils in no-till cropping systems have higher microbial populations, largely due to increased soil organic carbon, than soils under tillage. He also observed evidence for a shift in microbial community structure, with higher counts of denitrifiers and facultative anaerobes. A North Carolina study (Kirchner et al., 1993) evaluated the impacts of a crimson clover (*Trifolium incarnatum* L.) cover crop in a conventionally-tilled continuous corn system. Microbial biomass carbon was higher under clover than under a winter fallow system. Heterotrophic bacteria numbers were generally higher in the clover system as well. The clover cover crop also resulted in higher activities of alkaline phosphatase, arylsulfatase and β-glucosidase. Mullen et al. (1998) observed that cover crops significantly increased several enzyme activities relative to no cover in no-till corn. The application of cattle manure slurry has been demonstrated to increase soil microbial biomass (Acea and Carballas, 1988; Ritz et al., 1997). Applications of dairy-feedlot manure have also been shown to maintain or increase soil carbon and microbial biomass to a greater extent than that realized with chemical fertilization (Dormaar et al., 1988). Management of

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soil that leaves residue on the soil surface, such as in no-tillage, often results in higher concentrations of soluble organic carbon compounds (Alvarez et al., 1998), which may result in the enhancement of microbial properties.

One problem with characterizing changes in soil microbial communities has been the insensitivity associated with many methods. Standard plate count methods may recover only a few percent of the organisms present, with those isolated restricted to organisms suited to growth on the medium of choice. Measurements of soil microbial biomass give an indication of the standing crop of microbial life in the soil, but provide no indication of community structure. In short, traditional methodologies of microbiology lack the ability to quantitatively and comprehensively describe diverse communities, such as in soil. The application of molecular techniques to the study of microorganisms in situ overcomes a number of these deficiencies providing a better understanding of how bacterial mediated processes are affected by changing management practices. One such method is the measurement of phospholipid fatty acids (PLFA) in the soil.

Phospholipid lipid fatty acids can be used to describe viable microbial communities in terms of total biomass (Balkwill et al., 1988) and community composition (Vestal and White, 1989). Phospholipids are essential components of life making up the bulk of the matter of cell membranes. Since phospholipids are rapidly degraded by endogenous and exogenous phospholipases upon cell death, they are reliable measures of viable cell biomass (White et al., 1979). The extraction and purification methods used are optimized for phospholipid molecules, so 'fossil' fatty acids, such as free fatty acids or those attached to humic substances, are not detected. PLFA have also been shown to be sufficiently distinct to allow for the identification of individual species of bacteria (Guckert et al., 1991) as well as provide insight into the make-up of whole communities (Frostegård et al., 1993). Community composition can be inferred from a PLFA profile by relating the type of PLFA identified to different biosynthetic pathways utilized in fatty acid synthesis (Fredrickson et al., 1995) or to specific species or classes of bacteria which contain a unique signature PLFA biomarker (Vestal and White, 1989).

This study was part of a larger experiment to evaluate impacts of manure applications on surface and subsurface water quality and soil properties. Based upon past work and literature review, we hypothesized that manuring would increase the microbial biomass relative to the control and ammonium nitrate treatments, and cause shifts in the microbial community structure. Therefore, the objective of this study was to document the effect of several years of dairy manure applications on microbial biomass and community structure, using PLFA methods, in a no-till corn silage cropping system.

2. Materials and methods

2.1. Experimental plots

Eighteen no-till experimental plots were established in May 1991 at the Martin Agricultural Experiment Station in northwest Tennessee. Each plot was 9.1 m by 4.6 m wide. The experimental area had a 5% slope and the soil was a Loring silt-loam (Fine-silty, mixed, thermic Typic Fragiudalf). All plots were separated by earthen berms to prevent movement of water between plots and from above the plots. Corn (*Zea mays*, L. Merr; cultivar DeKalb 689) was no-till planted each May with 95 cm spacing between rows, and thinned to 49,500 plants ha^{-1} after germination. Corn was harvested for silage in August or September of each year. A forage crop of mixed crimson clover (*Trifolium incarnatum*, L.) and annual ryegrass (*Lolium multiflorum*, Lam.) was then planted for erosion control and harvested for hay in the spring.

Six treatments were applied to this cropping system from May 1991 until September 1996. These were a control with no fertilizer (*CT*), ammonium nitrate at 218 kg N ha^{-1} (*AN*), and manure N rates of 126, 252, 378 and 504 kg manure N ha^{-1}. The ammonium nitrate treatment was split with 168 kg N applied prior to corn planting (recommended N rate for corn in Tennessee), and 50 kg N applied in the fall with the clover-annual ryegrass cover. Manure treatments were also split, with two-thirds of the manure applied prior to corn planting and the remaining one-third applied prior to the cover crop in the fall. The day before manure application, total manure-N concentrations were determined to permit calculation of field application rates. The control plots received no applications of fertilizers, while the *AN* plots also received 35 kg of P and 66 kg K each spring. The manured plots did not receive any inorganic fertilization. There were three replicate plots for each treatment, and all treatments were arranged in a completely randomized design. For this study, only the control (*CT*), ammonium nitrate (*AN*) and two manure treatments, the 252 kg manure-N ha^{-1} (low manure treatment, *LM*) and the 504 kg manure-N ha^{-1} (high manure treatment, *HM*) treatments were used.

2.2. Soil sampling

Sampling occurred in March 1996, prior to the spring 1996 hay harvest and nutrient applications. Three replicate soil cores (5 cm diameter and 15 cm deep) were taken aseptically from each plot for lipid analysis. The core locations in each plot were chosen randomly using the PLAN procedure in SAS software (SAS Institute, Cary, NC). Cores were placed on sterile aluminum foil and undegraded plant material was removed from the core surface with a sterile knife. The cores were then divided into 0–5, 5–10 and 10–15 cm sections, placed in sealed plastic bags and frozen on-site using dry ice. Upon return to the laboratory, the soil samples were lyophilized and plant roots and stones were removed. After grinding and mixing with mortar and pestle, 5 g

sub-samples were removed for lipid analysis. Total soil C and N were measured by dry combustion using a LECO CNS 2000 analyzer (LECO, St. Joseph, MI) as described by Matejovic (1997). Soil pH was measured with a Corning pH meter 440 (Corning, NY) in a 1:2 soil-water paste.

2.3. PLFA analysis

Solvents were GC grade from Fisher Scientific, Fairlawn, NJ. Each lot was tested for purity before use. Silicic acid was Unisil (Clarkson Chemical Company). Potassium hydroxide and the internal fatty acid standard 19:0 were from Sigma Chemical Co., St. Louis, MO. Glassware for lipid analysis was freed of lipid contaminants by washing with non-phosphate containing soap and then heating in a muffle furnace at 450°C for 4 h.

Lyophilized soil was extracted with the single-phase chloroform-methanol-buffer system of Bligh and Dyer (1954), as modified (White et al., 1979). To 5 g soil samples, 5 ml of chloroform, 10 ml of methanol, and 4 ml of phosphate buffer (50 mm, pH = 7.4) were added, mixed, and allowed to equilibrate for 3 h. The single phase extractant was separated from the solid material by centrifugation at 2000 rpm for 20 min and decanting into another test tube. Five ml of chloroform was used to wash the pelletted solids, which were then re-centrifuged, and the chloroform added to the extract. An additional 5 ml of water was added to the extract to force the separation of the aqueous phase from the organic phases. After separation for approximately 12 h, the organic phase was pipetted into a new test tube and the solvent removed with a stream of dry nitrogen at 37°C.

The total lipid extract was fractionated into neutral lipids, glycolipids, and polar lipids by silicic acid column chromatography (Guckert et al., 1985). Pasteur pipets (1 cm diameter) partially blocked with a plug of glasswool were prepared and 0.5 g silicic acid added as a slurry in chloroform. The columns were pre-eluted with 2 ml of chloroform, and sample transferred to the column with 3–100 μl washes of chloroform. Neutral lipids were eluted with 5 ml chloroform, glycolipids with 5 ml acetone, and polar lipids with 5 ml of methanol. The solvent was removed from the polar lipids under a stream of dry nitrogen at 37°C. All results presented in this paper are for the polar lipid fraction.

The polar lipids were transesterified to the fatty acid methyl esters by a mild alkaline methanolysis (Guckert et al., 1985). The polar lipid extract was dissolved in 1 ml of 1:1 toluene/methanol, then 1 ml of methanolic KOH was added, and the mixture was heated at 37°C for 1 h. Fatty acids methyl esters (FAMES), were recovered from the organic fraction of the sample after adding 2 ml of hexane and 2 ml of water to break phase.

The FAMEs were analyzed by capillary gas chromatography with flame ionization detection on a Hewlett-Packard 5890 series 2 chromatograph with a 50 m non-polar column (0.2 mm I.D., 0.11 μm film thickness). The injector and detector were maintained at 270°C and 290°C, respectively. The column temperature was programmed from 60°C for 2 min then ramped at 10°C min^{-1} to 150°C, then ramped to 312°C at 3°C min^{-1}. Preliminary peak identification was by comparison of retention times with known standards.

Definitive identification of peaks was by gas chromatography/mass spectroscopy of selected samples using a Hewlett-Packard 5890 series 2 gas chromatograph interfaced to a Hewlett-Packard 5971 mass selective detector using the same column and temperature program previously described. Mass spectra were determined by electron impact at 70 eV. Methyl nonadecanonate was used as the internal standard, and the PLFA expressed as equivalent peak response to the internal standard.

Fatty acids are named according to the convention *X*:*Y*ω*Z*, where '*X*' stands for the number of carbon atoms in the chain, '*Y*' for the number of unsaturations, and '*Z*' the number of carbon atoms from the methyl end of the molecule to the first unsaturation encountered. So '20:4ω6' stands for a 20-carbon fatty acid with four unsaturations, with six carbons between the methyl end and the first unsaturation. Prefixes: '*i*' ≡ iso-branched, '*a*' ≡ anteiso-branched, '10*me*' ≡ methyl branch on the tenth carbon from the carboxylate end, '*Br*' ≡ branched at unknown location, and '*Cy*' ≡ cyclopropyl. The suffixes '*c*' and '*t*' stand for the *cis* and *trans* geometric isomers of the unsaturation, respectively. When different fatty acids had the same designation, they were distinguished by lower case letters suffixes—a, b, etc. The methyl group esterified to the carboxylate is not included in the carbon count for naming. A subtle ambiguity in this naming convention is that the number of carbons given for iso, anteiso, unknown branched, and cyclopropyl fatty acids includes all of the carbons in the molecule, but when the position of a mid-chain branch is known it is not added to the count. Therefore, 10*me*16:0 indicates a methyl group attached to a 16-carbon fatty acid at the ten position from the carbonyl (Gunstone and Herslöf, 1992).

2.4. Statistical analysis

As indicated previously, the experiment consisted of four treatments with three replicate plots in a completely randomized design. Three samples were taken from each plot, resulting in nine observations for each treatment in this study. PLFA profiles were analyzed with Statistica software (Statsoft, Inc., Tulsa, OK). A completely randomized design with nine replications per treatment was used. Only those PLFA that comprised at least 1% of any profile were included in the analysis; therefore fatty acids that may have been unreliably quantified were not included. The arcsine square root transformation was applied to the mole percent PLFA data. Three different classification algorithms (cluster analysis and two variations of factor analysis) were applied to the data to ensure the stability of the groups obtained. Hierarchical cluster analysis was performed on the treatment means from the transformed mole percent PLFA using Ward's method (Ward, 1963). Factor analyses of biomass and arcsine squareroot transformed PLFA data

were performed using both iterative and non-iterative extraction techniques. Factor analysis was also used as a data reduction method and in conjunction with hierarchical cluster analyses to assess changes in community structure (Rosswall and Kvillner, 1978). An ANOVA was performed on the factor scores and Tukey's Honest Significant Difference test (HSD) was used to identify significant differences between treatments, with the within-experiment family–wise error rate set at $\alpha = 0.05$.

3. Results

Applications of manure have resulted in differences in soil chemical properties over the 6 years of the study. Table 1 shows the effect of the treatments on total soil C and N, and on soil pH. For all treatments, soil C and N contents were significantly greater in the 0–5 cm depth than in the 5–10 and 10–15 cm depths. Application of ammonium nitrate for five years has resulted in a significant decrease in pH in the 0–5 cm depth relative to *CT*. The *HM* treatment also caused a downward shift in the 0–5 cm pH, although the change was only significantly lower than that observed for *LM*. The addition of manure (both *LM* and *HM*) to the soil surface also resulted in increased total C and N in the 0–5 cm depth relative to the *AN* and *CT* treatments, and the *HM* treatment also resulted in significantly higher C and N contents than the *LM* treatment.

Microbial biomass PLFA for all treatments was greatest in the 0–5 cm soil depth, with nearly twice as much total PLFA than in the 5–10 and 10–15 cm depths (Table 2). The manure treatments resulted in significantly higher biomass in the 0–5 cm depth than observed in the *CT* and *AN* treatments at that depth. The *HM* treatment also resulted in a significant increase in biomass in the 5–10 cm interval relative to the *CT* and *AN* treatments at 5–10 cm and all treatments at the 10–15 cm depth. If it is assumed that 1 pmole of PLFA is equivalent to 2.5×10^4 bacterial cells (Balkwill et al., 1988), then bacterial density in the 0–5 cm depth ranged from approximately 1.05×10^9 cells g^{-1} to 1.91×10^9 cells g^{-1} in the *CT* and *HM* treatments, respectively. Approximate cell numbers in the 5–10 cm depth ranged from 5.6×10^8 cells g^{-1} in *CT* to 9.64×10^8 cells g^{-1} in *HM*. The cell densities in the 10–15 cm segment were approximately 4.49×10^8 cells g^{-1} for *CT* and 5.21×10^8 for the *HM*.

PLFA analysis identified 72 fatty acids. However, of these, only 27 comprised greater than 1% of the total PLFA in any given treatment (data not shown). The types of fatty acids identified included normal saturates, iso- and anteiso-branched saturates, monounsaturates, mid-chain branched saturates, cyclopropyl, and polyunsaturated fatty acids. The proportions of the PLFA in each of these fatty acid groups for the 0–5 cm depth are provided in Fig. 1. Relative to the controls, percentages of total mono-unsaturated PLFA decreased approximately 15% under the *AN* treatment and increased 15 and 27% under the *LM* and *HM* treatments, respectively. In contrast, normal and mid-chain branched saturated PLFA were higher in the *AN* treatment than in both manure amended soils. The proportion of iso- and anti-iso-branched PLFA was also lower in the manure treatments. The proportion of polyunsaturated fatty acids was not significantly affected by treatment.

Exploratory multivariate statistics in the form of a hierarchical cluster analysis of arcsine squareroot transformed mole percentages was used to determine the clustering pattern of the soils (Fig. 2). There were two primary clusters, one that contained only the manure treatments *LM* and *HM* at the 0–5 cm depth, and another containing two secondary clusters. Of the two secondary clusters, one contained all depths of the ammonium nitrate treatment, while the other cluster was again divided into two additional tertiary clusters. The first cluster contained the 0–5 cm *CT* treatment and both 5–10 cm manure treatments; the second contained the remaining control and manure treatments.

Table 1
Total soil carbon and nitrogen and soil pH as affected by manuring and ammonium nitrate additions

Depth increment (cm)	Treatments[a]			
	CT	*AN*	*LM*	*HM*
Total carbon (g kg^{-1})				
0–5	15.2^{cb}	16.3^{c}	24.2^{b}	30.7^{a}
5–10	$6.6^{d,e,f}$	8.9^{d}	$6.9^{d,e,f}$	$8.1^{d,e}$
10–15	$4.4^{d,e,f}$	2.9^{f}	3.1^{f}	$3.9^{e,f}$
Total nitrogen (g kg^{-1})				
0–5	1.61^{c}	1.55^{c}	2.34^{b}	2.70^{a}
5–10	$0.83^{d,e,f}$	1.03^{d}	$0.82^{d,e,f}$	$0.94^{d,e}$
10–15	$0.67^{d,e,f}$	0.49^{f}	0.47^{f}	$0.56^{e,f}$
Soil pH				
0–5	$6.0^{a,b,c}$	5.4^{d}	$6.3^{a,b}$	$5.8^{c,d}$
5–10	$6.4^{a,b}$	$6.0^{a,b,c}$	6.5^{a}	$6.4^{a,b}$
10–15	$6.2^{a,b,c}$	$6.0^{a,b,c}$	$6.2^{a,b,c}$	$6.2^{a,b,c}$

[a] Treatment abbreviations: *CT* = control, *AN* = ammonium nitrate, *LM* = low manure (252 kg manure-N ha^{-1}), *HM* = high manure (504 kg manure-N ha^{-1}).

[b] Within a given soil parameter, means labeled with the same letter are not different as determined by Tukey's HSD at $\alpha = 0.05$.

Table 2
Soil microbial biomass PLFA for all treatments and soil depths

Depth (cm)	Treatments (nmol PLFA g^{-1} soil)[a]			
	CT	*AN*	*LM*	*HM*
0–5	42^{bb}	49^{b}	72^{a}	76^{a}
5–10	22^{c}	23^{c}	$30^{b,c}$	39^{b}
10–15	18^{c}	20^{c}	20^{c}	21^{c}

[a] Treatment abbreviations: *CT* = control, *AN* = ammonium nitrate, *LM* = low manure (252 kg manure-N ha^{-1}), *HM* = high manure (504 kg manure-N ha^{-1}).

[b] Means followed by the same letter are not different as determined by Tukey's HSD at $\alpha = 0.05$.

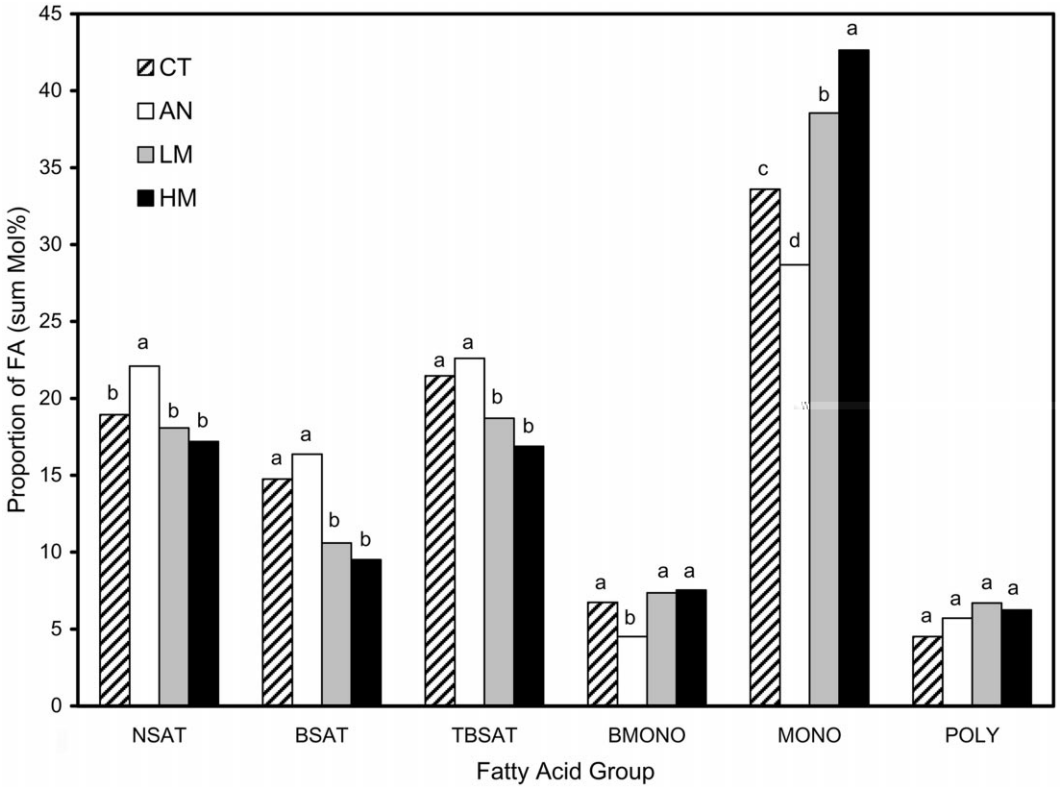

Fig. 1. Proportions of PLFA in different functional groups in the 0–5 cm depth. Mole percents within a treatment do not sum to 100 since some PLFA are in more than one functional group. Abbreviations: NSAT = normal saturates, BSAT = mid-chain branched saturates, TBSAT = terminally branched saturates, BMONO = branched monounsaturates, MONO = total monounsaturates, POLY = polyunsaturates. Treatment abbreviations: *CT* = control, *AN* = ammonium nitrate addition, *LM* = low manure addition (252 kg manure-N ha^{-1}), and *HM* = high manure addition (504 kg manure-N ha^{-1}). Bars within a group labeled with the same letter are not different as determined by Tukey's HSD at $\alpha = 0.05$.

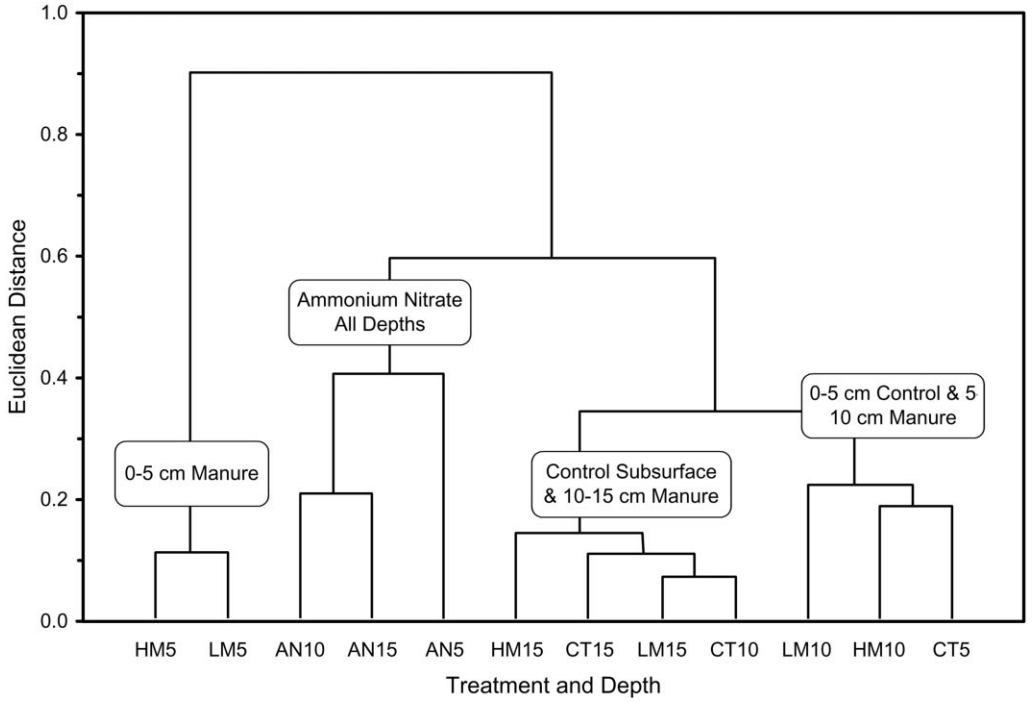

Fig. 2. Results of a hierarchical cluster analysis of PLFA mol percentages for treatment and depth. Treatment and depth abbreviations: *CT* = control, *AN* = ammonium nitrate addition, *LM* = low manure addition (252 kg manure-N ha^{-1}), and *HM* = high manure addition (504 kg manure-N ha^{-1}), 5 = 0–5 cm, 10 = 5–10 cm, and 15 = 10–15 cm.

Table 3
Major components (loading $>|0.7|$) of the two major factors contributing to treatment differences as determined by factor analysis. Factor analysis was done using arcsine squareroot transformed PLFA mol percent and biomass data. Positive and negative contributors and their loadings for each factor are given

Positive contributors		Negative contributors	
Variable	Loading	Variable	Loading
Factor 1: Effect of depth and manure on microbial community[a]			
Biomass (pmol g^{-1})	0.92	*i*10*me*16:0	−0.89
18:1ω9*c*	0.82	10*me*16:0	−0.86
		11*me*16:0	−0.78
		10*me*18:0	−0.77
		*a*17:0	−0.76
		12*me*18:0−0.71	−0.71
Factor 2: Effect of ammonium nitrate[b]			
*i*16:0	0.85	18:1ω5*c*	−0.81
10*me*17:0	0.76	16:1ω7*c*	−0.77
		20:4ω6	−0.75
		16:1ω5*c*	−0.73
		16:1ω9*c*	−0.73
		18:1ω7*c*	−0.71

[a] Positive contributors were biomass and the PLFA 18:1ω9*c* found in Gram-negative bacteria. Negative contributors dominated by mid-branched PLFA indicative of Gram-positive actinomycete bacteria.
[b] Positive contributors were mid-chain and terminally-branched saturated PLFA indicative of Gram-positive bacteria. Negative contributors were primarily monounsaturated or general Gram-negative PLFA.

Factor analysis identified PLFA that were highly correlated (loading $>|0.7|$) in describing similarities and differences between treatment PLFA profiles (Table 3). The first two factors accounted for 26 and 25% of the variance in the data. The third and fourth factors accounted for 17 and 10% of the variance in the sample set, and by examination of the scree plot, could have been considered important (Kim and Mueller, 1978). However, the third and fourth factors did not add to the analysis, and were not considered further. A plot of the first and second factors for all treatments and depths is shown in Fig. 3. The results show a clear segregation of the manured soils in the 0–5 cm depth relative to all other treatments. The *AN* treatment in the 0–5 cm depth resulted in a community structure that was also clearly resolved from the other treatments. As was observed in the hierarchical cluster analysis (Fig. 2), the 0–5 cm *CT* treatment and the 5–10 cm *LM* and *HM* treatments were very similar. Finally, the 5–10 and 10–15 cm *AN* and *CT* treatments and the 10–15 cm *LM* and *HM* treatments all segregated into a fourth grouping, indicating that the impact of the different fertilization regimes had not resulted in a differentiation of the microbial community at depth.

Table 4 shows which treatments are significantly different from the factor analysis, and whether factor 1 or 2 or both contribute to the differences. It is interesting to note that the *AN* 0–5 cm treatment was found to be significantly different from all other treatment and depth combinations.

4. Discussion

Based upon previous research, we hypothesized that manure addition would increase the microbial biomass

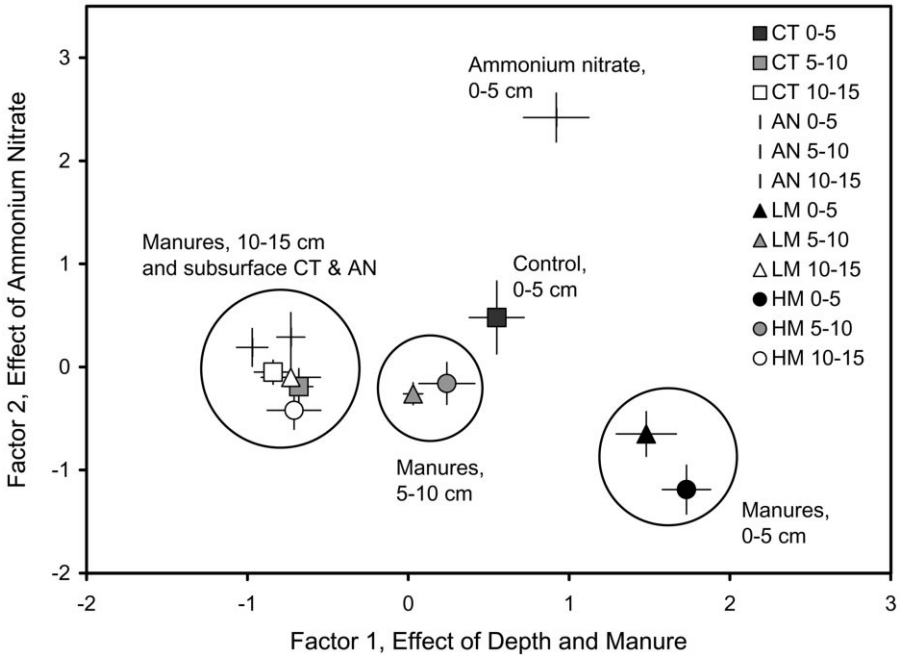

Fig. 3. Plot of the first and second factors extracted from factor analysis of PLFA and biomass data (see Table 3 for factor loadings). Numbers in legend indicate depth increment, and abbreviations are: *CT* = control, *AN* = ammonium nitrate, *LM* = low manure addition (252 kg manure-N ha^{-1}) and *HM* = high manure addition (504 kg manure-N ha^{-1}). Bars represent ±the standard error of the mean in the *x* and *y* direction.

relative to the control and ammonium nitrate treatments and that there would be a shift in the resulting viable microbial community structures. There were three primary effects on the microbial community in this experiment due to treatment as determined by factor analysis (Fig. 3). The first two effects, depth and manuring, were described by factor 1, while the effect of ammonium nitrate fertilization was described by factor 2.

The major positive contributors to factor 1 were biomass and the fatty acid 18:1ω9*c*. The effects of depth and manuring in factor 1 were demonstrated by the vertical stratification of C, N, pH and biomass (Tables 1 and 2). No-tillage cropping systems are characterized by accumulation of carbon, nutrients and microbial biomass near the soil surface (Haines and Uren, 1990). We observed significantly greater soil C, N, and microbial biomass PLFA in the surface 0–5 cm than in underlying layers. The addition of dairy manure to the soil surface accentuated the depth effect, resulting in significantly greater total C, N, and biomass content in the 0–5 cm manured treatments than in the *CT* and *AN* treatments. Differences in total C and N were not observed below the 0–5 cm depth for all treatments, however, biomass increased in the 5–10 cm depth with the *HM* treatment (Table 2). The enhanced microbial biomass in the manured soils at the 5–10 cm depth is probably due to the movement of soluble carbon below the 0–5 cm depth, providing readily utilizable C for biomass development. Applications of manure have been shown to significantly increase soluble organic C in soil (Bhogal and Shepard, 1997; Gregorich et al., 1998; Liang et al., 1998) and this soluble carbon can move downward in the soil profile (Bhogal and Shepard, 1997). Although not measured in this study, it is likely that soluble C is available over a longer part of the year under manure than in the inorganic systems. Consequently the manure provided a more stable and readily available stream of substrate to the underlying microbial community.

As discussed previously, manuring significantly increased PLFA biomass. The manured surface soils are enriched with bacterial monounsaturates (Gram-negative bacteria) and eukaryotic markers, with a relative decrease of actinomycete markers. The fatty acid 18:1ω9*c* is found in Gram-negative bacteria (Wilkinson, 1988), and is also a common eukaryotic marker found in soil (Frostegård et al., 1997). The minor positive contributors to factor 1 included the bacterial monounsaturates 18:1ω7*c*, 16:1ω7*c*, and 16:1ω5*c*, again typical of Gram-negative bacteria (Wilkinson, 1988), as well as the eukaryotic markers 20:4ω6 and 18:2ω6 (data not shown). Major negative contributors to factor 1 included five midchain-branched and one anteiso fatty acid, which together are indicative of the actinomycetes group within the Gram-positive bacteria (O'Leary and Wilkinson, 1988; Verma and Khuller, 1983). Several studies of soil PLFA have documented an increase in monounsaturated fatty acids with increases in the availability of organic substrates (Bååth et al., 1995; Bossio and Skow, 1998; Borga et al., 1994; Zelles and Bai, 1993). Manure applications typically result in increased soluble organic C in soil (Bhogal and Shepard, 1997; Gregorich et al., 1998; Liang et al., 1998), therefore, our observation of increased monounsaturated PLFA in the manured soils is consistent with published work.

The third effect on the microbial community was caused by the ammonium nitrate treatment; this effect was described as factor 2 in the factor analysis (Fig. 3). Examination of the PLFA by functional groups showed that *AN* treatment generally shifted the PLFA functional groups in the opposite direction from manuring (Fig. 1). Tukey's HSD test clearly showed that the *AN* 0–5 cm treatment differed from all other treatment and depth combinations (Table 4), while cluster analysis (Fig. 2) separated the *AN* treatments at all depths from the other treatments. The microbial community resulting from the *AN* treatment was typified by lower biomass than in the manured treatments, and a higher relative proportion of Gram-positive bacteria when compared to the *CT* and manured soils. The major positive PLFA contributors to factor 2 were 10*me*17:0 and *i*16:0. These midchain and iso-branched fatty acids are also consistent with the presence of Gram-positive bacteria (Verma and Khuller, 1983). Moreover five of the six negative contributors to factor 2 were monounsaturated PLFA, denoting a decrease in the relative proportion of Gram-negative bacteria in the *AN* 0–5 cm treatment. Since the *AN* treatment also resulted in a divergent community structure from the *CT* soil, the impact of fertilization is important in addition to the separation noted from manuring. Indeed, soil pH in the surface 0–5 cm was significantly lower in the *AN* treatment (Table 1), and it is likely that this had an impact on the microbial community.

We hypothesize that the reaction of the microbial community to the addition of manure is very similar to a rhizosphere response. The manured soils likely have higher levels of soluble organic C, therefore supporting higher levels of microbial activity. Kennedy (1998) describes the process by stating that many bacteria have a large rhizosphere to bulk soil ratio, indicating marked stimulation in the rhizosphere. Moreover nonsporulating rods, *Pseudomonas*, and other Gram-negative bacteria are especially competitive in the rhizosphere. In contrast, actinomycetes account for approximately 10–30% of total microflora in the rhizosphere and have a smaller rhizosphere/bulk soil ratio (Kennedy, 1998). In addition to the rhizosphere-like response, we also detected a rise in protozoan biomarkers with manuring. Protozoan populations in soil generally increase following an increase in bacterial numbers (Clarholm, 1981).

These results suggest that the use of organic vs inorganic nutrient sources results in widely diverging microbial communities with possibly different ecological functions. If the application of manures results in a rhizosphere-like response, then the microbial community in manured soils is likely to be more metabolically active, responding quickly to the input of fresh organic residues. Indeed, the use of

Table 4
Treatment differences and factors that cause the differences as determined by Tukey's HSD at $\alpha = 0.05$

	Treatments[a]										
	CT 0–5	*CT* 5–10	*CT* 10–15	*AN* 0–5	*AN* 5–10	*AN* 10–15	*LM* 0–5	*LM* 5–10	*LM* 10–15	*HM* 0–5	*HM* 5–10
C 5–10	1[b]										
C 10–15	1	ns									
AN 0–5	2	1,2	1,2								
AN 5–10	1	ns	ns	1,2							
AN 10–15	1	ns	ns	1,2	ns						
LM 0–5	1,2	1	1	2	1	1					
LM 5–10	ns	ns	1	1,2	ns	1	1				
LM 10–15	1	ns	ns	1,2	ns	ns	1	ns			
HM 0–5	1,2	1	1,2	1,2	1,2	1,2	ns	1	1,2		
HM 5–10	ns	1	1	2	1	1	1	ns	1	1	
HM 5–15	1	ns	ns	1,2	ns	ns	1	ns	ns	1	1

[a] Treatment abbreviations: *CT* = control, *AN* = ammonium nitrate, *LM* = low manure (252 kg manure-N ha^{-1}), *HM* = high manure (504 kg manure-N ha^{-1}); numbers are depth increments in cm (e.g. 0–5 cm).

[b] Factors from factor analysis contributing to differences. Factor 1 (effect of depth and manuring) = 1, and factor 2 (effect of ammonium nitrate) = 2. The notation 'ns' indicates that the treatments are not significantly different.

organic nutrient sources depends on microbial activities for the mineralization of plant available nutrients. Soil management methods that increase carbon inputs to the soil, such as no-tillage (Doran, 1980), use of cover crops (Kirchner et al., 1993; Mullen et al., 1998), and manuring (Acea and Carballas, 1988; Dormaar et al., 1988; Ritz et al., 1997; Witter et al., 1993), are often observed to enhance microbial biomass, populations and activities. While animal manures are typically applied to supply crop N, P and K needs, the impact on the soil, and consequently, crop growth and microbial activities, goes beyond the application of those nutrients. The long-term use of manure also supplies large amounts of readily available C, resulting in a more diverse and dynamic microbial system than in inorganically fertilized soil.

In conclusion, applications of dairy manure over a 5-year period resulted in significant increases in C, N, and soil microbial biomass, as well as changes in microbial community structure. Those practices that enhance soil carbon and provide slowly mineralizable nutrients may result in a larger and potentially more robust microbial community. The use of methods such as PLFA analysis, coupled with multivariate statistical analysis, allows for better characterization of changes that occur with different management schemes, suggesting insights into how these practices enhance long-term sustainability and productivity.

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References

Acea, M.J., Carballas, T., 1988. The influence of cattle slurry on soil microbial population and nitrogen cycle microorganisms. Biological Wastes 23, 229–241.

Alvarez, C.R., Alvarez, R., Grigera, M.S., Lavado, R.S., 1998. Associations between organic matter fractions and the active soil microbial biomass. Soil Biology & Biochemistry 30, 767–773.

Bååth, E., Frostegård, Å., Pennanen, T., Fritze, H., 1995. Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear cut or burned coniferous forest soils. Soil Biology & Biochemistry 27, 229–240.

Balkwill, D.L., Leach, F.R., Wilson, J.T., McNabb, J.F., White, D.C., 1988. Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface aquifer sediments. Microbial Ecology 16, 73–84.

Bhogal, A., Shepard, M., 1997. Effect of poultry manure on the leaching of carbon from a sandy soil as a potential substrate for denitrification in the subsoil. Journal of the Science of Food and Agriculture 74, 313–322.

Bligh, E.G., Dyer, W.J., 1954. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.

Borga, P., Nilsson, M., Tunlid, A., 1994. Bacterial communities in peat in relation to botanical composition as revealed by phospholipid fatty acid analysis. Soil Biology & Biochemistry 26, 841–848.

Bossio, D.A., Scow, K.M., 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35, 265–278.

Clarholm, M., 1981. Protozoan grazing of bacteria in soil—impact and importance. Microbial Ecology 7, 343–350.

Doran, J.W., 1980. Soil microbial and biochemical changes associated with reduced tillage. Soil Science Society of America Journal 44, 764–771.

Dormaar, J.F., Lindwall, C.W., Kozub, G.C., 1988. Effectiveness of manure and commercial fertilizer in restoring productivity of an artificially eroded dark brown chernozemic soil under dryland conditions. Canadian Journal of Soil Science 68, 669–679.

Fredrickson, J.K., McKinley, J.P., Nierzwicki-Bauer, S.A., White, D.C., Ringelberg, D.B., Rawson, S.A., Li, S-M., Brockman, F.J., Bjornstad, B.N., 1995. Microbial community structure and biogeochemistry of Miocene subsurface sediments: implications for long-term microbial survival. Molecular Ecology 4, 619–626.

Frostegård, Å., Tunlid, A., Bååth, E., 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Applied and Environmental Microbiology 59, 3605–3617.

Frostegård, Å., Petersen, S.O., Bååth, E., Nielsen, T., 1997. Dynamics of a microbial community associated with manure hot spots as revealed by phospholipid fatty acid analyses. Applied and Environmental Microbiology 63, 2224–2231.

Gregorich, E.G., Rochette, P., McGuire, S., Liang, B.C., Lessard, R., 1998. Soluble organic carbon and carbon dioxide fluxes in maize fields receiving spring-applied manure. Journal of Environmental Quality 27, 209–214.

Guckert, J.B., Antworth, C.P., Nichols, P.D., White, D.C., 1985. Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiology Ecology 31, 147–158.

Guckert, J.B., Ringelberg, D.B., White, D.C., Hanson, R.S., Bratina, B., 1991. Membrane fatty acids as phenotypic markers in the polyphasic taxonomy of methylotrophs within the Proteobacteria. Journal of General Microbiology 137, 2631–2641.

Gunstone, F.D., Herslöf, B., 1992. A Lipid Glossary. Oily Press, Dundee, p. 101.

Haines, P.J., Uren, N.C., 1990. Effects of conservation tillage farming on soil microbial biomass, organic matter and earthworm populations, in north-eastern Victoria. Australian Journal of Experimental Agriculture 30, 365–371.

Kennedy, A.C., 1998. The rhizosphere and spermosphere. In: Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., Zuberer, D.A. (Eds.). Principles and Applications of Soil Microbiology. Prentice Hall, Upper Saddle River, New Jersey, pp. 389–407.

Kim, J.-O., Mueller, C.W., 1978. Factor Analysis: Statistical Methods and Practical Issues. Sage Publications, Beverly Hills, CA.

Kirchner, M.J., Wollum, A.G., King, L.D., 1993. Soil microbial populations and activities in reduced chemical input agroecosystems. Soil Science Society of America Journal 57, 1289–1295.

Liang, B.C., Mackenzie, A.F., Schnitzer, M., Monreal, C.M., Voroney, P.R., Beyaert, R.P., 1998. Management-induced change in labile soil organic matter under continuous corn in eastern Canadian soils. Biology and Fertility of Soils 26, 88–94.

Matejovic, I., 1997. Determination of carbon and nitrogen in samples of various soils by dry combustion. Communications in Soil Science and Plant Analysis 28, 1499–1511.

Mullen, M.D., Melhorn, C.G., Tyler, D.D., Duck, B.N., 1998. Biological and biochemical soil properties in no-till corn with different cover crops. Soil and Water Conservation Journal 53, 219–224.

O'Leary, W.M., Wilkinson, S.G., 1988. Gram-positive bacteria. In: Ratledge, C., Wilkinson, S.G. (Eds.). Microbial Lipids, Vol. 1. Academic Press, London, pp. 117–201.

Ritz, K., Wheatley, R.E., Griffiths, B.S., 1997. Effects of animal manure application and crop plants upon size and activity of soil microbial biomass under organically grown spring barley. Biology and Fertility of Soils 24, 372–377.

Rosswall, T., Kvillner, E., 1978. Principal components and factor analysis for the description of microbial populations. Advances in Microbial Ecology 2, 1–48.

Verma, J.N., Khuller, G.K., 1983. Lipids of actinomycetes. Advances in Lipid Research 20, 257–310.

Vestal, J.R., White, D.C., 1989. Lipid analysis in microbial ecology—Quantitative approaches to the study of microbial communities. Bioscience 39, 535–541.

Ward, J.H., 1963. Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association 58, 236–244.

White, D.C., Bobbie, R.J., Heron, J.S., King, J.D., Morrison, S.J., 1979. Biochemical measurements of microbial mass and activity from environmental samples. In: Costerton, J.W., Colwell, R.R. (Eds.). Native Aquatic Bacteria: Enumeration, Activity, and Ecology, ASTM STP 695. American Society for Testing and Materials, Philadelphia, PA, pp. 69–81.

Wilkinson, S.G., 1988. Gram-negative bacteria. In: Ratledge, C., Wilkinson, S.G. (Eds.). Microbial Lipids, Vol. 1. Academic Press, London, pp. 299–489.

Witter, E., Martensson, A.M., Garcia, F.V., 1993. Size of the soil microbial biomass in a long-term field experiment as affected by different N-fertilizers and organic manures. Soil Biology & Biochemistry 25, 659–669.

Zelles, L., Bai, Q.Y., 1993. Fractionation of fatty acids derived from soil lipids by solid phase extraction and their quantitative analysis by GC-MS. Soil Biology & Biochemistry 25, 495–507.