Effects of Tillage Systems on Soil Microbial Community Structure under a Continuous Cotton Cropping System

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ABSTRACT

Soil management practices affect soil microbial communities, which in turn influence soil ecosystem processes. In this study, the effects of conventional and no-tillage practices on soil microbial communities were examined under 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 to 3, 3 to 6, 6 to 12, and 12 to 24 cm. The no-till treatment had significantly higher soil organic carbon and microbial biomass carbon contents in the surface layer than the conventional till treatment. Microbial community structure, as indicated by the phospholipid fatty acid (PLFA) profile, was analyzed using principal components analysis; analysis of variance (ANOVA) on the first two principal components (PCs) was performed to assess the effects of tillage and sampling time. PLFA profiles clearly shifted over time and along soil depths. ANOVA on PC 1 revealed that both month x depth and tillage x depth interactions were significant. The response of PC 1 was different for conventional till and no-till treatments, as well as for the late season and the two early season samples. The influential fatty acids to the first two PCs were 10Me16:0, *i*15:0, and *cy*19:0 which are signature bacterial PLFAs, suggesting that the observed differences may result from the shift of bacterial populations. These results indicate that microbial communities associated with conventional tillage and no-tillage continuous cotton systems were dissimilar and the tillage effect varied by soil depths and over time. The use of culture-independent methods, such as PLFA profile analysis, allows us to better characterize the changes of the microbial community under different management systems and may provide insights into how conservation tillage improves soil quality and sustainability.

KEYWORDS

Phospholipid fatty acid (PLFA) profile, soil carbon, microbial biomass carbon

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). Lately, more and more farmers have adopted conservation 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. Changes in soil physical and chemical properties associated with different tillage practices have been studied extensively (Blevins and Frye, 1993; Reeves, 1997); however, characterization of the soil microbial community lags behind.

There is increasing interest in the management of the biological component of soil to improve soil quality and sustainability. Amounts or types of organic inputs to soils, as well as the environmental conditions, can influence microbial biomass, population function, and community composition. In this study, we used the phospholipid fatty acid profile to characterize microbial communities developed under 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 soil microbial community structure, as indicated by phospholipid fatty acid (PLFA) profiles under continuous cotton systems.

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MATERIALS AND METHODS

Field experiment and soil sampling

Soil samples were collected from a long-term cotton tillage and rotation experiment located 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 type is a Decatur silt loam (fine, kaolinitic, thermic Rhodic Paleudults). The soil was sampled from two winter fallow continuous cotton (*Gossypium hirsutum* L.) treatments subjected to conventional tillage and no-tillage. Conventionally tilled plots were established in 1979 and no-till plots in 1988 from previously conventionally tilled plots. Conventional tillage involved chisel plowing in the fall 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.

The soil was sampled in February, May, and October of 2000. Ten 3.9-cm diameter soil cores (0-24 cm deep) were collected randomly from 1000 ft^2 (50' x 20') individual plots. The 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 a thorough mixing, subsamples were taken for water content, microbial biomass determination by the chloroform fumigation incubation method, and extraction of lipids. Field moist soil samples were stored at 4˚C for no more than 2 weeks before microbial biomass determination and no more than 4 weeks before lipid extraction.

Laboratory Analysis

Soil samples taken in February were air-dried and used for total carbon determination using a C/N analyzer (Fisons Instruments, Beverly, MA). Since there is no appreciable carbonate carbon in this inherently acidic soil, the total carbon content is equivalent to the soil organic carbon (SOC) content. Microbial biomass carbon (MBC) was determined by the fumigation-incubation method according to 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*., 1999).

Field moist soil samples were used for PLFA analysis according to a procedure modified after Findlay and Dobbs (1993) and Bossio and Scow (1998). Duplicate soil samples (4 g dry weight) were extracted in 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). The phospholipids were separated from neutral and glycolipids using silicic acid column chromatography and then subjected to a mild alkaline methanolysis to obtain the fatty acid methyl esters (FAME). Samples were dissolved in appropriate amounts of hexane containing 19:0 methyl ester as an internal standard and analyzed using a Hewlett Packard 5890 gas chromatograph equipped with a 25-m HP Ultra 2 capillary column and a flame ionization detector. 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). Identification of the FAMEs was confirmed by gas chromatography mass spectrometry using a Varian Saturn 4 Ion Trap GCMS system.

PLFA compositions were analyzed with SAS software using principal components analysis (PCA). All samples were analyzed for PLFA profiles using a set of 22 fatty acids indicative of various taxonomic groups of soil microorganisms. Analysis of variance (ANOVA) on the first two principal components was performed to assess the effects of tillage, soil depth, and sampling time.

RESULTS AND DISCUSSION

The tillage treatments greatly affected soil organic carbon and microbial biomass carbon (Table 1). SOC content was more than twice as high in the surface layer of the no-till

Table 1. Soil organic carbon and microbial biomass carbon from conventional and no-till plots of a long-term cotton tillage and rotation experiment in Belle Mina, Alabama.

Tillage		Depth			
treatment		0-3 cm	3-6 cm	6-12 cm	12-24 cm
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
Biomass carbon, µg g^{-1}					
Conventional					
	February	236.7	181.8	117.8	73.9
	May	266.2	155.5	101.5	66.8
	October	221.2	164.5.	113.2	62.9
No-till					
	February	380.3	161.7	96.8	78.5
	May	632.9	184.8	107.9	73.2
	October	387.8	187.3	104.1	74.8
$LSD_{(0.05)}$		35	35	35	35

treatment compared to the conventional-till treatment (18.8 vs. 8.3 mg g^{-1}). The differences in SOC between the two tillage treatments were not significant at three lower depths. SOC in no-till plots decreased sharply with increasing soil depth. There was a significant increase in SOC at the second sampling depth compared to the surface layer (9.3 vs. 8.3 mg g^{-1}) for conventional-till plots; thereafter, soil organic carbon declined linearly with depth. The increase in SOC at the second sample depth may reflect the density of cotton root growth and/or buried residues with plowing. These results support the findings that no-till practice results in increased SOC at the surface layer (Edwards *et al*., 1992; Wander *et al*., 1998; Motta *et al*., 2001; Ding *et al*., 2002).

Microbial biomass carbon ranged from 63 to 266 µg g^{-1} in conventionally tilled soils and 73 to 633 µg g^{-1} in no-till soils for all sampling depths and months (Table 1). The percentages of SOC as biomass carbon ranged from 1.17 to 3.21% in conventionally tilled plots and 1.20 to 3.37% in no-till plots and the values decreased as soil depth increased. No-till soils contained significantly higher amounts of MBC than conventionally tilled soils at the surface layer for all sampling months (Table 1). Surface MBC content under no-till treatment was 61, 138, and 75% greater than under conventional till treatment in February, May, and October, respectively. Under both tillage systems, the highest MBC content was observed in May, probably due to the combined effect of nitrogen fertilizer application in the spring and the rhizodeposition of cotton roots. MBC contents decreased with increasing soil depths, as did SOC (Table 1). The largest changes occurred between the surface layer and lower depth, irrespective of the sampling month. Change in biomass carbon was most pronounced for the no-till treatment at the surface layer sampled in May, which was at least twice as large as for other months. Our results agree with previous reports that higher levels of MBC are found near the soil surface under no-tillage compared with conventional tillage and similar or lower levels at lower depths (Granastein *et al*., 1987; Franzluebbers *et al*., 1994; Motta *et al*., 2001).

PLFA profiles of 22 fatty acids were analyzed using principal components analysis. The first two principal components (PCs) accounted for 65% and 11% of the total observed variance. The PCA plot of the first two PCs showed that October data formed a cluster, whereas data points for February and May were intermixed (data not shown). PLFAs 10Me16:0, *cy*19:0, 18:1ω9*c*, 18:1ω7*c*, 18:2ω6c, and i15:0 were influential fatty acids to PC 1 with 10Me16:0 having the largest loading of 0.72 (Table 2). The PLFA with the highest loading (-0.71) for PC 2 was *i*15:0; other major contributors included *cy*19:0, 18:1ω9*c*, 18:1ω7*c*, *a*15:0, and 10Me16:0 (Table 2). PLFAs 10Me16:0, *cy*19:0, 18:1ω7*c*, i15:0, and *a*15:0 have been reported as marker PLFAs for bacteria with 10Me16:0, i15:0, and *a*15:0 being indicators of Gram-positive bacteria and *cy*19:0 and 18:1ω7*c* of Gram-negative bacteria (Paul and Clark, 1996; Findlay and Dobbs, 1993). PLFAs 18:1ω9*c* and 18:2ω6c have been identified as signature PLFAs for fungi (Paul and Clark, 1996; Findlay and Dobbs, 1993). The relative abundance (mole percentage) of these PLFAs was comparable under no-till and conventional till systems (data not shown). The ratio of *cy*19:0 to 18:1ω7*c,* which describes community response to anaerobic conditions (Guckert *et al*., 1986), increased with increasing soil depths and was higher in no-till soil at lower depths. This suggests that microbial community structure shifted as its surrounding physical and chemical environment was altered by the tillage system.

Table 2. PLFAs receiving loadings > |±0.2| on the first two principle components. The principal component analyses were carried out using 22 marker PLFAs. Soil samples were taken at four depths in February, May, and October 2000.

PC 1		PC 2	
Fatty acid	Loading	Fatty acid	Loading
10Me16:0	.72	*i*15:0	-.71
*cy*19:0	.38	*cy*19:0	. 35
18:1ω9*c*	-.26	18:1ω9*c*	.26
18:1ω7*c*	-.26	18:1ω7*c*	.26
18:2ω6*c*	-.23	*a*15:0	-.24
*i*15:0	. 20	10Me16:0	.21

ANOVA of PC 1 revealed that both month x depth and tillage x depth interactions were significant at P≤d 0.1 (data not shown). There was no significant tillage x depth x month interaction. The response of PC 1 was different for conventional till and no-till treatments. There was a strong linear response in PC 1 to depth for conventional tillage, whereas the response was nonlinear for no-tillage (Fig. 1A). The month x depth graph shows clearly that the late season (October) samplings differed from the two early-season (February and May) samplings (Fig. 1B). PC 1 showed a strong relationship with depth, and thus could be renamed the "depth response" variable indicating the cause of the observed variation. The only significant effect revealed by ANOVA for PC 2 was month ($P = 0.047$); therefore, PC 2 could be called the "time variable". PLFAs with dominant loadings for both PC 1 and PC 2 were Gram-positive bacterial markers (10Me16:0 and *i*15:0), suggesting that

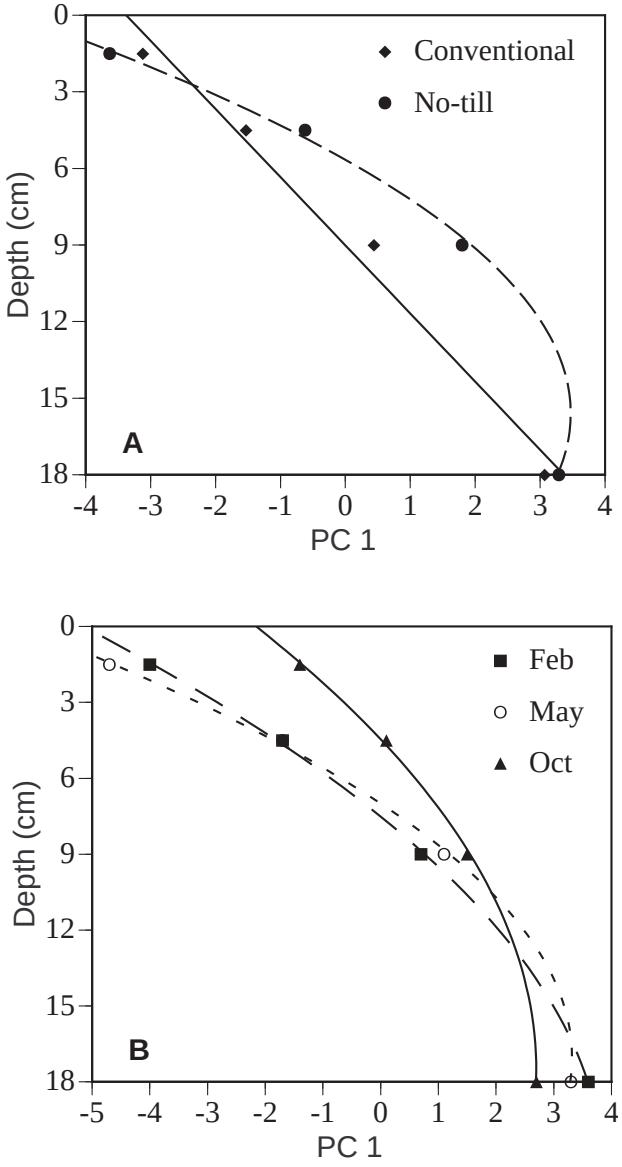

Fig. 1. Responses of the first principal component to increasing soil depth for tillage treatments (A) and sampling months (B) from a continuous cotton field. The principal components analysis was based on all three sampling dates and 22 fatty acids. The regression equations for tillage treatments are: PC 1 = 0.368 depth - 3.317 (R^2 = 0.981) for conventional tillage; PC 1 = -0.0352 depth^2 + 1.097 depth – 5.08 (R^2 = 0.997) for no-tillage (quadratic). The regression equations for sampling months are: PC 1 = -0.019 depth^2 + 0.82 depth -5.11 (R^2 = 0.999) for February; PC 1 = -0.034 depth^2 + 1.16 depth - 6.37 (R^2 = 0.999) for May; PC 1 = -0.016 depth^2 + 0.57 depth - 2.18 (R^2 = 0.998) for October.

differences in microbial community structure between tillage systems and sampling months may result from the shifts of bacterial populations. These results support previous observation of eubacterial groups affected by tillage (Calderon *et al*., 2001). Drijber *et al*. (2000) observed that for wheat-fallow cropping system, marker PLFA for arbuscular mycorrhizal fungi (16:1ω5) was important in discriminating no-till and plow treatments.

CONCLUSIONS

No-till practice resulted in significant increases in soil organic carbon and microbial biomass at the surface layer, as well as changes in the soil microbial community. The tillage effect on microbial community varied by soil depths and over time. The use of culture independent methods, such as PLFA profile analysis, allows us to better characterize the changes of the microbial community under different management systems and may provide insights into how conservation tillage practice improves soil quality and sustainability.

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