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The functional significance of the microbial biomass in organic and conventionally managed soils

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Abstract

In order to achieve sustainability in managed ecosystems we must understand management impacts on soil processes and clarify the regulatory role of the microbial community on these processes. Crop rotation and organic management practices are thought to have positive impacts on the microbial biomass; however, the specific impacts of crop rotation organic management on soil microbial ecology are largely unknown. The effect of organic management on soil microbial ecology was investigated using soils collected from the Rodale Institute Research Center's long-term Farming Systems Trial (FST) experiment. The FST, begun in 1981, included a manured and a cover cropped organic rotation and a conventionally managed grain based rotation. Soil respiration rates and ^{13}C-isotope fate in a companion study suggest that the biomass characteristics of the FST treatment soils were different in November 1991. However, direct measurement of the microbial community at this time using Phospholipid Fatty Acid Analysis (PLFA) did not identify statistically significant treatment based differences in soil biomass characteristics. Variability among the PLFA profiles of treatment replicates was as great as variability between farming systems. Treatment based trends were observed among selected PLFAs, particularly those present in large amounts, that were consistent with indirect biomass and biomass-dependent measures. Overall, PLFA profiles, soil respiration rates and ^{13}C-cycling suggested that the organic cover cropped soil had the Largest and most heterogeneous microbial population while the biomass of the organic-manure amended soil was the least heterogeneous, and the most metabolically active.

Introduction

Estimates suggest that 25 to 50% of the land cultivated today has lost 2.5 to 6 kg of C per square meter since the 19th Century (Bolin, 1977). Soil disturbance and soil organic matter (SOM) losses are believed be associated with reduced soil biodiversity (Stewart, 1991); however, direct evidence of this is lacking. The specific impacts of agronomic management on and soil biodiversity depend upon the soil's inherent properties and upon the management practices employed (McGill et al., 1981). Mixed cropping patterns, crop rotations, and organic management practices may be tools that can off set the negative impacts of agriculture on biologically active SOM by increasing the size (Anderson and Domsch, 1989) activity (Bolton et al., 1985), and diversity (Hassink et al., 1991; Stewart, 1991) of the microbial community. Management-based enhancement of the soil biomass may increase productivity and the rate of nutrient cycling (Doran et al., 1987), reduce the risk of soil erosion by improving aggregation (Angers et al, 1992), macroporosity and pore continuity (Lal, 1991), and accordingly, reduce nutrient leaching loss (Elliott, 1988). While the biomass is believed to play a major regulatory role in soils (Paul, 1990), the specific relationships between management practices, the soil biomass, biologically active SOM, and soil processes, are not well characterized.

Table 1. Total soil C and N contents of FST plots sampled in November 1991. Soil collected from the top 10 cm of plots in blocks 1, 2, 3, and 5, that were planted to corn in 1991. Different letters following means within columns indicate values were significantly different at the 5% level (n=4)

Rotation	Carbon	Nitrogen
	—mg kg^{-1} soil—	
Animal	22.9 a b	3.50 a
Cover Crop	25.0 a	3.40 a
Conventional	21.3 b	3.30 a

In order to achieve sustainability in managed items we must understand management impacts on seasonally and spatially variable soil processes, clarify the regulatory role of the microbial community on these processes, and identify functionally important biomass and organic matter pools.

We examined the effects of 10 years of organic and conventional management on the soil biomass size and structure by using ester-linked phospholipid fatty acid (PLFA) profiles. In the past, methods available for microbial characterization provided only limited information about the in situ characteristics of the soil biomass. Less than one percent of the microbial cells in many environments, including surface soils, can be isolated and grown in culture (Colwell et al., 1985). Biochemical methods, such as the PLFA approach, are increasing in popularity as a means to characterize microbial communities. Biochemical methods avoid the problems associated with microbial isolation and culturing techniques that have limited direct counting methods (Jenkinson and Ladd, 1981) and can provide much more accurate and precise estimates of the viable microbial biomass than chloroform fumigation (Balkwill et al., 1988; Tunlid and White, 1992). Furthermore, PLFA profiles contain detailed information on lipid structure (i.e. the location of double bonds, lipid geometrys, and side groups) that can be used to investigate microbial community structure and metabolic condition (Guckert et al., 1986; Hedrick et al., 1991; Nichols and White, 1989; Vestal and White, 1989. While PLFA profiles do not reveal species-level information directly, lipid analysis provides a fingerprint of microbial diversity present at the time of sampling.

Results from previous FST studies

This work was done in collaboration with the Rodale Institute Research Center on the long-term Farming Systems Trial (FST) experiment comparing two organic, and one conventionally managed farming systems (Liebhardt et al., 1989; Peters et al., 1992). At present, the two organic systems are competitive agronomically with the conventionally managed system (Hanson et al., 1990; Liebhardt et al., 1989; Peters et al., 1992). A deeper understanding of soil and/or microbial characteristics that are associated with the different farming practices could promote the successful implementation of organic and reduced-input techniques at other sites. In the first ten years of the FST experiment there were small, statistically significant increases in the C content of the surface soil in the organic cover crop based farming system (Table 1). As early as 1982, management induced changes in the size and activity of the soil microbial biomass were suggested by Doran et al. (1987) who used the chloroform fumigation and soil incubation method of Jenkinson and Powlson (1976), and quantified ammonium released from autoclaved soils (Doran, 1987), to estimate soil biomass and potentially mineralizable nitrogen, respectively. Studies conducted throughout the 1985 and 1988 seasons, which also used chloroform fumigation and soil incubation based methods, linked soil biological activity to rotation characteristics (Doran et al., 1987; Harris et al., 1994). During early season samplings, soil biomass size estimates were significantly greater in the recently cover cropped soil than in the other two treatment soils. At the end of the season there were no treatment based differences. Harris et al. (1994) found that N mineralization was greatest in the manure amended soil, suggesting that in this treatment, the viable biomass was either larger in size or more metabolically active. In 1990, soil respiration rates, NH_4 and NO_3 in fresh soil extracts, and net N mineralization rates in two week aerobic incubations also indicated that the size and/or rate of biological activity were greatest in the manured, intermediate in the cover cropped, and least in the conventionally managed soil (Wander, 1993; Wander et al., 1994).

Previous studies also suggest that management practices have led to changes in soil physical characteristics. Water infiltration rates were greater in the two organically managed soils than in the conventionally managed soil (Peters et al., 1994). In addition, enhanced soil aggregation has been observed in the organically managed soils following the production of

Table 2. Farming Systems Trial cropping sequence

Rotation	Entry[a] Year 1[b]	Crops Produced Each Year Year 2	Year 3	Year 4	Year 5
		——————1981-1990——————			
Animal	1 Wh/Hay.....[c]	.....	.../Corn	Soy	Corn-si
Animal	2 Corn	Soy	Corn-si/Wh	.../Hay	.....
Animal	3 Corn-si/Wh	./Hay.....	.....	.../Corn	Soy
		——————1981-1985——————			
C. Crop	1 Oats/C.Crop.....	.../Corn	Oats/C.Crop.....	.../Corn	Soy
C. Crop	2 Soy	Oats/C.Crop.....	.../Corn/Wh.....	.../C.Crop.....	.../Corn
C. Crop	3 Corn	Soy	Oat5/C.Crop.....	.../Corn	Oats/C.Crop...
		——————1986-1990——————			
C. Crop	1 Oats/C.Crop.....	.../Corn	Bar/Soyd /Wh.....	.../C.Crop.....	.../Corn
C. Crop	2 Bar/Soy/Wh.....	.../C.Crop	.../Corn	Bar/Soy/Wh.....	Oats/C.Crop...
C. Crop	3 C.Crop/Corn/Wh.	.../Soy/Wh.....	Oats/C.Crop.....	.../Corn	Bar/Soy
		——————Beginning in 1981——————			
Conv.	1 Corn	Corn	Soy	Corn	Soy
Conv.	2 Soy	Corn	Corn	Soy	Corn
Conv.	3 Corn	Soy	Corn	Corn	Soy

[a]Year 1 corresponds to 1981, '86, and '91, Year 2 to 1982, and '87, Year 3 to 1983, and '88, Year 4 to 1984, and '89, and Year 5 to 1985, and 90.

[b]Entry designates the crop entry-point in the organic animal based (mal), organic cash grain based (C. Crop). and conventional cash grain based (Conv.) rotations. After two cycles of the organic animal based rotation the crop sequence was altered. The crop sequence was modified every five years in the organic cash-grin based rotation and the sequence remained unchanged in the conventional cash-grin based rotation.

[c] Crops followed by "..." are grown throughout the winter or following season and tilled in or harvested during the spring of the crop season marked "..../". Wh=Wheat, Hay = Red Clover ('81-'85) h ('88-'0), and Red Clover + Alfalfa 86 & '87). Soy-Soybean, Corn-si = Corn silage, Baser= Barley, C.Crop = Red Clover ('81-'83) and (85-'90), and Hairy Vetch (1984).

[d]Soybeans were not seeded in 1988.

legume hay and cover crops (Werner, 1988; Friedman, 1993).

Considered together, these results suggest that management has led to differences in the microbial communities of the FST soils. The objectives of this study were to characterize the microbial communities of the FST soils directly and to determine whether organic and conventional management practices led to differences in the size and/or composition of the microbial community.

Materials and methods

The Farming Systems Trial

The Rodale Institute Research Center is located in eastern Pennsylvania, near Kutztown. The plots are located on Typic Fragiudalf soils with large inclusions Typic Dystrochrept and Typic Hapludalfs. The site had been farmed conventionally prior to the experiment's initiation in 1981. Experimental treatments included a five year organic animal based rotation (Animal) that simulated a cattle based operation (Table 2). The Animal treatment received cattle manure and produced dinitrogen-fixing clover hay as its sources of fertility. The FST also included a four year organic cover crop based rotation (Cover Crop) that produced a cash grain every year. It received N fertility from leguminous cover crops. Finally, the FST included a conventional cash-grain rotation (Conventional) that produced corn and soybeans and used mineral fertilizer. In addition to differences in fertility and residue inputs, the rotations varied in their pest control strategies and planting patterns. The organically managed rotations relied on cultivation and multi-species crop mixes for weed and pest control (Edwards et al., 1992; Vandeemer, 1989), while the Conventional rotation received synthetic amend-

Table 3. Comparison of Farming system trial rotations

	Rotation		
	Animal	Cover Crop	Conventional
Cops grown[a]	Corn-grain,corn-silage, soybeans, wheat, legume hay	Corn-grain, soybeans, wheat, oats, barley, legume green manure	Corn-grain, soybeans
Surface condition of soil from 1/86 12/90[b]	Bare 7%, live plants 73%, dead residue 20% of time	Bare 8%, live plants 69%, dead resides 23% of time	Bare 8%, live plants 42%, dead residue 50% of time
Primary tillage	Moldboard plow: 4 times per 5 years	Moldboard plow: 5 times per 5 years, spring or fall	Moldboard plow: every spring
Weed control	Rotary hoe, cultivate corn and soybeans	Rotary hoe, cultivate corn	Herbicides applied to corn and soybean
Insect control	Rotation	Rotation	Insecticide is applied to second year corn
Nitrogen fertility	Beef manure applied to coqn (poultry manure applied in 1986), residual hay	Legume (red clover/alfalfa) green manure	34 kg ha^{-1} urea starter fertilizer, side dress N 112 kg ha^{-1} ammonium nitrate
Potassium fertility	139 kg ha^{-1} K added as K2SO4 in 1989, ≈ 56 kg ha^{-1} K added per year in manure	139 kg ha^{-1} K added as K2SO4 in 1989	139 kg ha^{-1} K added as K2SO4 in 1989
Phosphorus fertility	≈45 kgha^{-1} added annually in manure	none	14.6kg ha^{-1} added as starter fertilizer each year
Lime	3360 kg ha^{-1} of Ca limestone added in 1989	3360 kg ha^{-1} of Ca limestone added in 1989	8960 kg ha^{-1} Mg limestone added in 1982
Additional features	Frost seeded legumes into small grains	Frost seeded legumes into small grain, relay cropped soybeans into small grain	None

[a] Table modified from Peters et al. (1992).
Assume that the soil surface is bare for one month following plowing and planting a new crop. When a legume crop is established in the fall the winter period is considered to be under live cover even though the plants are dormant.

ments (herbicides and insecticides) as recommended by Pennsylvania State University. All rotations were moldboard plowed to the same depth (approximately 25 cm). A complete comparison of management practices is listed in Table 3. The experiment was a randomized block that included eight replications of the three 18 m by 91 m rotation treatment main plots. This experiment is more fully described by Liebhardt et al. (1989), and Peters et al. (1992).

Soil sampling

Plots coming out of the corn production phase in all rotations were sampled in November 1991. Surface soils (0-10 cm) were collected using a 10 cm by 5 cm bucket auger from blocks 1, 2, 3, and 5. Beginning 5 m into the plot, ten cores were collected from center rows of 90 m beds. Cores were spaced 8 m apart. Soil samples were combined to form twelve plot composites (3 rotation treatments by 4 replicates). To avoid lipid contamination the soil auger was rinsed with methanol:water (80:20 v/v) between sampling

plots and then 'soil rinsed' by repeatedly forcing the auger into the soil of the plot to be sampled. Immediately after sampling, cores were bagged and plunged into liquid nitrogen. During transport, soil was kept on ice. Samples for direct biomass characterization were immediately lyophilized and sieved using a 2 mm sieve. Samples collected for indirect biomass and available N characterization, which were not frozen, were maintained field moist and sieved (< 2 mm).

Microbial characterization

PLFAs were extracted from 30 g soil samples using a chloroform-methanol-water extraction phase ratio based on the Bligh and Dyer (1959) method as modified by White et al. (1979). After the extraction was complete solids were removed and chloroform and water (1:1, v/v) were added to bring the total solvent ratio to(1:1:0.9 v/v). The total lipid extract was separated into neutral lipid, glycolipid, and polar lipid fractions on silicic acid columns using organic solvents chloroform, acetone, and methanol, respectively (Guckert et al., 1985). The polar lipid fractions was derivatized via methanolysis (using methanolytic KOH in toluene and methanol (1:1 v/v) to fatty acid methyl esters (FA). The FAME fraction was purified using thin layer chromatography, recovered in hexane, and characterized with capillary gas chromatography (Hewlett Packard 588 OGC, 60 m nonpolar cross linked methyl silicone column) and flame ionization detection (Bobbie and White, 1980). Samples were introduced to the GC in hexane with methylnonadecanoate (Cl9:0) as an internal standard. Uniform peak component response was assumed within the detected range of microbial FAME (12:0-24:0). The chromatographic output was processed with a Nelson Analytical 760 series programmable data system processor using equivalent chain length analysis (ECL) for peak identification and quantification after Christie (1989). This technique is based on the linear relationship between the retention times of a homologous series of straight chain saturated FAME against the number of carbons in the FAME chain. PLFAs were quantified by determining the areas under each sample peak. To normalize peaks to a per gram sample basis, the output in nmol lipid per unit volume were determined using the area of the peak and the area and concentration of the internal standard.

To characterize available N in freshly sieved soils, inorganic NO_3^- and NH_4^+ were determined by extraction of 7 g of freshly sieved field moist soil (about 5.3 g oven dry wt) in 60 mL centrifuge tubes with 33 mL 2*M* KCl. After shaking 1 hr on a reciprocal shaker, the extracts were removed and filtered through rinsed Whatman no. 50 filter paper. NO_3^- and NH_4^+ were quantified colorimetrically using a Lachat auto analyzer (Lachat Instruments, Milwaukee WI.).

Soil respiration rates of homogenized soils were assayed to compare in situ soil biological activity (Anderson, 1982). Following sieving, which generally stimulates soil respiration rates, 10 g (dry wt basis) of field moist soil was placed into 117-cm^3 incubation vials. Soil was brought to 40% water holding capacity based on the mass of water remaining after saturated soil freely drained for 24 h. Vials were then sealed with rubber septa and covered with two layers of parafilm. Incubations were initiated two days after the samples were collected. Carbon dioxide in the vial head space was sub-sampled throughout a 2-week period using a Varian Model 3700 Gas Chromatograph (Varian, Sunnyvale CA) equipped with a Chromosorb 102 column and a thermal conductivity detector. Gas sample injections were made directly from incubation vials. Sample incubation vials were opened and sparged following each sampling to prevent oxygen depletion of head space gases. Random sampling of re-sealed vials confirmed that their gas composition matched ambient air.

Data analysis

The samples' fatty acid profiles were statistically analyzed after arcsine square root transformation of mole percents (Winer, 1971). PLFA mole percents were clustered using both the l-Pearson's r correlation coefficient and Euclidian metrics, and four linkage methodscentroid, average, complete (nearest neighbor), and single (farthest neighbor) using Systat statistical package (Systat, Inc. San Diego, CA). Eight combinations of methods were used together to determine whether the clusters obtained were robust with respect to the method used.

Results

Biomass results

The microbial structures of the three communities are described by the average mole percent of each of 72 fatty acids with chain lengths ranging between C: 14 and C:23 (Table 4). Forty-eight of the 72 lipid structures were identified. Unidentified lipid peaks represented

Table 4. Mean mole % (n=4) and standard deviation of each Fatty Acid Methyl Ester identified in extracts of Farming Systems Trial soils. Fatty acid nomenclature follows the form A:B C, where A is the number of carbon atoms, B is the number of double bonds, and C is the location of the unsaturation closest to the molecule's aliphatic end. The suffixes 'c' and 't' designate cis and trans isomerization, and prefixes 'i, a, br, and Cy 'indicate iso, anteiso, and methyl-branching, al cyclopropyl rings that are present in the molecule, respectively (Kates, 1986). The prefix 'Mc', preceded by a number indicates the location of a methyl branch.

Type PLFA	Phospholipid fatty acid (PLFA) % mole fraction		
	Animal	Cover Crop	Conventional
i14.0	0.635 ± 0.096	0.600 ± 0.183	0.575 ± 0.096
14:7ωc	0.125 ± 0.050	0100 ± 0.000	0.050 ± 0.058
14:0	0.525 ± 0.126	0.475 ± 0.126	0.475 ± 0.050
i15:0	6.325 ± 0.830	6.575 ± 0.718	6.725 ± 0.556
a15:0	4.125 ± 0.568	4.225 ± 0.395	4.525 ± 0.263
15:0	0.350 ± 0.058	0.375 ± 0.050	0.350 ± 0.058
br15:0a	0.375 ± 0.050	0.375 ± 0.096	0.325 ± 0.050
br15:1	0.575 ± 0.050	0.650 ± 0.058	0.625 ± 0.050
10Me15:0	0.200 ± 0.000	0.250 ± 0.058	0.200 ± 0.000
i16:0	2.050 ± 0.300	2.300 ± 0.1 15	2.300 ± 0. 141
16:1 9c	1.550 ± 0.191	1.675 ± 0.050	1.700 ± 0.082
16:1 7c	6.925 ± 0.974	7.125 ± 0.287	7.675 ± 0.189
16 :1 7t	0.375 ± 0.050	0.375 ± 0.096	0.300 ± 0.082
16: 1 5c	11.200 ± 12.33	4.500 ± 0.082	4. 150 ± 0. 129
16:0	6.450 ± 4.102	9.400 ± 0.337	9.150 ± 0.443
br16:0a	0.500 ± 0.141	0.750 ± 0.129	0.900 ± 0.356
soi17:1	3.150 ± 0.311	3.125 ± 0.299	3.475 ± 0.222
10Me16:0	5.025 ± 0.670	6.375 ± 0.386	6.325 ± 0.275
br16:0b	0.850 ± 0.173	0.975 ± 0.050	1.000 ± 0.000
br16:0c	0.175 ± 0.050	0.275 ± 0.096	0.200 ± 0.000
soi17:0	1.650 ± 0.238	1.800 ± 0.000	1.800 ± 0.141
a17:0	1.800 ± 0.216	2.075 ± 0.096	2.100 ± 0.141
17:1 6c	0.425 ± 0.126	0.475 ± 0.171	0.520 ± 0.150
cy17:0	2.325 ± 0.386	2.275 ± 0.150	2.300 ± 0.200
17:0	0.375 ± 0.050	0.425 ± 0.050	0.400 ± 0.000
br17:0	1.050 ± 0.208	1.300 ± 0.115	1.375 ± 0.171
18:3 6	0.800 ± 0.082	0.750 ± 0.100	0.775 ± 0.096
18:02	0.225 ± 0.096	0.275 ± 0.050	0.275 ± 0.050
18:2 9	2.950 ± 1.348	2.550 ± 0.661	2.000 ± 0.589
18:3 3/soil	0.800 ± 0.183	0.650 ± 0.058	0.750 ± 0.191
18:1 9c	6.375 ± 0.793	6.675 ± 0.479	6.725 ± 0.222
18:1 7c	8.100 ± 1.117	9.475 ± 0.411	9.050 ± 0.742
18:1 7t	0.250 ± 0.058	0.225 ± 0.050	0.275 ± 0.050
18:1w5c	1.250 ± 0.265	1.350 ± 0.058	1.325 ± 0.050
18:0	1.675 ± 0.320	1.950 ± 0.129	1.550 ± 0.507
br19:1a	0.775 ± 0.126	0.775 ± 0.050	0.675 ± 0.263
br19:1b	0.375 ± 0.150	0.300 ± 0.000	0.625 ± 0.650
10Me18:0	1.800 ± 0.082	1.925 ± 0.320	1.400 ± 0.678
11Me18:0	0.425 ± 0.050	0.425 ± 0.050	0.400 ± 0.141
19:1 6	0.350 ± 0.100	0.475 ± 0.050	0.400 ± 0.000
cy19:0	3.500 ± 2.045	4.725 ± 0.486	4.650 ± 0.705
20:4 6	0.900 ± 0.141	1.050 ± 0.208	0.925 ± 0.096
20:5 3	0.550 ± 0.100	0.750 ± 0.436	0.450 ± 0.058
20:2 3/3	0.175 ± 0.050	0.175 ± 0.050	0.125 ± 0.050
20:1 9c	0.475 ± 0.050	0.475 ± 0.096	0.450 ± 0.058
20:0	0.850 ± 0.191	0.650 ± 0.058	0.550 ± 0.100
21:0	0.425 ± 0.050	0.375 ± 0.096	0.375 ± 0.096
22:0	0.325 ± 0.050	0.275 ± 0.050	0.225 ± 0.050

Table 4. continued

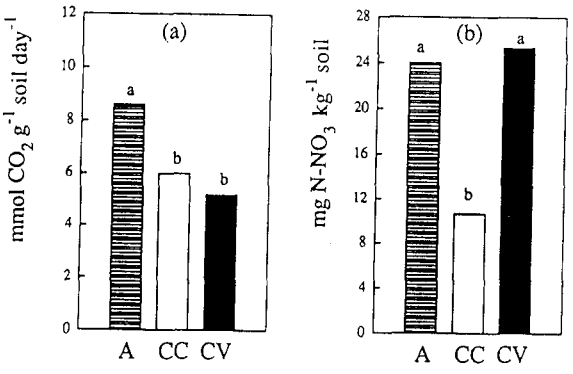

Fig. 1. Soil CO_2 evolution rates (**a**) in two week aerobic incubations and available nitrate (**b**) 4 in fresh soil from the A= organic manure amended, CC= organic cover cropped, and CV = the 5 conventionally managed rotations.

only 5, and 6.3% of the total mole % PLFA in the Animal, Cover Crop and Conventional extracts respectively. Short chain FAMEs (<14) were not identified; low recovery rates may have been due to their volatilization during rotatary evaporation (Zelles and Bai, 1993). Zelles and Bai (1993) recovered more than twice the number of fatty acids than were recovered in our study. The greater number of individual FAMEs recovered in their study, compared to ours, may reflect greater diversity in the microbial populations sampled and/or their methods, which included fractionation, derivatization, and detection methods selected specifically to retain and detect as many fatty acids as possible.

FAME profiles of treatment replicates were heterogeneous: sample standard deviations were 20% of the mean (n = four plots). Over half of the lipid mole fraction was assigned to eight lipid structures (identified as i15:0, a15:0, 16:1ω7c, 16:1ω5c, 16:0, 10Me16:0, 18:1ω9c, 18:1ω7c). Several of these PLFAs are common eubacterial signatures. PLFAs i15:0), 10 Me16:0, and 18:1ω9c are indicators of gram positive (O'Leary and Wilkinson, 1988), and 18:17c of gram negative bacteria (Wilkinson, 1988). The identification of

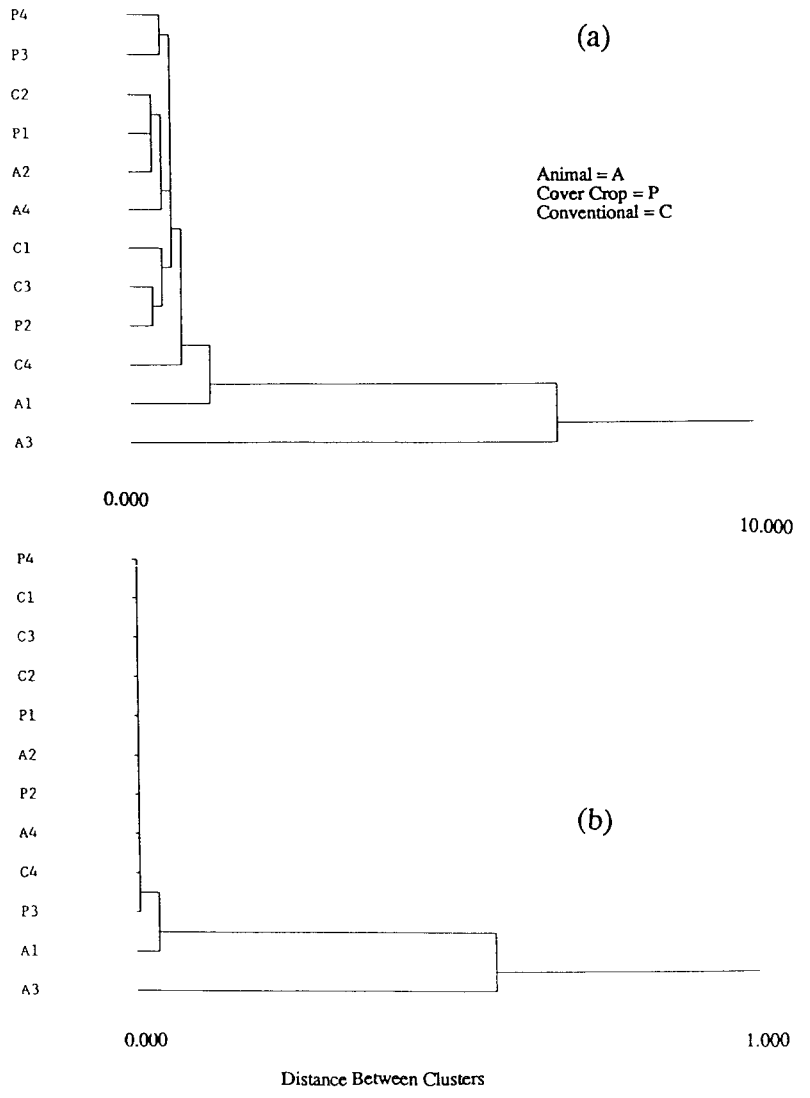

Fig. 2. Cluster analysis of mole percent FAME: (**a**) sample clustering using Euclidean distance metrics and the complete linkage method, and (**b**) sample clustering using the 1-Pearsons's r correlation coefficient metric and single linkage method.

16:0 lipid suggested the presence of sulfate reducing bacteria (Dowling et al., 1986); based on treatment means and their standard deviations, there was less 10Me16:0 in the Animal than in the Cover Crop and Conventional treatment soil extracts. The presence of some actinomycetes is indicated by methyl branched (10Me18:0) FAME (Kroppenstedt, 1985). Polyenoic PLFAs with chain length greater than 20 suggest that microeukaryotes were part of the soil biomass (Smith et al., 1986). PLFAs 18:2ω6 and 18:3ω6 are biomarkers of fungi, which typically synthesize saturated even-chain length and polyenoic acids (Federle, 1986). The 18:2ω9 PLFAS may have been 18:2ω6 (it is difficult to separate these without 6C/MS verification), indicating that fungi and protozoa are of some importance in these soils.

Figure 2 includes examples of cluster analysis showing the greatest (Fig. 1a) and least (Fig. 1b) extents of sample clustering. These results and analysis of variance of mole percentages indicated that there were no statistically significant differences in microbial community structures that could be linked to management at the time soils were sampled. This result does not mean that management induced differences in microbial communities did not exist, just that differences between replicate plots of the same management

Table 5. Summary of the quantity and distribution of PLFAs. Individual PLFAs listed in Table 4 were ranked by assigning the treatment with the lowest PLFA contents a 0, with intermediate contents a 1, and with the highest mol percent of individual PLFAs a 2. The total ranking is the sum of ranks 1 and 2 mutiplied by rank score

Farming System	Lowest (0)	Intermediate (1)	Highest (2)	Total
	———sum of PLFA ranking———			
Animal	24	14	10	34
Cover Cop	7	27	14	55
Conventional	15	17	13	44

system were as important as differences among the treatments themselves.

The total biomass (nmol PLFA g^{-1} soil), expressed as the average of four sample replicates, also did not vary significantly among the treatment soils: Animal (29.17 ± 6.46, Cover crop: 33.00 ± 10.98, and Conventional 26.60 ± 7.99 nmol PLFA). Assuming a conversion factor of 5.9×10^4 cells per picomol PLFA (based on *E. coli.*, see Findlay et al. (1989), the Animal, Cover Crop and Conventional soils had 1.72×10^9, 1.95 × 109, and 1.57×10^9 cells g^{-1} soil. When the number of microbial cells per g soil was normalized by the soil C content of the sample (Table 2), the numbers of microbial cells per g soil C were: 7.51×10^8, 7.8×10^8, and 7.3×10^8, respectively. This suggests that the proportion of C allocated to biomass may be slightly larger on average in the Cover Crop treatment. Using the straight chain fatty acid 16:0 to estimate biomass, after Baird and White (1985), the data suggests that the biomass size is 16% greater in the Cover crop and 11% greater in the Conventionally managed soils than in the Animal soil. This conflicts with the measured soil respiration rates, which were significantly greater in the Animal soils than in the other two treatment soils (Fig. 2a), suggesting that the viable biomass was larger and/or more active in the manure amended soil than in the other two soils.

The uniqueness of the microbial ecology of the manured amended soil and relative similarity between the biomass characteristics of the Cover Crop and Conventional soils is supported by the distribution of FAMEs among several of the most abundant structures. The proportion of 16:1ω5c and 18:23ω9 are greater in the Animal soil than the other two soils; while, its' contents of 16:1ω7c, 10 Mel6:0, al7:0, 18:17c and cy19:0 are less than the Cover Crop and Conventional soils. Overall, the manured soil had relatively large amounts of PLFAs occurring in fewer structures than either of the cover cropped and conventionally managed soils (Table 5). Twenty-seven of the 72 FAMES were found in highest concentration in the Cover Crop soil, far more frequently than found in the other two soils. This result may indicate diverse that the biomass in the cover cropped soil was more diverse than the biomass in the other soils.

Several other measures suggested that the microbial ecology of the cover cropped soil differed from the other two soils. Findings from a companion study investigating the effects management on the fate of newly added organic compounds suggested the Cover Crop soil retained significantly more ^{13}C from applied ^{13}C-labeled acetate than the Animal and Conventional soils (Wander et al., 1995). Could the greater retention of ^{13}C in the Cover Crop soil have been linked to N limitation? The available soil NO_3^- contents of the Cover Crop soil were significantly lower (by one-half) than the $N0_3^-$ contents of the other two treatment soils (Fig. 2b). In addition, solid state CPMAS ^{13}C-NMR spectra of whole soils showed that the cover cropped soils had not only retained more ^{13}C, but had also relocalized more ^{13}C into new C structures. This occurred despite indications that the Cover Crop soil had a larger, more diverse biomass, as suggested by the PLFA data. It is possible that much of the biomass was metabolically active. During the first two weeks of the incubation, the proportional activity of the microbial cells (based upon soil $C0_2$ evolution rates divided by the number of viable cells per g soil) was 1.6:1.0:1.1 for the Animal: Cover Crop: Conventional soils. This is consistent with the fate of acetate derived-^{13}C, with more ^{13}C being respired from more metabolically active soils.

Conclusions

Direct PLFA based characterization of the FST soils' microbial biomass size and composition suggested that management had not affected their microbial ecology in a way that was readily detected by PLFA characterization. In fact, PLFA profiles suggest that the viable microbial communities were generally very similar in the three farming systems; even though, $C0_2$ evolution rates, $N0_3^-$ contents, and ^{13}C- fate varied significantly among treatment soils collected in November 1991. These findings do not necessarily contradict those of Zelles et al. (1992), who showed that FAME profiles could distinguish between soils under different management practices. Their study compared PLFA profiles of composite samples collected from eight cropping systems and had analytical replication, but no field replication. Our results from the Rodale FST site are, to our knowledge, the first results comparing PLFA profiles of management treatment replicates. We found treatment based differences could be associated with the relative quantities of selected lipid structures; however, differences between replicate samples was notable. The general similarity between our PLFA profiles was probably associated with the fact that our samples were collected in November, when microorganisms are fairly dormant, from plots in all treatments that had been in corn. Other workers have found that the time of sample collection influenced their ability to document treatment based differences in biomass and biomass dependent soil properties (Friedman, 1993; Harris et al., 1994). We submit that climate and geographic conditions occurring within small areas (in our case 6.1 ha) determine the general character of microbial populations within fields, while fine scale spatial (meters to microns) and temporal (days to weeks) factors generate localized microbial heterogeneity. Accordingly, management practices like tillage and crop rotation, that increase local resource heterogeneity, should increase microbial diversity. This assertion is consistent with the findings of Zelles and Bai (1993) and Kennedy and Petersen (1993).

Both soil respiration and ^{13}C fate were determined subsequent to soil disturbance, while the biomass characteristics reflected by PLFAs represents microbial ecology prior to disturbance. Disturbance and acetate amendment appear to have elicited different responses from the biomass in the FST soils. The soil biomass can be extremely dynamic. Bott and Kaplan (1985) have observed bacterial turnover rates as high as 375 generations per year in some ecosystems. Accordingly, sampling of the PLFA profiles after the acetate had been added may have provided very different results, which may have been linked to management. However, those results would not reflect the relationship between in situ microbial ecology and processes occurring in natural soils. The microbial ecology and related soil function (mineralization and compound fate) depended upon soil biotic competence and nutritional constraints present in natural soils. Research must determine how and when to characterize biologically active and inactive microorganisms in order to sort out the relationships between microbial heterogeneity, metabolic competence and soil processes. PLFA characterization of soil microbial communities will provide a powerful means to characterize soil microorganisms once researchers identify when, where and how to sample functionally important biomass pools.

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