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Soil Biology & Biochemistry 35 (2003) 1693–1703

Soil Biology & Biochemistry

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Soil microbial communities under conventional-till and no-till continuous cotton systems

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Received 30 July 2002; received in revised form 22 July 2003; accepted 13 August 2003

Abstract

Soil management practices affect soil microbial communities, which in turn influence soil ecosystem processes. In this study, the effects of conventional- (fall disking, chiseling and spring disking, field cultivation) and no-tillage practices on soil microbial communities were examined under long-term continuous cotton (*Gossypium hirsutum* L.) systems on a Decatur silt loam soil. Soil samples were taken in February, May, and October of 2000 at depths of 0–3, 3–6, 6–12, and 12–24 cm. Compared to the conventional-till treatment, the no-till treatment increased soil organic carbon and total nitrogen contents in the surface layer by 130 and 70%, respectively. Microbial biomass C content under no-till treatment was 60, 140, and 75% greater than under conventional-till treatment in February, May, and October, respectively. Principal components analysis of phospholipid ester-linked fatty acid (PLFA) profile indicated soil microbial communities shifted over time and with soil depth. This change appeared to be driven primarily by soil bacterial populations as indicated by the major PLFA contributors (i.e. fatty acids 16:0, 10Me16:0, cy19:0, 16:1 2OH, and *i*15:0) to the first two principal components. Tillage treatment differences were revealed by analysis of variance on the first principal components (PC 1), which accounted for 62% of the total sample variance, and by the relative abundance of selected PLFAs and PLFA ratios. The impact of tillage practices was significant in February and May, but not in October. During the growing season, changes in the microbial community may be primarily determined by soil conditions responding to cotton growth and environmental variables such as moisture and temperature; during fallow or prior to cotton establishment, community changes associated with tillage practices become more pronounced. These findings have implications for understanding how conservation tillage practices improve soil quality and sustainability in a cotton cropping system.

Keywords: Microbial community; Phospholipid ester-linked fatty acid; No tillage; Conventional tillage; Cotton

1. Introduction

Soil management practices affect soil microbial communities, which mediate many processes essential to the productivity and sustainability of soil. Until very recently, conventional tillage has been the predominant method of land preparation in the southeastern US where continuous cotton has been grown for decades on soils with low inherent fertility, susceptible to aggregate disruption, crusting formation, and erosion (Miller and Radcliffe, 1992; Reeves, 1994). The primary reasons for tillage were to: (1) provide a smooth and suitable seedbed given the type of seeding equipment available; (2) (temporarily, at least) reduce soil compaction to promote crop rooting; (3) incorporate fertilizers and lime (which is not necessary in most situations); (4) control weeds before the advent of herbicides, and later to incorporate some residual soil herbicides; (5) bury crop residues for certain types of plant disease control; (6) warm the soil in humid and cooler regions; and finally; (7) follow tradition (Gebhardt et al., 1985).

The improvement of seeding equipment and availability of pesticides and alternative weed/pest control strategies have reduced the need for conventional tillage practices. Although it temporarily reduces soil compaction, conventional tillage promotes a vicious cycle of soil loosening and reconsolidation that results in denser soils with the loss of

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0038-0717/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2003.08.016

organic matter and repeated traffic. Soils in humid thermic regimes are more sensitive to degradation from repeated tillage due to erosion and loss of soil organic matter. Thus, they are better candidates for adoption of no-tillage systems. It is well-known that no-till practices increase soil organic matter content in the surface layer, improve soil aggregation, and preserve the soil resources better than conventional till practices. Increased soil organic matter content associated with no-till practices not only improves soil structure and water retention, but also serves as a nutrient reservoir for plant growth and a substrate for soil microorganisms.

Different tillage systems have been reported to impact soil microbial and biochemical properties (Doran, 1980). Although much information exists regarding effects of tillage practices on gross microbial response (e.g. microbial biomass and respiration) and numbers of total or culturable microorganisms, characterization of the soil microbial diversity using culture-independent and community-level approaches did not appear until recently. These new approaches, such as nucleic acid-based methods and phospholipid ester-linked fatty acid (PLFA) analysis, are advantageous for 'fingerprinting' the soil microbial community at a finer resolution to monitor changes of community structure resulting from different management practices. To date, there are only four reported studies that examined the tillage effects on soil microbial communities as indicated by PLFA profiles (Bossio et al., 1998; Calderón et al., 2001; Drijber et al., 2000; Ibekwe et al., 2002)

Cotton is the most important row crop in Alabama, USA, contributing 30% of total crop cash receipts annually (Alabama Agricultural Statistics Service, 2002). Lately, more and more cotton farmers have adopted conservation tillage systems. Changes in soil physical and chemical properties associated with different tillage practices have been studied extensively (Edwards et al., 1992; Reeves, 1997). Biological changes brought about by conversion from conventional- to no-tillage practices in the region have not been evaluated. In this study, we used the phospholipid fatty acid profile to characterize microbial communities developed under long-term conventional-till and no-till treatments in a kaolinitic soil cropped to cotton. The objective of the study was to determine the effects of conventional and no-tillage practices on the size and composition of the soil microbial community under continuous cotton systems over time and at various soil depths.

2. Materials and methods

2.1. Field experiment and soil sampling

The long-term cotton tillage and rotation experiment is located on a Decatur silt loam (fine, kaolinitic, thermic Rhodic Paleudults) at the Tennessee Valley Research and Extension Center, Belle Mina, Alabama, USA. The experiment is a randomized complete block design with four blocks and nine treatments. The soil was sampled in 2000 from two winter fallow continuous cotton treatments subjected to conventional tillage and no-tillage. Conventional-till plots were established in 1979 and no-till plots in 1988 from previously conventionally tilled plots. Conventional tillage involved disking and chisel plowing in the fall and disking and field cultivation in the spring prior to planting. No-till cotton was planted into the cotton stubble of the previous year. Fertilizers, insecticides, herbicides, and defoliants were applied according to Auburn University recommendations (Adams et al., 1994). On average, cotton yields for the no-till treatment were 4% higher than for the conventional-till treatment from 1988 to 2000. No-till cotton yield was 1.7% higher than conventional-till cotton in 2000.

The soil was sampled on February 10, May 4 (13 days after cotton planting), and October 2 (12 days after cotton harvest) of 2000. Ten 3.9 cm diameter soil cores (0–24 cm depth) were collected randomly from 93 m^2 (50′ × 20′, 15.2 m × 6.1 m) individual plots. Soil cores were divided into four depths (0–3, 3–6, 6–12, and 12–24 cm), composited by depth, and passed through a 4–mm sieve. After thorough mixing, subsamples were taken for water content, microbial biomass determination, and extraction of lipids. Field-moist soil samples were stored at 4 °C for no more than 1 week before microbial biomass determination and no more than 3 weeks before lipid extraction. The concentrations of fatty acids generally do not change significantly over a 7-week storage period at 4.5 °C (Petersen and Klug, 1994). Samples for soil bulk density (D_b) determination were collected in April of 2000.

2.2. Laboratory analysis

2.2.1. Soil analysis

Soil samples collected in February were air-dried, passed through a 2-mm sieve, and used for soil chemical analyses. Total carbon and nitrogen contents were determined by dry combustion using a C/N analyzer (Fisons Instruments, Beverly, MA). Since there is no appreciable carbonate C in this inherently acid soil, the total carbon content is equivalent to the soil organic carbon (SOC) content. The pH was measured in a 1:1 soil/water suspension (Page et al., 1982). Microbial biomass C was determined using the fumigation–incubation method with a 5-day pre-incubation period (Horwath and Paul, 1994). Biomass carbon was calculated using a conversion factor of 0.41 without the subtraction of a control (Voroney and Paul, 1984; Franzluebbers et al., 1999a).

2.2.2. Phospholipid fatty acid (PLFA) analysis

Duplicate field-moist soil samples were used for PLFA analysis according to a procedure modified after Findlay and Dobbs (1993), and Bossio et al. (1998). Briefly, 4 g (dry weight) soil was extracted using 19 ml of a single-phase

mixture (1:2:0.8 v/v/v) containing chloroform, methanol and citrate buffer (0.15 M, pH 4) for 2 h on a rotator in the dark. After centrifugation for 10 min at 2500 rev min^{-1}, the supernatant was decanted to a fresh tube. The soil was then vortexed with an additional 8 ml of extractant and the supernatant was combined with the first extraction. More buffer (5.7 ml) and chloroform (7.1 ml) were added to the extraction mixture to break phase and the phases allowed to separate overnight. The chloroform layer was then transferred to a fresh tube and dried under nitrogen at 37 °C. Phospholipids were separated from neutral and glycolipids using packed activated silicic acid (0.5 g, Unisil 100–200 mesh) columns. The column was conditioned with 3-ml chloroform; lipids were transferred to the column with 4 × 150 μl of chloroform. Neutral lipids, glycolipids, and phospholipids were eluted with 5 ml chloroform, 10 ml acetone, and 5 ml methanol, respectively. Methanol eluates were collected and dried under nitrogen at 37 °C. The phospholipids were then subjected to a mild alkaline methanolysis. Samples were dissolved in 1 ml 1:1 methanol:toluene and 1 ml 0.2 M NaOH in methanol, heated at 37 °C for 15 min, and cooled to room temperature. After addition of 2 ml of deionized water and 0.3 ml of 1 M acetic acid, fatty acid methyl esters (FAMEs) were extracted twice with 2 ml of hexane by vortexing for 30 s. Hexane fractions were combined and dried under nitrogen at 37 °C. Prior to GC analysis, samples were dissolved in appropriate amounts of hexane containing 19:0 methyl ester as an internal standard.

2.2.3. GC analysis

FAMEs were analyzed using a Hewlett Packard 5890 gas chromatograph with a 25-m HP Ultra 2 capillary column and a flame ionization detector. The column temperature was 170 °C initially, increased to 270 °C at 5 °C min^{-1}, and finally ramped to 315 °C at 2 °C min^{-1}. The injector and detector temperatures were held at 250° and 300 °C, respectively. Fatty acid peaks were identified using the MIDI peak identification software (MIDI, Inc., Newark, DE) and bacterial FAME standards (Matreya, Inc., Pleasant Gap, PA). Identification of the FAMEs was confirmed by gas chromatography mass spectrometry using a Varian Saturn 4 Ion Trap GCMS system.

2.3. Nomenclature of fatty acids

Fatty acids are designated according to the convention X:YωZ, where 'X' indicates the total number of carbon atoms in the molecule (except for molecules with a mid-chain branch), 'Y' indicates the number of double bonds, 'Z' indicates the position of the 1st double bond or cyclopropane ring, and ω indicates the position counted from the methyl end of the molecule. The prefix '*i*' indicates *iso* branching, '*a*' indicates *anteiso* branching, '10Me' refers to methyl branching on the 10th carbon from the carboxyl end, and 'cy' stands for cyclopropane ring. Suffixes '*c*' and '*t*' indicate the *cis* and *trans* configuration, respectively. The number before an OH refers to the location of a hydroxy group relative to the carboxyl end of the molecule. When branching occurs in the middle of the carbon chain, the branching C is not added to 'X' (Gunstone and Herslof, 1992).

2.4. Statistical analysis

The mole percentage distribution of PLFAs was analyzed with SAS software using principal components analysis (PCA). Analysis of variance on the first principal component (PC 1) was conducted to assess the effects of tillage, sampling time, and soil depth. PCA was performed on combined PLFA data of all three sampling dates as well as PLFA data of each sampling date. All samples were analyzed for PLFA profiles using a set of 46 fatty acids that were present in most of the samples.

3. Results

3.1. Soil physical and chemical properties

Tillage treatments greatly affected soil physical and chemical properties (Table 1). Soil organic C content was more than twice as high in the surface layer of the no-till treatment compared to the conventional-till treatment. Differences in SOC between the two tillage treatments were not significant at the three lowest depths. The SOC in no-till plots decreased sharply with increasing soil depth.

Table 1
Selected chemical and physical properties of a silt loam soil from conventional-till and no-till cotton plots

Tillage treatment	Depth (cm)			
	0–3	3–6	6–12	12–24
Soil organic carbon (mg g^{-1})				
Conventional	8.3	9.3	6.4	5.4
No-till	18.8	10.0	6.5	6.1
$LSD_{(0.05)}$	0.9	0.9	0.9	0.9
Total nitrogen (mg g^{-1})				
Conventional	1.0	1.0	0.9	0.8
No-till	1.7	1.0	0.9	0.8
$LSD_{(0.05)}$	0.1	0.1	0.1	0.1
Bulk density (g cm^{-3})				
Conventional	1.27	1.38	1.46	1.59
No-till	1.22	1.55	1.58	1.56
$LSD_{(0.05)}$	0.03	0.03	0.03	0.03
pH				
Conventional	6.5	6.4	6.7	6.7
No-till	6.7	6.7	6.6	6.4
$LSD_{(0.05)}$	0.2	0.2	0.2	0.2

Soil samples were collected in April 2000 for bulk density determination and in February 2000 for other analyses.

There was a significant increase in organic carbon at the 3–6 cm sampling depth compared to the surface layer (9.3 vs. 8.3 mg g^{-1}) for conventional-till plots; thereafter, SOC declined linearly with depth. The decline in organic carbon for no-till plots was best described with a polynomial response function in log-linear space (% SOC = 2.4–2.9 log(depth) + 2.4 [log(depth)]^2; $R^2 = 0.95$). Total soil N was 70% higher in no-till compared to conventional-till plots (1.7 vs. 1.0 mg g^{-1}) at the surface layer (Table 1). While there was no change in soil N between the 0–3 and 3–6 cm depths for conventional-till plots, total N for no-till plots declined sharply at the 3–6 cm sampling depth and stabilized at approximately 0.9 mg g^{-1}. Additionally, no-till plots had significant lower bulk density at the surface layer compared to the conventional-till plots (Table 1). In the latter, bulk density increased linearly with soil depth ($D_b = 0.02$ depth + 1.27, $R^2 = 0.96$) whereas the bulk density in the former increased from 1.22 in the surface layer to 1.55 g cm^{-3} in the second sampling depth and did not change any further. There was little change in soil pH for either conventional-till or no-till treatments (Table 1).

3.2. Microbial biomass C and total PLFAs

Microbial biomass C ranged from 63 to 266 μg g^{-1} in conventional-till soils and 73–633 μg g^{-1} in no-till soils for all sampling depths and months (Fig. 1). The percentages of SOC as biomass C ranged from 1.17 to 3.21% for conventional-till treatments and 1.20–3.37% for no-till treatments, and the values decreased as soil depth increased. The total amounts of PLFAs ranged from 13 to 47 nmol g^{-1} in conventional-till soils and 12–147 nmol g^{-1} in no-till soils. The ratios of total PLFAs (in nmol) to microbial biomass C (in mg) averaged 189 in conventional-till soils and 181 in no-till soils. No-till soils contained significantly higher amounts of microbial biomass C and total PLFAs than conventional-till soils at the surface layer for all sampling months (Fig. 1). Both microbial biomass C and total PLFAs decreased with increasing soil depths. The changes for conventional-till plots were fitted through a second order polynomial response curve, whereas the changes were modeled on log (depth) for no-till plots (Table 2). The largest changes occurred between the 0–3 and 3–6 cm depths, irrespective of the sampling month. Differences in both microbial biomass C and total PLFAs between the two upper depths were most pronounced for the no-till treatment sampled in May; these changes were at least twice as large as for other months. The linear correlation coefficient between microbial biomass C and total PLFAs was 0.73 ($n = 96$).

3.3. Soil microbial community structure as indicated by PLFA profiles

PLFA analysis identified a total of 66 different fatty acids. Forty-six of them were consistently present in the samples and were used for data analysis. These 46 fatty acids ranging in carbon chain length from C12 to C20 consisted of saturated, mono- and polyunsaturated, methyl-branched, hydroxylated, and cyclopropane fatty acids. PLFA mole percents were analyzed using PCA and the first two principal components (PCs) accounted

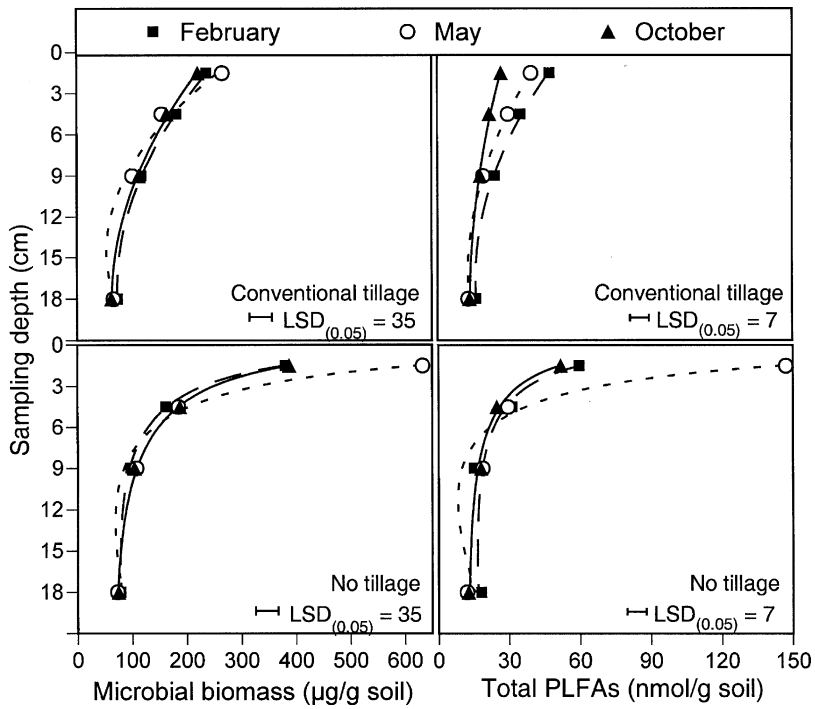

Fig. 1. Microbial biomass C and total PLFAs for conventional-till and no-till treatments at four soil depths sampled in February, May, and October 2000.

Table 2
Coefficients (SE: standard error) from the regression of microbial biomass and total PLFAs on depth from soils of conventional-till and no-till plots

	Conventional tillage				No-tillage			
	Intercept (SE)	Depth (SE)	Depth^2 (SE)	R^2	Intercept (SE)	Log (depth) (SE)	(Log(depth))^2 (SE)	R^2
Microbial biomass								
February	53.6 (1.34)	−4.5 (0.37)	0.14 (0.018)	0.95	77 (7.8)	−103 (25.8)	44 (17.9)	0.94
May	45.4 (0.07)	−4.0 (0.02)	0.12 (0.001)	0.99	206 (20)	−375 (66.2)	178 (45.7)	0.96
October	29.1 (0.99)	−1.6 (0.27)	0.04 (0.013)	0.96	65 (3.0)	−79 (9.9)	31 (6.8)	0.98
Total PLFAs								
February	269.9 (1.73)	−22.8 (0.47)	0.66 (0.023)	0.99	493 (4.4)	−696 (14.5)	291 (10.1)	0.95
May	306.6 (31.6)	−34.8 (8.62)	1.20 (0.417)	0.92	859 (51.7)	−1420 (172)	678 (119)	0.97
October	248.5 (6.63)	−20.3 (1.81)	0.56 (0.087)	0.98	490 (9.1)	−617 (29.9)	227 (20.7)	0.99

Data from conventional-till plots were regressed on depth, whereas no-till data were fitted on log(depth).

for 62% and 9% of the total sample variance. The PCA plot of the first two PCs (Fig. 2) showed that PLFA profiles varied primarily by depth and by sampling month, but not by tillage treatment. Principal component 1 separated PLFA profiles by depth with 11 of the 12 data points for 0–3 and 3–6 cm depths having negative scores and 11 of the 12 data points for 6–12 and 12–24 cm depths having positive scores. The PCA plot also showed that October data formed a cluster and had negative scores for PC 2, whereas data points for February and May were intermixed and 13 of the 16 data points had positive scores for PC 2 (Fig. 2). This

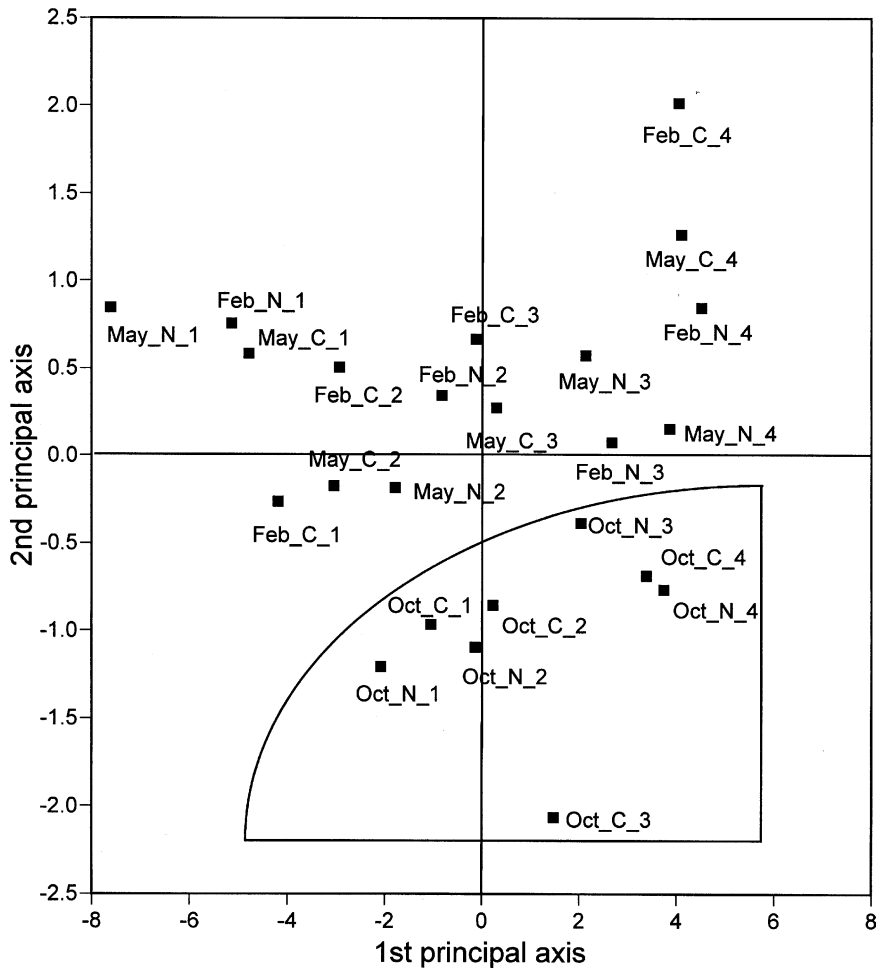

Fig. 2. Plot of the first two principal axes from the principal components analysis of PLFA profiles for conventional-till and no-till treatments at four soil depths sampled in February, May, and October 2000. Labels for data points stand for Month_Tillage treatment_Depth (C: conventional tillage; N: no-tillage; 1: 0–3 cm; 2: 3–6 cm; 3: 6–12 cm; 4: 12–24 cm).

suggests that microbial community composition was similar at the two earlier sampling times.

Since PC 1 explained 62% of the total sample variance, ANOVA on the first PC was performed using a complete $2 \times 4 \times 3$ factorial model with tillage, depth, and month as sources of variation. There was a significant tillage × depth interaction ($P < 0.01$); the responses of PC 1 to depth were different for conventional-till and no-till systems (Fig. 3a). The response was linear for conventional till system (PC $1 = -3.82 + 0.49$ depth, $R^2 = 0.99$) but log linear for no-till system (PC $1 = -6.37 + 8.54$ log (depth), $R^2 = 0.99$). The depth effect was less pronounced with conventional tillage than no-tillage as indicated by the slopes of the regression lines. The ANOVA also revealed a significant month × depth interaction ($P < 0.01$). Fig. 3b indicates that microbial communities in the late season (October) samples differed from those in two early season samples, which is also shown in Fig. 2. There was no significant tillage × depth × month interaction.

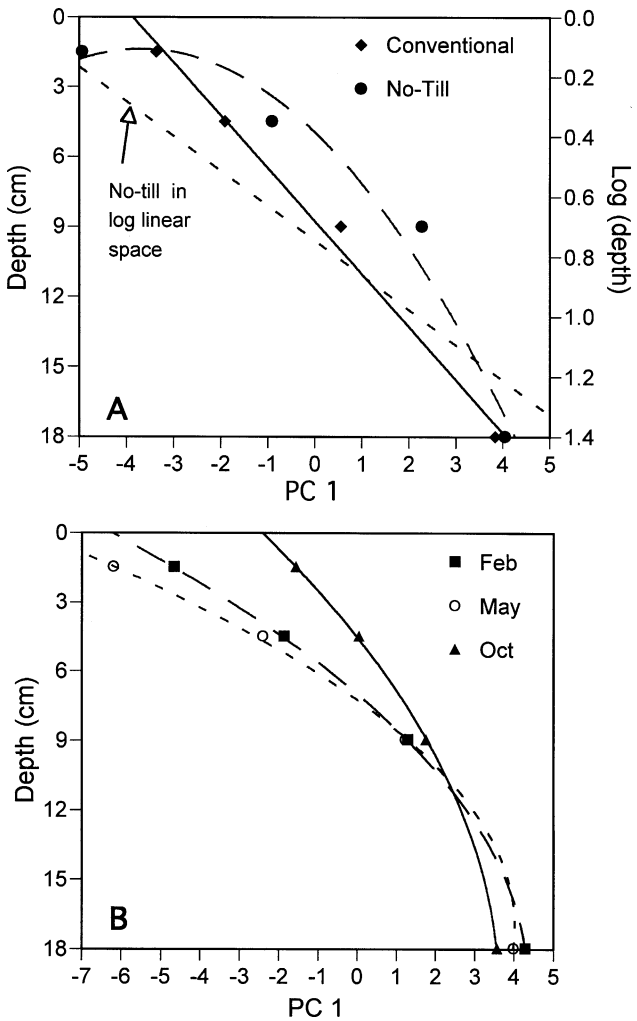

Fig. 3. Responses of the first principal component (PC 1) to increasing soil depth for tillage treatments (A) and sampling months (B) from a continuous cotton field. The principal components analysis was based on all three sampling dates. The regression equations for tillage treatment are: PC $1 = 0.436$ depth $- 3.823$ ($R^2 = 0.991$) for conventional tillage; PC $1 = -0.048$ depth$^2 + 1.474$ depth $- 6.89$ ($R^2 = 0.996$) for no-tillage (quadratic); and PC $1 = 8.539$ depth $- 6.37$ ($R^2 = 0.992$) for no-tillage (log-linear). The regression equations for sampling months are: PC $1 = -0.0279$ depth$^2 + 1.085$ depth $- 6.218$ ($R^2 = 1$) for February; PC $1 = -0.0423$ depth$^2 + 1.436$ depth $- 8.178$ ($R^2 = 0.999$) for May; PC $1 = -0.015$ depth$^2 + 0.6$ depth $- 2.41$ ($R^2 = 0.998$) for October.

PCA also identified fatty acids that were important in explaining the variability in PLFA profiles. The PLFAs 16:0, 10Me16:0, *cy*19:0, 18:1ω9*c*, and 18:1ω7*c* were influential fatty acids to PC 1 whereas 16:1 2OH, *i*15:0, and 18:1ω9*c* were the major contributors to PC 2 (Table 3). Fig. 4 shows the depth distributions of selected influential PLFAs in mole percent averaged across sampling months. The PLFAs 16:0 and 16:1 2OH have not been associated with any particular groups of microorganisms. The fatty acid 16:0 is present in almost all microorganisms (Ratledge and Wilkinson, 1988). It constituted the largest mole fraction of all fatty acids in the samples and correlated well with total PLFAs and microbial biomass C. 10Me16:0 and *i*15:0 are Gram-positive bacterial biomarkers and their mole percentages increased as soil depths increased in both conventional-till and no-till soils (10Me16:0 in Fig. 4a; *i*15:0 not shown). 10Me16:0 may also occur in sulfate-reducing bacteria (Findlay and Dobbs, 1993). The PLFAs *cy*19:0 and 18:1ω7*c* are signature fatty acids of Gram-negative bacteria; 18:1ω7*c* is the precursor molecule of *cy*19:0. The ratio of *cy*19:0/18:1ω7*c* increased with soil depths and was 17.8 and 23.6% higher in no-till than conventional-till soils at the two middle depths (Fig. 4b). The relative abundance of PLFA 18:1ω9*c* decreased with increasing soil depth under both tillage treatments and was lower in no-till plots (Fig. 4c).

The mean ratio of fungal to bacterial PLFAs (the sum of mole percent for 18:2ω6,9*c*, 18:1ω9*c*, 18:3ω6*c*, and 20:1ω9*c* to that for *i*15:0, *i*16:0, 10Me16:0, *a*15:0, *cy*17:0, 18:1ω7*c*, *cy*19:0, 14:0, 15:0, 16:1ω9*c*, 16:1ω7*c*, 16:1ω5*c*, *a*17:0, *i*17:0, 17:0, and 18:0) decreased with increasing soil depth. The fungal to bacterial PLFA ratio was similar at

Table 3
PLFAs receiving scores $> | \pm 0.20|$ on the first two principle components

Fatty acid	Score	Specificity as a biomarker[a,b]
PC 1		
16:0	−0.60	Nonspecific
10Me16:0	0.57	Gram-positive bacteria, sulfate-reducing bacteria
cy19:0	0.31	Anaerobes, Gram-negative bacteria
18:1ω9*c*	−0.21	Fungi, aerobic bacteria
18:1ω7*c*	−0.20	Aerobic bacteria, Gram-negative bacteria
PC 2		
16:1 2OH	−0.87	Nonspecific
*i*15:0	0.32	Gram-positive bacteria
18:1ω9*c*	−0.21	Fungi, aerobic bacteria

[a] The principal component analysis was carried out using 46 PLFAs. Soil samples were taken at four depths in February, May, and October 2000.
[b] Source: Ratledge and Wilkinson (1988), Findlay and Dobbs (1993) and Paul and Clark (1996).

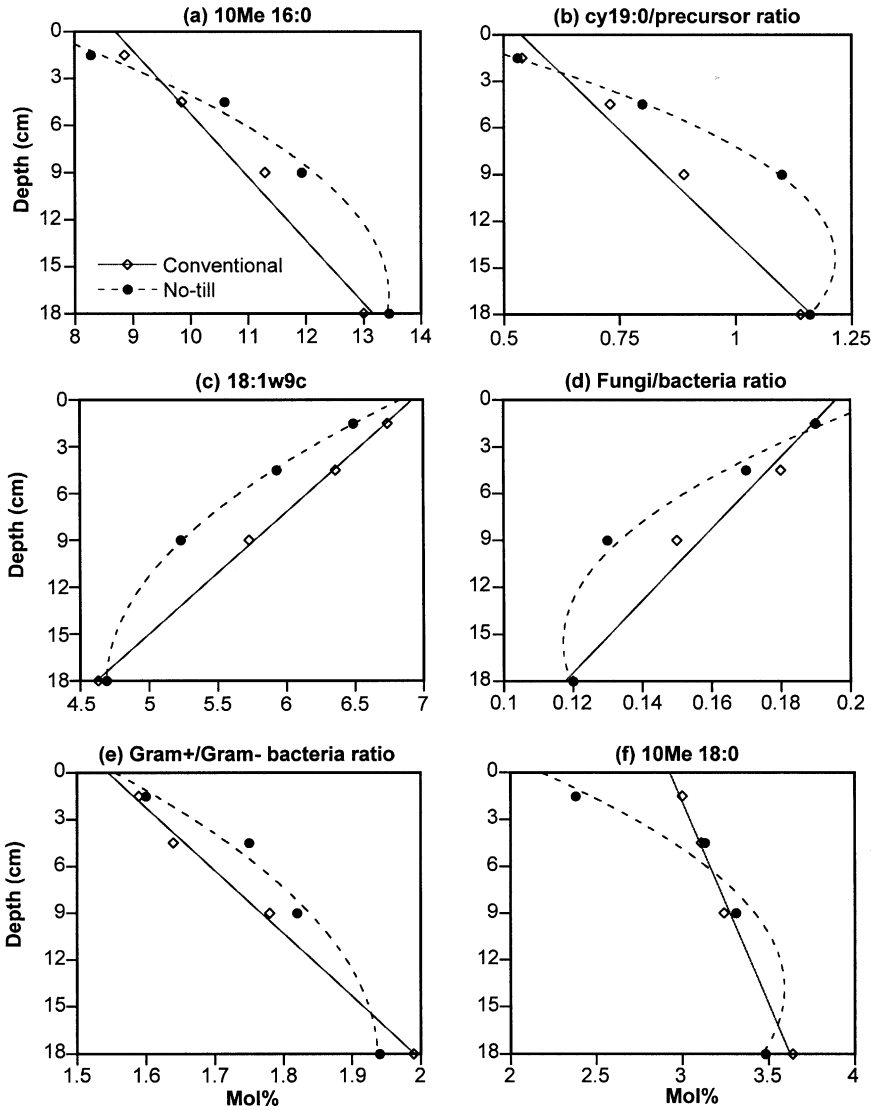

Fig. 4. Depth distributions of several PLFAs and PLFA ratios for conventional-till and no-till treatments. Values given are means averaged across sampling dates.

the surface layer for both tillage treatments and 10% lower in no-till plots than conventional-till plots at the 6–12 cm depth (Fig. 4d). The ratio of Gram-positive to Gram-negative bacterial PLFAs (the sum of mole percent of *i*15:0, *i*16:0, 10Me16:0, *a*15:0 to that for cy17:0, 18:1ω7*c*, *cy*19:0) increased with soil depths (Fig. 4e), suggesting that Gram-positive bacterial population increased with soil depth. The average mole percent of actinomycete PLFA 10Me18:0 increased with soil depth and was 26% higher in conventional-till soil than in no-till soil at the surface layer (Fig. 4f). For the two mid-chain branched fatty acids, the mole percentages of 10Me 16:0 was 3–4 times higher than 10Me 18:0 averaged across sampling months and depths. Fig. 4 also shows that changes in relative amounts of PLFA and PLFA ratios by depth were linear for conventional-till treatment and nonlinear for no-till treatment, indicating dissimilar compositions of the soil microbial community under different tillage treatments.

When principal component analysis was performed for each sampling month, PC 1 accounted for 60, 61, and 56% of the total variance for February, May, and October, respectively. The influential fatty acids identified in PCA of combined data were also major contributors identified in PCA of each month. ANOVA of PC 1 revealed significant tillage × depth interactions ($P < 0.01$) for February and May but not for October samples ($P = 0.93$).

4. Discussion

Conservation tillage systems improve soil chemical, physical, and biological properties of previously tilled soil, especially in the surface layer. In this study, implementation of no-till practices for 12 years in a continuous cotton field has resulted in significantly greater soil carbon and nitrogen, microbial biomass C, and total PLFAs in no-till plots than

conventional-till plots for the 0–3 cm depth. These results support the findings that no-till systems result in the surface enrichment of SOC (Edwards et al., 1992; Wander et al., 1998; Motta et al., 2001; Ding et al., 2002) and microbial biomass C (Granatstein et al., 1987; Franzluebbers et al., 1994; Motta et al., 2001). Microbial biomass responds rapidly to conditions that eventually result in changes of soil organic matter contents and has been used as an sensitive and early indicator of soil quality (Powlson et al., 1987; Sparling, 1997; Franzluebbers et al., 1999b).

Microbial biomass C determined by chloroform-fumigation incubation was well correlated with the total PLFAs that are quantitative measures of the viable biomass (White and Macnaughton, 1997). Our observation is consistent with previous reports of significant correlation between total PLFAs and microbial biomass (Zelles et al., 1995; Yao et al., 2000). Calderón et al. (2001), however, reported the lack of correspondence of the microbial biomass C and total PLFA data in a study of short-term dynamics of soil microbial properties after tillage. They suggested that a close correlation between microbial biomass C and total PLFA should not always be expected. The ratios of total PLFAs to microbial biomass C found in this study fall with the range of 49–340 nmol/mg for agricultural and forest soils (Hill et al., 1993).

4.1. Tillage induced changes

We detected three primary effects on the microbial community structure in this study, i.e. the presence of temporal, vertical, and tillage management-induced changes in the composition of the microbial communities (Figs. 2 and 3). A significant tillage × depth interaction revealed by ANOVA of PC 1, as well as the different depth response curves (Figs. 3 and 4), suggests that microbial community structure shifted as its surrounding physical and chemical environment was altered by tillage system. Our finding is consistent with those reported by Drijber et al. (2000) and Calderón et al. (2000). Soils under no-till treatment have greater crop residues and higher soil organic matter contents in the surface layer, which result in increased infiltration and higher water holding capacity than soils under conventional treatment. Thus, no-till soils are not only high in available substrate, but also wetter, cooler and fluctuate less in moisture and temperature (Doran, 1980). These conditions stimulate the growth and activity of soil microorganisms. Further, there is also evidence indicating that the chemical composition and structure of soil organic matter are different under conventional and conservation tillage system (Ding et al., 2002). Surface soils (0–5 cm) under conservation tillage are high in light fraction material (Ding et al., 2002) as well as particular organic matter (Motta et al., 2001). Humic acid in conservation tilled soil contained more reactive functional groups than in conventionally tilled soil (Ding et al., 2002).

When data were analyzed for each sampling month, ANOVA of PC 1 revealed significant tillage x depth interactions ($P < 0.01$) for February and May but not for October samples ($P = 0.93$). This suggests that the effect of tillage treatments were more pronounced during winter fallow (February) and when influence of cotton root growth was minimal (May). Our result supports the findings of a long-term wheat-fallow management study (Drijber et al., 2000). Drijber et al. (2000) compared plots remaining in mixed prairie sod and those cropped to wheat or left fallow under no-till, sub-till or plow management. Although PLFA profiles differentiated wheat and fallow systems by tillage, discrimination among tillage treatments was expressed most strongly during fallow. They suggested that during the wheat cycle the soil microbial community was influenced mostly by inputs from wheat, while during fallow, it responds to the physicochemical environment resulting from tillage management. Petersen et al. (2002) conducted studies during the growing season of spring wheat (May through September) and showed limited tillage effects on soil microbial communities. It is probably because the shift of the microbial community was regulated mainly by soil environmental conditions responded to wheat growth rather than long-term tillage treatments.

4.2. Temporal variation

Temporal changes in soil microbial communities are likely due, in part, to plant root growth as well as environmental conditions, such as soil moisture and temperature. The PLFA profiles for October was dissimilar to those for February and May. October samples were taken right after cotton harvest. The soil microbial community at this time may be dominated by the organisms responding to cotton growth during the growing season. It is well known that the plant is able to affect rhizosphere microorganisms and stimulate the growth of zymogenous populations. The rhizosphere usually harbors more Gram-negative bacteria and fewer Gram-positive and Gram-variable bacteria (Paul and Clark, 1996). In addition, the drier soil and higher soil temperature at the October sampling time may have also played important roles. Year 2000 precipitation in the area was 60% of the normal level, primarily due to low rainfall in the summer months (Fig. 5). Soil moisture content in October was the lowest of all three sampling months. Moisture content of the conventional-till soil at the surface layer was 41 and 31% lower in the October sample compared to the February and May samples, respectively. For no-till soil at the 0–3 cm depth, soil moisture content of the October sample was 46 and 47% lower than the February and May samples, respectively. Moreover, the daily mean soil temperature at 10 cm depth 30 days prior to sampling averaged 6.8, 16.9, and 27.4 °C for February, May, and October sampling dates, respectively (Fig. 5).

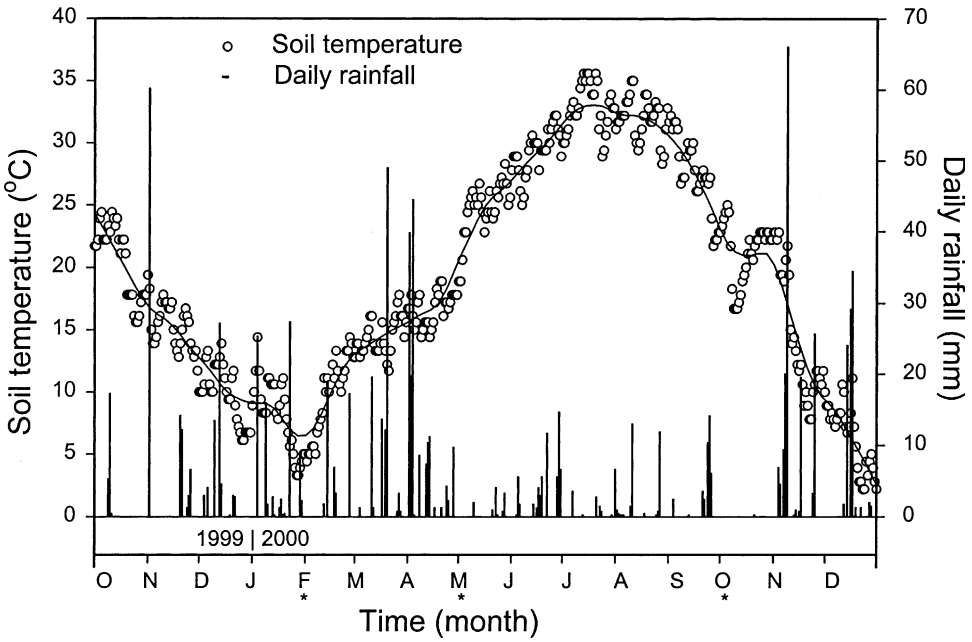

Fig. 5. Daily average soil temperature (bare soil at 10 cm depth) and daily rainfall totals at the Tennessee Valley Research and Extension Center from October 1999 to December 2000. The asterisks indicate sampling months.

These environmental factors affect the growth and activities of microorganisms and may have contributed in changes of the soil microbial communities. Bossio et al. (1998) found that, for a given soil, microbial community changes over time were of greater magnitude than changes associated with management regimes such as inputs of manure, cover crops, and mineral fertilizers.

4.3. Soil depth effect

The shift in microbial communities by soil depths may result from the interactions of soil moisture, temperature, aeration, and substrate availability collectively. With increasing soil depths, both rhizosphere effect and SOC contents decrease, as well as the composition of the humic material. Ding et al. (2002) characterized the humic acid from a Norfolk soil under long-term (20 years) conventional and conservation tillage management. The aliphatic C content decreased with increasing depth (0–15 cm) for both conventional and conservation tillage treatments whereas the more recalcitrant aromatic C content increased. The increases in the relative abundance with depth of two influential fatty acids 10Me16:0 and *i*15:0, which are Gram-positive bacterial biomarkers, as well as actinomycete marker 10Me18:0 (Fig. 4), support the above findings. Actinomycetes are Gram-positive filamentous bacteria known to utilize many resistant compounds. Since 10Me 16:0 is also found in sulfate-reducing bacteria, its increase in the relative abundance may also indicate increasing sulfate-reducing bacterial populations as well as anaerobic conditions with soil depth.

4.4. Significance of biomarker fatty acids

Formation of *cy*19:0 from its precursor 18:1ω7*c* has been associated with the development of anaerobic conditions in the subsoils and cell stasis or stress (Guckert et al., 1986; White and Macnaughton, 1997). Increases in the PLFA *cy*19:0 with soil depth suggest the development of anaerobic conditions in the subsoils. Additionally, greater *cy*19:0/18:1ω7*c* ratios at lower depths may also reflect the effects of progressive decreases in available substrates as soil depth increased. Drijber et al. (2000) reported that the ratio of *cy*19:0/18:1ω7*c* was greatest under fallow-plowed and sod treatments, indicating that a greater proportion of the bacteria in fallow-plowed and sod soils was in stasis. An increase in *cy*19:0 in tilled soil was noted by Calderón et al. (2001), due to increased anaerobic microsites after tillage. Petersen et al. (2002) found that the ratios of cyclopropyl fatty acids (*cy*17:0 and *cy*19:0) to their precursors increased with time (from May to September), which was attributed to the influences of the hot and dry summer period.

The proportion of the microbial biomass composed of fungi under no-till practices has been shown to be greater than that under conventional-till practices in surface soil (Beare, 1997; Frey et al., 1999). Frey et al. (1999) reported that the ratios of fungal to bacterial biomass determined using microscopy method are above 1 in 5 out of 6 no-till soils for the 0–5 cm depth increment. In our study, however, the fungal to bacterial PLFAs ratios in no-till soil were similar to conventional-till soil, ranging from 0.11 to 0.18. Several other studies also reported that the fungal to bacterial biomass ratios determined by PLFAs are less than 1 under various natural or managed systems (Bardgett et al., 1999; Bardgett and McAlister, 1999; Zeller et al., 2001).

This may be due to the fact that different groups of microorganisms sharing overlapping PLFAs and that phospholipids concentrations in fungi are lower than in bacteria (White et al., 1980; Petersen et al., 1991).

In our studies, no-till systems resulted in significant increases in SOC, total nitrogen, and microbial biomass at the surface layer, hence, improved soil quality. Temporal, vertical, and tillage practice-induced changes in soil microbial community structure were also detected. The tillage effect on microbial community varied by soil depth and over time. During the growing season, the soil microbial community responded to the addition of root exudates and prevailing environmental conditions (e.g. moisture and temperature). Thus, the change of microbial community is primarily determined by these factors and not by soil conditions modified by tillage practices. The impacts of tillage treatments were more pronounced during the fallow period and early in the growing season. Recently, there has been increasing interest in the management of the biological component of soil to improve soil quality and sustainability. This requires better understanding on how management systems as well as the environmental conditions influence microbial biomass, community composition, and population function. Cultural independent methods (e.g. PLFA profile analysis) allow us to better characterize the changes of microbial community under different management systems and may provide insights into how conservation tillage practices improve soil quality and sustainability.

Acknowledgements

The authors wish to thank John McInroy for performing GC analyses, John Mattice and Guangyao Sheng for carrying out GC-MS analyses, and Pingsheng Ji, Bin Lin, and Gang Wu for their assistance in laboratory analyses.

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