Soil Microbial Community Dynamics as Influenced by Composted Dairy Manure, Soil Properties, and Landscape Position

Dexter B. Watts,[1] H. Allen Torbert,[1] Yucheng Feng,[2] and Stephen A. Prior[1]

Abstract: Manure applications can benefit crop productivity by adding required nutrients and organic matter to soil. There is a paucity of information on how soil microbial community dynamics will be altered by the application of manure to different landscape positions. Thus, an *in situ* field study was conducted during the summer and winter months to evaluate microbiological properties of three soil types that have evolved because of different landscape positions in an agricultural field. The three Coastal Plain soils investigated were Bama (sandy loam), Lynchburg (loam), and Goldsboro (loam) representing the landscape position of a summit, drainageway, and sideslope, respectively. Composted dairy manure was incorporated into *in situ* soil cores at a rate of 350 kg N ha^{-1} and compared with unamended controls. Soil microbial biomass N and dehydrogenase enzyme activity were determined to evaluate changes in the microbial biomass size and activity, whereas phospholipid fatty acid analysis was used as an indicator of the microbial community structure. Addition of composted dairy manure increased microbial activity and N immobilization, representing a shift in microbial response resulting from changes in substrate availability. This was most evident during summer months, with the composted dairy manure increasing dehydrogenase enzyme activity 21% and microbial activity 20% compared with without manure, suggesting that seasonal timing of application will influence microbial activity. Microbial properties were also impacted by landscape position. The drainageway landscape position soil, a loam, had the highest microbial biomass and microbial activity. Changes in microbial community structure using phospholipid fatty acid profiles were evaluated with canonical discriminate analysis. This analysis indicated that a shift in microbial community structure occurred between season, manure application, and landscape position. Findings from this study suggest that changes in soil variability from landscape positions and season can impact the growth and dynamics of the microbial community when manure is applied to agricultural fields.

Key words: Microbial community structure, landscape position, manure, PLFA.

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Because soil microbial communities are the driving force behind regulating soil processes such as organic matter (OM) decomposition and nutrient cycling, it is imperative to have a better understanding of the factors that regulate its size, activity, and structure (Zeller et al., 2001). The role that microbial activity plays in ecosystem processes is significant because approximately 80% to 90% of soil processes are mediated by microorganisms (Nannipieri and Badalucco, 2003). Soils containing a high microbial diversity are characteristic of a healthy agroecosystem, whereas those with low microbial diversity are characterized as an unhealthy soil that often hardly responds to environmental changes (Mader et al., 2002). Garbeva et al. (2003) reported that in healthy agroecosystems, the microbial community dynamics is governed by interactions between plant type, climate, and management practices. In addition, the soil microbial biomass in agroecosystems responds more quickly to management practices than OM and is often used as an indicator of soil quality and health. Therefore, a better understanding of the microbial size and diversity in a soil ecosystem may also provide useful information on the environmental and fertility impacts of agronomic practices.

The use of manure as low-cost nutrient source for crop production has increased in recent years. Addition of this nutrient source to soil increases microbial activity. The stimulation of microbial activity from manure addition has been attributed to increased inputs of organic carbon, nutrients, and microorganisms, thereby affecting the soil microbial population. In essence, animal manure addition may increase biodiversity in soil, thereby causing alteration in composition, size, and activity of soil microorganisms and extracellular enzyme activities. Because microbiological activities are important in regulating soil properties (Dick, 1992) as a result of the integral role that they play in biogeochemical cycles, a better understanding of the structure and functions of microbial communities in soils amended with manure is needed. Thus, understanding the impact manure additions to soil have on microbial properties and functions is key to improving soil fertility (Kennedy, 1999; Buckley and Schmidt, 2003) while identifying areas that may potentially cause nutrient loss to the environment.

When land applying manure during the growing or nongrowing season, producers usually apply manure at a uniform rate without considering variability in soil texture and landscape positions that are present in most agricultural fields (Delgado, 2002). The landscapes' topography can affect both the microclimate and hydrologic conditions of agronomic fields (Rowe, 1984). For instance, topography has been shown to influence water movement, thereby affecting the redistribution of materials carried within the water. This influences the type and the rate of soil microbial processes occurring within a landscape (Huggett, 1975, Pennock et al., 1994), resulting in spatial patterns of soil OM, soil texture, soil moisture, redox potential, bulk density, N mineralization, N immobilization, denitrification, and microbial respiration observed in agricultural fields (Goovaerts and Chiang, 1993; Pennock et al., 1992; van Kessel et al., 1993). Thus, as soil environments change resulting from different landscape positions in agricultural fields, it is likely that distinct microbial communities will change in composition (Bossio et al., 2005). However, little information has been reported on the influence that spatial variability has on microbial activity and community structure in agronomic fields with differing landscapes and soil types in the southeast United States. The change in microbial activity and microbial community structure caused by spatial variability in

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soil moisture, nutrient redistribution, and soil texture could affect microbial transformations (altering nutrient cycling processes), especially after manure application.

Thus, more information is needed on the relative differences in metabolic activities from microbial communities inhabiting different landscape positions in Coastal Plain soils. This information will be useful in providing insight into how these microbes could affect the fertility status of the soil environment. Therefore, the objective of this study was to determine the effects of manure application on three different soils located in close proximity to each other but differing in landscape position and soil textural class on microbial properties and community structure during two different seasons.

MATERIALS AND METHODS

Soil and Manure Description

Soil samples were collected from an ongoing precision agriculture experiment established in 2000 at Auburn University's E.V. Smith Experiment Station located in Macon County, AL. The site was extensively evaluated, and three different soil management and landscape positions were selected and characterized as the summit (elevated, relatively flat, well-drained area), sideslope (sloping, eroded, well-drained, high clay content area), and drainageway (somewhat poorly drained, depositional, relatively high in OM area) within a 9-ha agricultural field. The soil series consisted of Bama (summit), a fine-loamy, siliceous, subactive, thermic Typic Paleudults; Goldsboro (sideslope), a fine-loamy, siliceous, subactive, thermic Aquic Paleudults; and Lynchburg (drainageway), a fine-loamy, siliceous, semiactive, thermic Aeric Paleaquults. These three Coastal Plain soils, formed from the same parent material, were chosen because they proximate to one another within an agricultural field but are different in texture. The farming practice was a cotton (*Gossypium hirsutum* L.)-corn (*Zea mays* L.) rotation under conventional tillage with inorganic fertilizer application. Soils selected for this study were from plots that have not received manure within the last 10 years. Detailed description of the field can be found in Terra et al. (2004).

Composted dairy manure used in this study consisted of a dairy manure and sawdust bedding mixture from Auburn University's Dairy Research Unit. The composted dairy manure was collected in December 2003, air-dried, and stored until use in the experiment. Composting practices consisted of windrowing the manure and bedding mixture collected from the dairy cow's feeding area for approximately 8 months to 1 year with frequent turning (monthly). Composted dairy manure composition on a dry weight basis for C, N, P, and K was 114 g kg^{-1}, 7.7 g kg^{-1}, 7.9 g kg^{-1}, and 2.9 g kg^{-1}, respectively.

Initial Laboratory Analysis

Initial soil chemical and physical analysis was performed on soil before manure application. Total C and N for the soil and composted dairy manure were determined by dry combustion (CN LECO 2000 analyzer). Phosphorus and K concentrations in the composted dairy manure were determined with the dry ash procedure (Donohue, 1983). Soil pH, soil effective cation exchange capacity (CEC), and particle size analysis were measured by the Auburn University Soil Testing Laboratory using methodology described by Hue and Evans (1986).

In situ Microbial Study

Microbial properties were evaluated with *in situ* microplot cylinders used as part of a larger nationally coordinated USDA-ARS research project according to procedures described by Honeycutt et al. (2005). The study was conducted by inserting polyvinyl chloride plastic cylinders, 6.25 cm in diameter and 20.32 cm in length, into the soil surface. Treatments consisted of three soil types, two fertility treatment (with and without composted dairy manure), and six replications in a completely randomized design. Briefly, intact cores were collected by driving the polyvinyl chloride cylinder into the top 20 cm of the soil profile using an air hammer core sampler. For each landscape position soil, a total of 90 cylinders (84 for treatments, three for monitoring temperature, and three for monitoring soil moisture) were collected. Vegetation was removed from the surface portion, and roots were severed to prevent N loss to plant uptake. Soil core samples were collected and brought to the laboratory. The top 4 cm of soil in the microplot cylinders was removed, and an appropriate amount of composted dairy manure was added and thoroughly mixed to give 350 kg N ha^{-1} (18.9 g dry wt) applied based on a 15-cm depth furrow slice, mimicking manure application to an agricultural field for crop production. The soil was gently packed back into the microplot cylinder. The unamended soil was treated in the same manner, except for the addition of manure, to subject the soil to the same disturbance. Soil cores were then transported back to the field and reinserted into the respected holes from which they had been removed. Temperature and moisture probes were placed in the soil cores used to measure continuous soil temperature and moisture. These data were collected over the course of the study using data loggers (HOBO Weather Station, Onset Computer Corporation, Pocasset, MA). Soil moisture was measured by placing soil probes (254 × 32 × 1.0 mm; ECH_{20}) in the top of the soil core to a depth of 25.4 cm. Soil temperature was measure by inserting temperature probes (6 × 32 mm; S-TMA-002) at a soil depth of approximately 7.62 cm, by drilling 0.35-mm-diameter hole into the side wall of the tube. The tubes were placed in the ground on January 12, 2004 (winter study) and May 25, 2005 (summer study). Twelve soil cores (6 with and 6 without manure) from each of the landscape position soils were randomly collected from each plot and transported back to the laboratory on days 0, 7, 14, 21, 28, 49, and 70 after manure application. Upon returning to the laboratory, all of the soil within the core was removed and homogeneously mixed for analysis. Soil analysis was performed for microbial biomass N and dehydrogenase activity on each sampling day. Phospholipid fatty acid analysis was determined on day 70.

Microbial Biomass N

Microbial biomass N was determined in a manner similar to Runion et al. (2004) using the chloroform fumigation extraction method, as described by Horwath and Paul (1994). For each soil, a subsample (30 g of fresh soil) was either fumigated with chloroform or not fumigated and incubated (25°C) for 24 h. After incubation, samples were extracted with 0.5 *M* K_2SO_4, and N was determined using standard Kjeldahl procedures (Bremner and Mulvaney, 1982). Microbial biomass N was calculated as the difference between N in fumigated samples and N in unfumigated samples expressed as microgram N per gram of dry soil weight.

Dehydrogenase Activity

Dehydrogenase activity, a measure of microbial respiration and a reliable index of microbial activity in soil (Stevenson, 1959), was determined using a procedure modified from that used by Tabatabai (1982). One gram of sieved soil was placed in each test tube (15 × 100 mm), covered with 1 mL of 3% (wt/vol) 2,3,5-triphenyltetrazolium chloride, and stirred with a glass rod. After a 96-h incubation period (27°C), 10 mL of methanol was added to each test tube, and the suspension was vortexed for

30 sec. Tubes were incubated for an additional 4 h to allow suspended soil to settle. The resulting supernatant (5 mL) was carefully transferred to clean test tubes using Pasteur pipettes. Absorbance was read using a spectrophotometer at 485 nm, and formazan concentration was calculated using a standard curve produced from known concentrations of triphenyl formazan.

Phospholipid Fatty Acid Analysis

Field moist soil samples were used for phospholipid fatty acid (PLFA) analysis, as described by Feng et al. (2003) using a modified procedure of Findlay and Dobbs (1993) and Bossio and Scow (1998). This involves extraction of total lipids from soil, separation of PLFA from total lipids, using silicic acid column chromatography, derivatization of fatty acids to form fatty acid methyl esters (FAME), and gas chromatography analysis of FAME. Briefly, field moist soil samples (8 g dry weight) were extracted using a single-phase citrate-buffered chloroform-methanol solution (Feng et al., 2003). Phospholipids were separated from neutral and glycolipids using packed activated silicic acid columns. The phospholipids were then subjected to a mild alkaline methanolysis at 37°C, and the resulting FAME were extracted by hexane and dried under nitrogen. Before GC analysis, samples were dissolved in appropriate amounts of hexane containing 19:0 methyl ester as an internal standard.

A Hewlett Packard 5890 gas chromatograph (GMI, Inc., Ramsey, MN) equipped with a 25-m HP Ultra 2 capillary column and a flame ionization detector was used to analyze the FAME. Column temperature of this device initially started at 170°C and increased to 270°C at 5°C min^{-1}. The injector and detector temperatures were maintained at 250°C and 300°C, respectively. Fatty acid peaks were identified using the MIDI peak identification software (MIDI, Inc., Newark, DE) and bacterial fatty acid methyl ester standards (Matreya, Inc., Pleasant Gap, PA). The nomenclature for fatty acids, as described by Feng et al. (2003), was used.

Statistics

The treatments of the *in situ* study consisted of a total of three soils × 2 manure additions (with and without) × 6 replications × 7 sampling dates × 2 seasons for a total of 504 experimental units. The experiment was analyzed as a completely randomized factorial design, with three landscape position soils amended with and without manure. Statistical analysis was performed using a GLM procedure of SAS (SAS institute, 1985), and means were separated using least significant difference (LSD) at an *a priori* 0.10 *P* level to determine significant differences average over the seven sampling time per season. To access specific effects of season (winter vs. summer), landscape position soil, and manure application on microbial community structure, canonical discriminant analysis (CDA) was performed using mole percentage distribution of PLFA with SAS software version 9.13. Canonical discriminate analysis was performed on the PLFA data from winter 2004 and summer 2005. The PLFA profiles of all samples were analyzed using a set of 33 fatty acids that were present in most of the samples.

RESULTS AND DISCUSSION

This study focuses on the influence that landscape variability of soil amended with manure during the summer and winter seasons have on microbial dynamics. Information presented in this study is of particular value because there is a scarcity of data on the impacts that changes in landscape positions of an agricultural field has on soil microbes in southeastern United States. Soil physical and chemical properties collected before the initiation of *in situ* study are shown in Table 1. Soil moisture and temperature continuously collected throughout the course of the *in situ* incubation period are present in Figs. 1 and 2. Although the soil-types evaluated in this study were from three different topographic landscapes representing a summit, sideslope, and drainageway, rarely did these soils remain at field capacity for prolonged periods.

TABLE 1. Soil Physical and Chemical Properties for the *In Situ* Field Studies

Soil Properties	Bama (Summit)	Lynchburg (Drainageway)	Goldsboro (Sideslope)
Initial soil physical properties			
BD, g cm^{-3}	1.68	1.64	1.61
Sand, %	66.25	46.25	33.75
Silt, %	21.25	41.25	12.5
Clay, %	12.5	12.5	17.5
Winter 2004 soil chemical properties†			
pH	6.31	6.1	6.24
CEC, cmol kg^{-1}	5.84	5.46	6.09
Total C, g kg^{-1}	4.42	5.57	3.77
Total N, g kg^{-1}	0.48	0.51	0.41
C:N ratio	9.21	10.92	9.2
Summer 2005 soil chemical properties†			
pH	6.26	6.25	6.86
CEC, cmol kg^{-1}	5.7	7.79	5.12
Total C, g kg^{-1}	3.77	6.12	4.02
Total N, g kg^{-1}	0.39	0.58	0.54
C:N ratio	9.67	10.56	7.41

†Soil chemical properties are reported on a dry weight basis.

Dehydrogenase

Dehydrogenase is an enzyme that occurs in all intact viable microbial cells. These soil enzymes function as a measurement of the metabolic state of soil microorganisms by relating it to the presence of viable microorganisms and their oxidative capacity. Therefore, dehydrogenase can be used as a measure of microbial respiration and a reliable index of microbial activity in soil (Tejada et al., 2008; Trevors, 1984, Stevenson, 1959). In general, an increase in dehydrogenase activity was observed for soil containing composted manure compared with soil without manure ($P < 0.1$) when averaging across each sampling day during the winter and summer months (Table 2, Fig. 3). These results suggest that changes in the size of microbial populations and respiratory activity occurred in response to the increase in available substrate. In addition, an increase in available substrate corresponds to more readily available C and N pools, which were most likely disproportionally enhanced as a result of manure addition. Season also greatly impacted dehydrogenase activity ($P < 0.001$). Dehydrogenase activity measured during the summer was almost double that measured during the winter months (Table 2 and Fig. 3). The increase in activity during the summer compared with winter months suggests that greater microbial biomass occurred with a change in season. In addition, higher dehydrogenase enzyme activity, which is a representation of microbial activity, was probably a result of higher soil temperature, which has been shown to stimulate microbial activity. This is similar to the finding of previous research that has reported that temperature and moisture are the two most important abiotic factors affecting

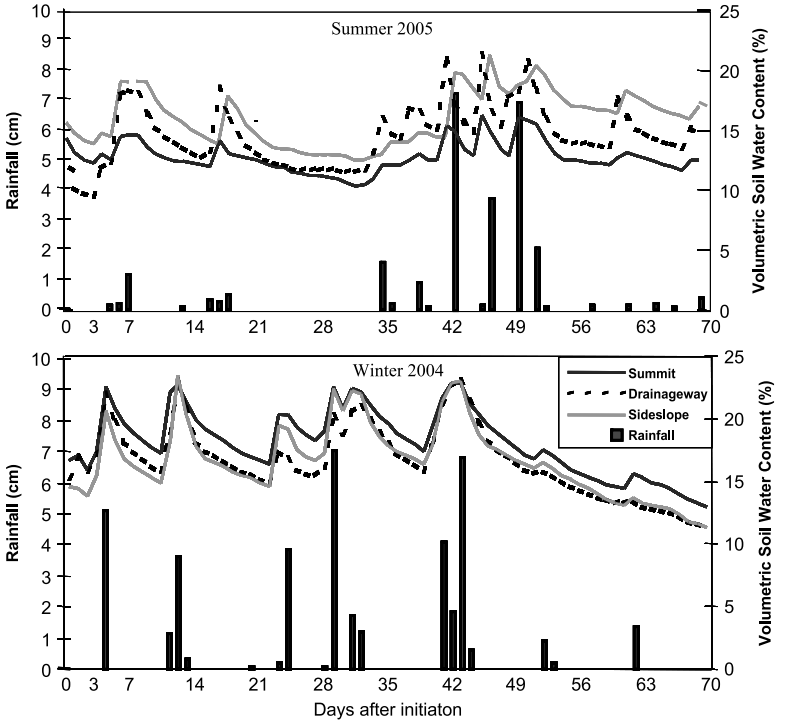

FIG. 1. Daily mean soil moisture in the microplot cylinders and average rainfall (winter 2004 and summer 2005) for three landscape position soils during the season. Scales of figure are adjusted to show treatment differences between seasons.

microbial activity (Katterer et al., 1998). Although moisture was slightly higher during the winter (Figs. 1 and 2), soil temperature was probably the overriding factor affecting microbial activity.

Dehydrogenase activity was also greatly affected by the soil's landscape position. Differences for landscape position soil amended with manure were observed in the winter and summer seasons, as evidenced by the landscape X amendment interaction effect ($P < 0.084$). The drainageway landscape position soil with manure produced the highest microbial activity compared with the other soils. The soil at the drainageway position had the

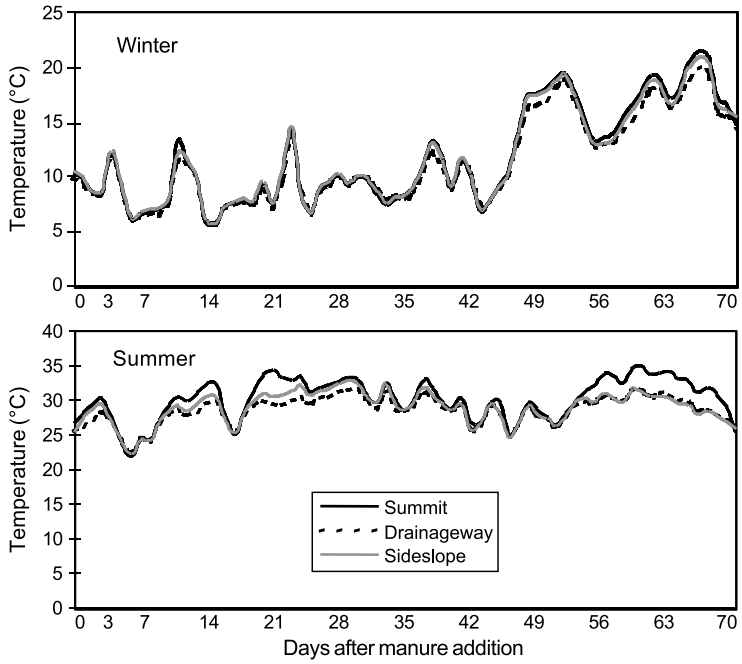

FIG. 2. Daily mean soil temperature (winter 2004 and summer 2005) in the microplot cylinders for three landscape positions soils during the season. Scales of figure are adjusted to show treatment differences between seasons.

TABLE 2. Mean Dehydrogenase Enzyme Activity and Microbial Biomass N Average Over Each Day

	μg Formazon/g Dry Soil	μg Nitrogen/g Dry Soil
Winter		
Summit	15.68 (6.23)[†]	35.29 (13.73)
Summit manure	17.06 (5.00)	46.63 (16.52)
Drainageway	15.59 (4.68)	26.92 (12.99)
Drainageway manure	18.81 (6.94)	40.00 (21.65)
Sideslope	14.73 (10.62)	49.88 (17.12)
Sideslope manure	14.39 (4.92)	64.06 (19.28)
$LSD_{(0.10)}$	2.40[‡]	6.17
Summer		
Summit	43.21 (18.86)	43.21 (18.86)
Summit manure	58.31 (18.85)	58.31 (18.85)
Drainageway	58.34 (24.01)	58.89 (23.99)
Drainageway manure	72.94 (20.87)	72.73 (21.08)
Sideslope	28.73 (12.55)	28.74 (12.55)
Sideslope manure	34.99 (19.50)	34.99 (19.50)
$LSD_{(0.10)}$	7.01	7.00

[†]Numbers in brackets indicate S.D., $n = 7$.
[‡]LSD: least significant difference.

highest organic C and N contents. Because this soil was located in a depression area, this was most likely attributed to nutrient transportation from water movement, thus, resulting in higher OM and CEC. For instance, during high rainfall events, the soil OM in the surface soil of the summit and sideslope has the potential to be lost through erosion when surface water runoff occurs. The eroded sediment settles in depressed areas of agronomic fields, thus increasing the OM content. Soils with higher OM tend to have larger microbial biomass pools. These microbial biomass pools in soil are sensitive to environmental changes such as land use or modification in available substrate. Thus, as result of manure application, the drainageway landscape position soil was more responsive to addition of available substrate. Kanchikermath and Singh (2001) reported that, generally, enzyme activities in the soil are closely related to the OM level. This is especially true for the dehydrogenase enzyme, in that, its activity is directly related to the oxidation of OM (Dick et al., 1996), which helps explain our results in this study. The drainageway position soil with manure had the highest dehydrogenase from day 0 to day 14 during the winter and was the highest on each summer sampling day except day 7 (Fig. 3). A shift in the order of microbial activity was observed among landscape position soils during the winter between day 28 and day 70. The sideslope landscape position soil had the highest microbial activity during this period. This was probably attributable to the soil having the highest clay content and lowest sand content.

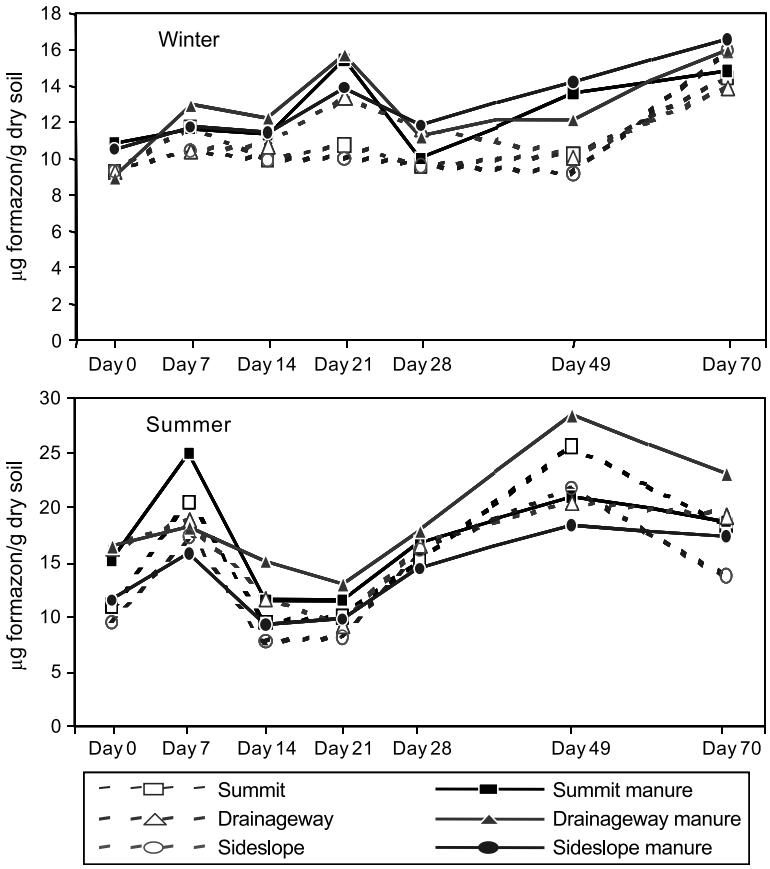

FIG. 3. Dehydrogenase enzyme activity interactive effects of landscape position and composted dairy manure for the summer and winter season. Scales of figure are adjusted to show treatment differences between seasons.

Thus, the microbes were probably better protected in the micropores of the clay soil compared with that of the more sandy soils.

Soil Microbial Biomass N

Although the microbial biomass in the soil represents approximately 1% to 5% of the total OM (Jenkinson and Ladd, 1981), it can be used as an early indicator of soil quality resulting from different land management practices (Powlson, 1994). Microbial biomass has also been used to evaluate the microbial transformations of plant nutrients as well as for their ability to act as a source or sink for N, P, S, and C (Anderson and Domsch, 1980; Paul and Voroney, 1980). Similar to dehydrogenase activity, microbial biomass N also increased after the application of composted dairy manure (Table 2, Fig. 4). It is well known that changes in microbial biomass concentrations in soil correspond to changes in the availability of decomposable substrate. Thus, the manure addition in this study provided the soil microbes with more readily available C and N, increasing the microbial biomass. This is consistent with the finding of Bohme et al. (2005) who reported that microbial biomass was greater in soil after the application of farmyard manure. The same trend was also shown for landscape X season interaction effect. During the summer season, a higher microbial biomass N content was observed compared with the winter months ($P < 0.0016$). This is consistent with dehydrogenase activity, suggesting that as microbial activity increased, more N was immobilized into microbial cells. In addition, the greatest microbial biomass N was observed on day 0 (day of manure application) and decreased thereafter until approximately day 14 or day 21 for both the summer and winter months (Fig. 4), respectively. This trend may represent a temporary storage of N in the microbial cells (immobilization) at day 0, which decreased by day 7, representing release of N into the soil environment as a result of cell lysing (N mineralization).

A comparison of landscape position shows that differences were observed on every sampling date for the winter and summer seasons ($P < 0.001$). In the drainageway landscape position soil, which had the highest initial soil organic C and N contents, microorganisms were more efficient at immobilizing the N into their cells during both seasons, suggesting that topography of a landscape could cause changes in soil C and N cycling rates and accumulation of OM (Chen and Stark, 2000). Microbial biomass N was the lowest in the sideslope landscape position soil. This means that less N was being immobilized into the microbial cells. The reduction observed in the microbial biomass N concentration for the sideslope landscape position soil could be attributed to a greater rate of nitrification and less immobilization. A lower C:N ratio was also observed in the sideslope landscape position soil, suggesting that although this soil had the highest clay content, microbial biomass N was more closely related to the C, N, and C: N ratio of the soil. In essence, a lower C:N ratio means that more mineralization was occurring and less immobilization was occurring. As stated earlier, there was a landscape X season interaction effect ($P < 0.0016$). No significant amendment X season interaction effect ($P < 0.7163$) was observed, suggesting that the interaction between the landscape and season

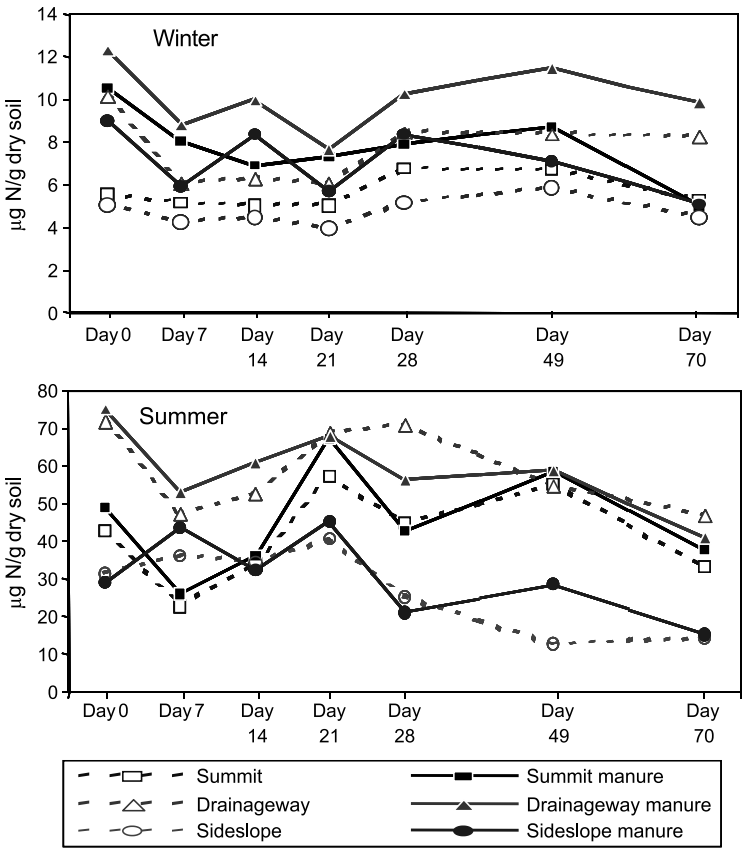

FIG. 4. Microbial biomass N interactive effects of landscape position soil and composted dairy manure for the summer and winter season. Scales of figure are adjusted to show treatment differences between seasons.

was greater than the effect between manure and season. In essence, the landscape position effect was the major determinant toward increasing the immobilization of N into the microbial biomass.

Microbial Community Composition

The response of the total viable microbial community composition and microbial community structure to differences in landscape position, manure application, and season were determined using mole percentage of the PLFA most common to all samples. Observations in this study showed that among the PLFA identified, total PLFA (in nanomoles) were present in significantly lower concentrations during the winter months ($P < 0.0001$) than in the summer months (Fig. 5). This suggests that an increase in total PLFA concentration corresponded to higher microbial composition, probably resulting from increased soil temperatures. These findings are in agreement with those of Ritz and Robinson (1988) and Ross et al. (1995). Total PLFA were also significantly higher ($P < 0.002$) in the manure treatments compared with no manure treatments for both the winter and summer seasons, suggesting that manure application caused the soil microbial populations to increase in size compared with control plots. This increase in total PLFA represents a change in relative proportions, probably as a result of increased nutrients as well as microbial biomass contained in the manure. An increase in total PLFA ($P < 0.10$) was also observed to result from differences in landscape position, implying that changes in soil microbiological characteristics have occurred in these areas. Total PLFA was the highest in the drainageway landscape position. Large inputs of nutrients have accumulated in this soil over the years, resulting in greater changes in microbial community composition. The drainageway landscape position soil was subjected to an accumulation of carbonaceous nutrients with water movement that caused the microbial biomass to increase, thereby affecting soil characteristics and influencing greater changes in microbial community population. Therefore, changes in landscape position should be taken into account when applying manure to soil with a varying degree of topographies to maximize the benefits that microorganisms have on soil functions.

Soil Microbial Community Structure

In this study, PLFA analysis identified 48 fatty acids; however, of these, only the 33 that were common to all samples were used for data analysis to prevent the PLFA that were sporadically detected or unreliably quantified from influencing the analyses (Bossio and Scow, 1998). The PLFA identified consisted mainly of saturated and unsaturated fatty acids. The saturated fatty acids consisted of three subgroups: normal straight chain saturated fatty acids (NSFA): 14:0, 15:0, 16:0, 17:0, 18:0, 20:0; mid-chain branched saturated fatty acids (MBFA): 10 Me 18:0 and terminally branched saturated fatty acids - i14:0, i15:0, a15:0, i17:0, and a17:0. The unsaturated fatty acid group is composed also of three subgroups (CYCLO): cy17:0 and cy19:0, monounsaturated fatty acids (MONO): i15: 1, i16: 1, 16:1ω9c, 16:1ω5c, 16:12OH, 17:1, 18:1 ω9c, 18:1ω5c, 20.1ω9c; and polyunsaturated fatty acids (POLY): 18.3ω6c. Proportions of these PLFA identified from each fatty acid group are provided in Fig. 6.

The specific biomarkers obtained from the PLFA profiles were used to quantify the relative abundance and changes in microbial groups (Fig. 7) that have occurred because of treatment. The mean ratio of monounsaturated to saturated fatty acids (Table 3) increased in soil receiving composted dairy manure

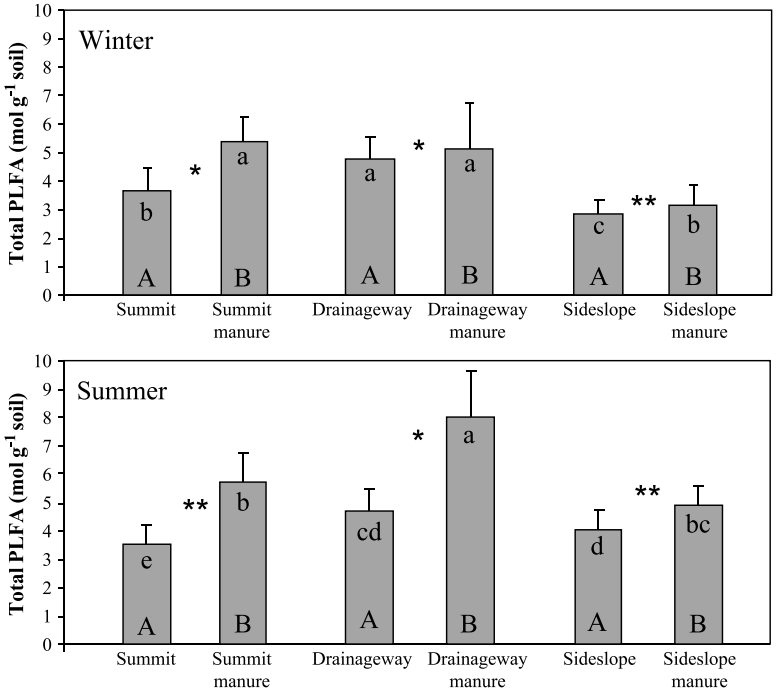

FIG. 5. Total phospholipids fatty acids (PLFA) for samples collected on the last day of the incubation for the *in situ* soil cores. Bars with same uppercase letter are not significantly different within a landscape position soil. Bars with same lower case letter are not significantly different among individual treatments Bars of the landscape position soils (with and without manure soil averaged together) with the same type of * are not significantly different.

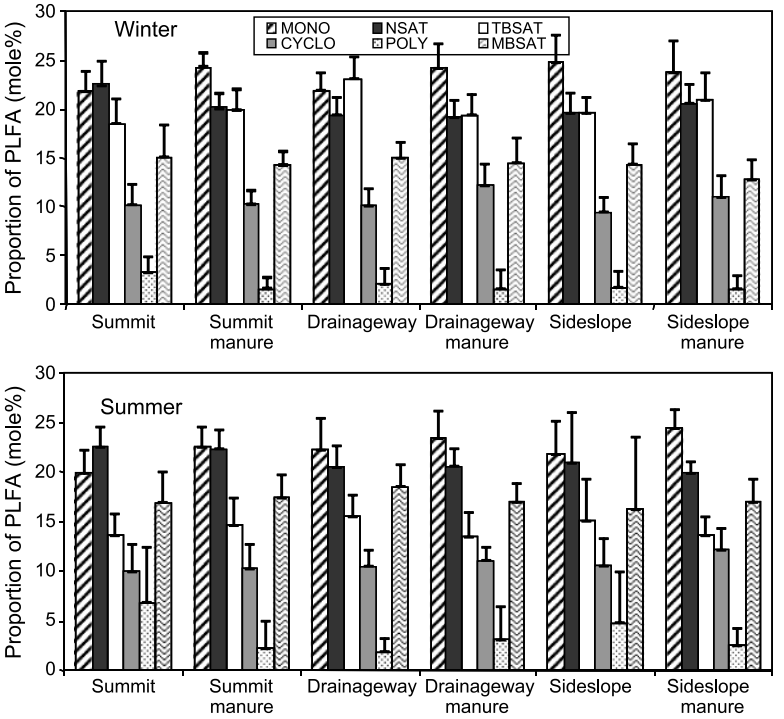

FIG. 6. The relative microbial community composition of different functional groups of biomarkers for the summit, drainageway, and sideslope with and without composted dairy manures for the winter and summer months.

during the winter ($P < 0.003$) and summer ($P < 0.02$) seasons, suggesting that gram-negative bacteria (commonly associated with monounsaturated fatty acids) increased in population as a result of composted dairy manure addition. This is similar to the finding of Bossio et al. (1998) who reported that farming practices that increase organic inputs also increase monounsaturated PLFA profiles. Although significant differences were not observed among landscape position soils, the drainageway and sideslope soils (both are loam soils) contained higher amounts of monounsaturated fatty acids compared with the summit soil (sandy loam). This suggests that soil-types varied resulting from differences in landscape positions could also affect the

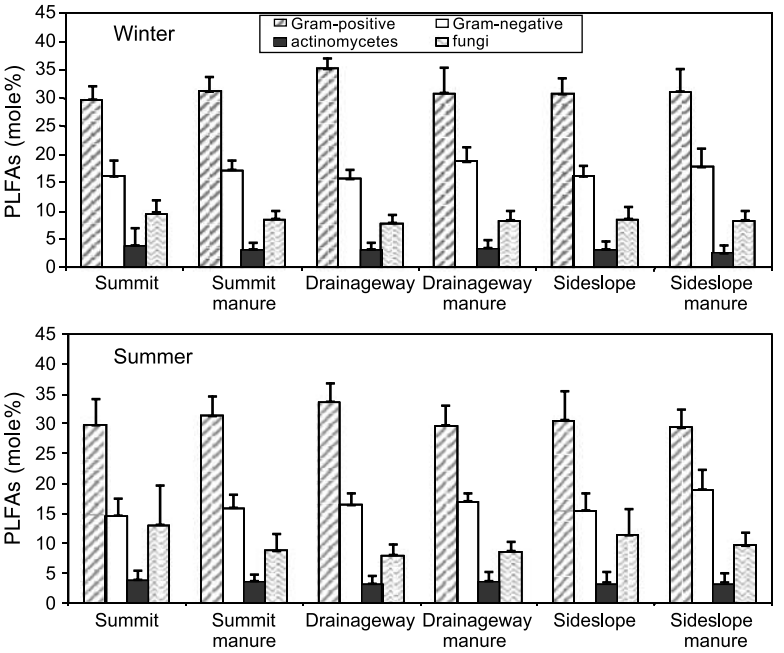

FIG. 7. The relative microbial community composition of different functional groups for gram-positive and gram-negative bacteria, fungi, *Actinomycetes* for the summit, drainageway, and sideslope with and without composted dairy manure for the winter and summer months.

TABLE 3. Proportional Distribution of Microbial Groups Identified Using PLFA Biomarkers for the Winter and Summer Months

Soil Landscape Position	Saturated, mol (%)	Unsaturated, mol (%)	Monounsaturated-to-Saturated Ratio	Gram-Positive-to-Gram-Negative Ratio	Fungi-to-Bacteria Ratio	Cyclopropyl-to-Monoenoic Ratio
Winter						
Summit	56.09 (1.41)	35.00 (1.37)	0.39 (0.03)	1.86 (0.23)	0.19 (0.03)	1.60 (0.12)
Summit manure	54.45 (1.16)	36.26 (0.56)	0.45 (0.10)	1.83 (0.13)	0.17 (0.15)	1.51 (0.12)
Drainageway	57.51 (1.50)	37.36 (0.69)	0.44 (0.22)	2.24 (0.95)	0.15 (0.10)	1.71 (0.08)
Drainageway manure	53.23 (2.61)	37.35 (1.08)	0.45 (0.02)	1.66 (0.35)	0.17 (0.03)	1.71 (0.09)
Sideslope	53.64 (2.13)	35.66 (0.47)	0.46 (0.02)	1.90 (0.20)	0.17 (0.03)	1.37 (0.09)
Sideslope manure	54.30 (2.39)	36.26 (1.65)	0.44 (0.05)	1.76 (0.29)	0.16 (0.02)	1.62 (0.31)
$LSD_{0.10}$	2.29	1.25	0.03	0.27	0.02	0.18
Summer						
Summit	53.05 (3.01)	36.61 (3.39)	0.37 (0.03)	2.06 (0.21)	0.25 (0.13)	2.22 (0.43)
Summit manure	54.34 (2.92)	34.00 (2.63)	0.40 (0.04)	2.02 (0.29)	0.16 (0.04)	1.87 (0.08)
Drainageway	54.32 (2.09)	34.45 (1.98)	0.41 (0.05)	2.07 (0.15)	0.15 (0.02)	1.81 (0.29)
Drainageway manure	50.89 (2.45)	37.59 (2.57)	0.46 (0.05)	1.75 (0.16)	0.17 (0.23)	1.88 (0.21)
Sideslope	52.05 (1.48)	37.12 (2.61)	0.43 (0.03)	2.04 (0.25)	0.22 (0.03)	2.24 (0.12)
Sideslope manure	50.51 (5.38)	39.22 (4.48)	0.49 (0.09)	1.59 (0.47)	0.20 (0.08)	1.84 (0.27)
$LSD_{0.10}$	3.62	3.47	0.06	0.31	0.07	0.29

[†]Numbers in brackets indicate S.D., $n = 7$.
[‡]LSD: least significant difference.

concentration of monounsaturated PLFA profiles, thus, further implying that changes in soil quality have occurred within the different landscape positions.

The PLFA markers used to quantify the relative abundances of specific gram-positive to gram-negative bacteria ratio were as follows: i14:0, i15:0, a15:0, i16:0, 10Me16:0, i17:0, and a17:0 for gram-positive bacteria, and cy17:0, cy19:0, 16:1ω9c, 18:1ω9c, 15:1ω4c, 18:1ω7c, and 17:1ω9c for gram-negative bacteria (O'Leary and Wilkinson, 1988; Zelles et al., 1994; White et al., 1996; Zelles, 1997, Fierer et al., 2003). The PLFA markers for fungi to bacteria ratio are as follows: 18:2 ω6,9c, 18:1 ω9c, 18:3 ω6c, and 20:1 ω9c for fungi, and i15:0, i16:0, 10Me16:0, a15:0, cy17:0, 18:1 ω7c, cy 19:0, 14:0, 15:0, 16:1 ω9c, 16:1 ω7c, 16:1 ω5c, a17:0, i17:0, 17:0, and 18:0 for bacteria (Frostegard et al., 1993; Zelles, 1997; Fierer et al., 2003; Feng et al., 2003). The ratios of the relative abundance of the calculated cyclopropyl fatty acids/monoenoic precursors (cy17:0 + cy 19:0/16:1 ω7c + 18:1 ω7c) have been previously used by other researchers as indicators of nutritional stress in bacterial communities (Knivett and Cullen, 1965; Kieft et al., 1997; Bossio and Scow, 1998; Fierer et al., 2003).

The mean ratio of gram-positive to gram-negative bacteria significantly decreased in soil containing composted dairy manure compared with soil without composted dairy manure for the winter ($P < 0.10$) and summer ($P < 0.008$) months (Table 3). This was mainly attributed to the increase in gram-negative bacteria, thus suggesting that the addition of organic C to soil from the composted dairy manure provided a more stable and readily available substrate for supporting higher levels of microbial activity for gram-negative bacteria (Peacock, 2001). Others have also reported that gram-negative bacteria are mainly associated with monounsaturated fatty acids, which corresponds to increases in OM content and high substrate availability (Böhme et al., 2005; Zelles et al., 1992; Bossio et al., 1998). Populations of actinomycetes were not significantly impacted by manure addition. On the other hand, populations of fungi decreased in the summit and sideslope landscape position soils and increased in the drainageway soil with composted dairy manure addition attributing to a lower fungi/bacteria ratio. These results show that microbial community structure can change with respect to manure addition and soil types that are characteristic of different landscape positions.

Differences in the landscape X amendment interaction effect for the fungi/bacteria ($P < 0.0211$) and gram-positive/gram-negative ($P < 0.0268$) ratios were observed. In general, the drainageway landscape position soil displayed an increase in the fungi/bacteria, whereas the summit and sideslope decreased, although generally not significantly. In addition, the drainageway landscape position soil resulted in a decrease in the gram-positive/gram-negative ratio during both the summer and winter seasons, although the sideslope landscape position soil decreased only during the summer. These differences are most likely a result of the inherent differences in available substrate between the soils. Under most conditions, the addition of manure increases the more readily decomposable compounds; these are mainly decomposed by soil bacteria, whereas fungi decompose the more recalcitrant and insoluble materials. The drainageway landscape position soil has received a more continual addition of readily available substrate, thus suggesting that historically, the substrate received from water disposition has allowed the long-term potential for simultaneous development of bacteria and fungi (Griffiths et al., 1999). As for gram-positive bacteria, they are often found in environments where there is plenty of available substrate. The drainageway landscape position soil periodically receives OM from runoff water deposition, allowing the soil to support a large population

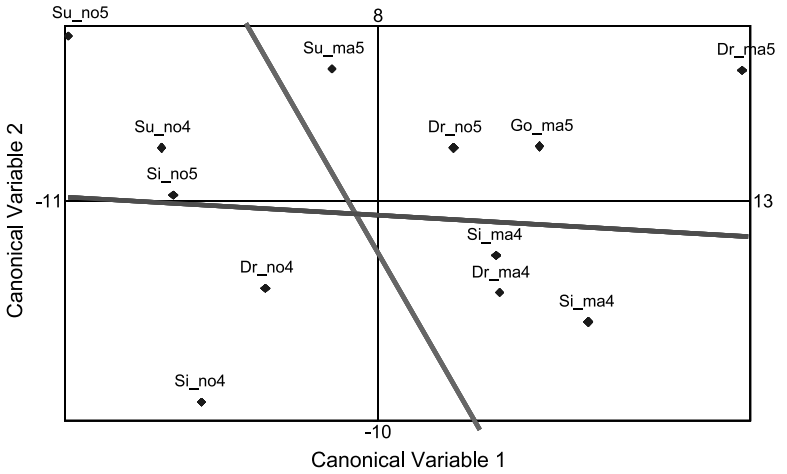

FIG. 8. Canonical discriminant analysis (CDA) of phospholipid fatty acid profiles for the canonical variates (CV). Plot of ordination of CV1 against CV2 during the summer (5) and winter (4) months for the summit (Su), drainageway (Dr), and sideslope (Si) soil with (ma) and without composted dairy manure (no).

of gram-positive bacteria. However, when composted dairy manure was added to the drainageway landscape position soil, a decrease in the amount of gram-positive bacteria was observed, whereas gram-negative bacteria increased, resulting in a change in microbial community structure. The other two soils, located on the summit and sideslope, historically have been subjected to erosion and translocation of soil nutrients compared with nutrient deposition in the drainageway landscape position soil, thus suggesting why the gram-positive bacterial response was more subtle.

The cyclopropyl/monoenoic precursor ratio can be used as an indication of the physiological status of the microbial population. Low cyclopropyl/monoenoic ratio is an indication of actively growing cells. There was a significant season effect ($P < 0.001$), more stressful conditions were observed during the summer compared with the winter (Table 3). This increase in stress could be attributable to an increase in the soil temperature during the summer compared with the winter. This is similar to the finding of Petersen et al. (2002) who also observed higher stress during summer months. Petersen et al. (2002) attributed the increased stress to more extreme environmental conditions resulting from a hot and drier climate observed during the summer. A lower cyclopropyl-to-monoenoic precursor ratio was observed in the composted dairy manure treatments during the summer months. This suggests that composted dairy manure addition helped decrease the stress ratio by promoting a more viable growing microbial population.

Cannonical discriminant analysis, using PLFA mole percentages, was performed to determine differences between treatment effects and microbial groups. The first four canonical discriminant variates (CDV) accounted for 90% of the total variance with the first two CDV accounting for 48% and 25% of the total sample variance, whereas the third and fourth CDV explained 16% total. Canonical discriminant analysis plots of the three soil series with and without composted dairy manure for the summer and winter seasons are shown in Figs. 8 and 9.

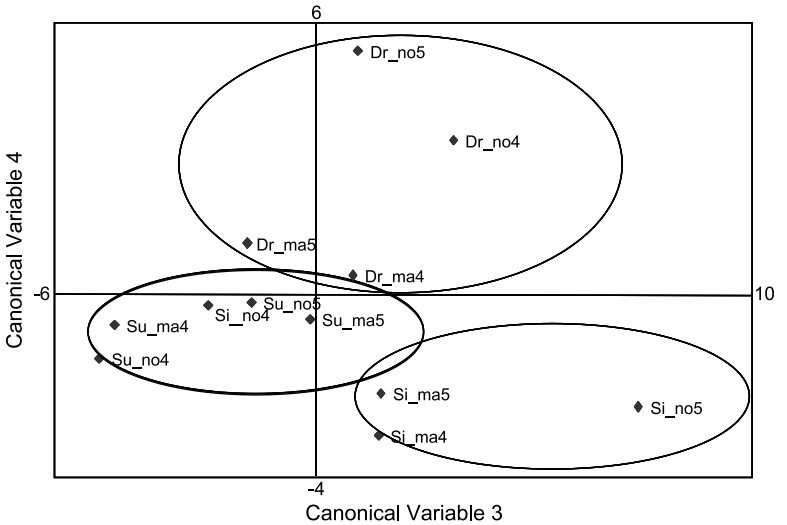

FIG. 9. Canonical discriminant analysis (CDA) of phospholipid fatty acid profiles for the canonical variates (CV). Plot of ordination of CV3 against CV4 during the winter (4) summer and (5) months for the summit (Su), drainageway (Dr) and sideslope (Si) soil with (ma) and without composted dairy manure (no).

Canonical discriminant analysis of PLFA profiles indicated that there were significantly different microbial communities among treatments. In general, a clear discrimination was observed among those with and without composted dairy manure treatments on the CDV1 (Fig. 8), with manure plots having higher ordinates values. The unamended drainageway landscape position soil from the summer of 2005 was intermixed with the soils containing composted dairy manure; this unusual occurrence could not be explained and is probably a result of experimental error. The PLFA profiles were also affected by season; the effect of season was separated by CDV2 (Fig. 8). The summit (no manure) and sideslope (manure) landscape position soils during the summer months had the highest ordinates. The PLFA profiles representing microbial community were affected by season. The warmer soil temperatures experienced during the summer probably lead to an apparent increase in the C substrate available for microbes because of a temperature-dependent change in growth efficiency or diffusional processes (Ellert and Bettany, 1988; MacDonald et al., 1995). In addition, higher temperature causes stimulation in microbial activity. Similar to results obtained for microbial community composition, the PLFA profiles suggest that a shift in microbial community structure occurred with changes in season resulting from higher soil temperature. The fourth CDV (Fig. 9) clearly separated the landscape position soils into distinct groups except for one treatment, suggesting that the microbial communities resulted from the characteristics of each landscape. Canonical discriminant analysis identified fatty acids that were important in explaining the variability observed within the PLFA profiles. The PLFA 16.1ω5c, 18:3ω6c, 18:1ω7c, cy19:0, 20:4ω6,9,12 were identified by CDA as influential biomarkers for the CV1 and 16:1ω7c / i15:0 2OH, 18:1ω7c, 18:0, 18:3ω6c for CV2, respectively (Table 4). The PLFA 14:0, i15:0 a15:0, 18:0 16:0 10 methyl were influential biomarkers for CV4. The PLFA 16.1ω5c was associated with monounsaturated fatty acids, which have been shown to increase with manure addition as previously stated. In addition, 16.1ω5c, 18:1ω7c, and cy19:0 are gram-negative bacteria that are associated with an increased readily available substrate. On the other end of the spectrum, 18:3ω6c and 20:4ω6, 9,12 are associated with fungi and was shown to decrease with the addition of available substrate. The PLFA identified for the second CV (16:1ω7c, 18:1ω7c) accounted for most of the discrimination. Fatty acid 16:1ω7c is associated with monounsaturated fatty acids and 18:1ω7c is associated with gram-negative bacteria both of which increased with the addition of manure as previously stated. The biomarker 18:0 is a nonspecific fatty acid, which probably affected the PLFA concentrations, thereby causing a shift in lipid composition between seasons. The PLFA identified for the fourth CV 14:0, a biomass of all organisms, i15:0 and 15:0, a gram-negative bacteria, 18:0, a biomass of all organisms, 16:0 10 methyl actinomycetes were all found in more abundance in the drainageway and sideslope landscape position soil, which are both loam soils. The drainageway landscape position soil had a higher concentration of the PLFA during the winter months, and the sideslope landscape position soil had a higher abundance during the summer months. The data observed from the CDA suggest that microbial community structure varied according to season, manure, amendment, and soil type, resulting from landscape position. These results help support the previous observation made in the study with microbial biomass N and dehydrogenase, total PLFA.

TABLE 4. PLFA of the First Five Scores Accounting for the Variance of the First Four Canonical Axes

Fatty Acid	Score	Specificity as a Biomarker
Canonical variable 1		
16.1 ω5c	0.82	Bacteria (gram-positive and gram-negative)
18:3 ω6c	−0.43	Fungi
18:1ω7c	0.42	Aerobic bacteria, gram-negative
cy19:0	0.40	Anaerobes, gram-negative bacteria
20:4ω6,9,12	0.39	Fungi
Canonical variable 2		
16:1ω7c/i15:0 2OH	−0.70	Nonspecific
18:1ω7c	−0.53	Aerobic bacteria, gram-negative
18:0	0.50	Biomass all organisms
a15:0	−0.38	Gram-positive bacteria
18:3ω6c	0.37	Fungi
Canonical variable 3		
i17:0	0.38	Gram-positive bacteria
a18:0/18:2ω6,9c	−0.42	Gram-positive/fungi
16:1 20H	0.37	Nonspecific
cy17:0	0.37	Gram-negative
17:0 10 methyl	0.36	*Actinomycetes*
Canonical variable 4		
14:0	0.47	Biomass all organisms
i15:0	0.46	Gram-positive bacteria
a15:0	0.42	Gram-positive bacteria
18:0	−0.32	Biomass all organisms
16:0 10 methyl	0.30	*Actinomycetes*

CONCLUSIONS

Soil microorganisms are the driving force behind maintaining the long-term fertility of an agroecosystem. Through their processes, soil formation and nutrient cycling occur. Availability of substrate in soil determines the microbial population's size, activity and biomass, which is instrumental to controlling the structure of the microbial community. Therefore, evaluation of key microbial indicators such as microbial activity, microbial biomass, and community structure may provide insight into the long-term fertility status of the soil ecosystem processes. Microbial indicators evaluated in the study suggest that season, addition of manure, and topography of a landscape can greatly affect the microbial community structure in soil. The addition of composted dairy manure changed the microbial community structure probably by increasing the soluble C in the soil. Season also increased the microbial indicators, resulting in increased metabolic activity during the summer compared with the winter. Soil landscape positions that contain higher OM were also observed to alter the microbial community. Significant microbial indictors were observed from increases in microbial biomass N, dehydrogenase (microbial activity), total PLFA, and changes in microbial community structure. Canonical discriminant analysis clearly discriminated PLFA profiles by season, manure addition, and soil type and landscape position, thus confirming that changes in microbial community structure changed as a result of the agronomic management practices evaluated. This study also suggests that accumulation of OM in depression areas can cause an increase in microbial activity. Addition of manure to soil increases microbial activity, which plays a prominent role in immobilizing and mineralizing plant nutrients. Therefore, consideration for landscape variability and its effects on soil microbial functions should be taken into account when developing management systems that use organic fertilizer sources such as manure.

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