Conservation Management in Cotton Production: Long-Term Soil Biological, Chemical, and Physical Changes

Martin A. Locke*
USDA-ARS
National Sedimentation Lab.
Water Quality & Ecology Research Unit
598 McElroy Drive
Oxford, MS 38655

Robert M. Zablotowicz
USDA-ARS
Crop Production Systems Research Unit
P.O. Box 350
Stoneville, MS 38776

Robert W. Steinriede, Jr.
Sam Testa
USDA-ARS
National Sedimentation Lab.
Water Quality & Ecology Research Unit
598 McElroy Drive
Oxford, MS 38655

Krishna N. Reddy
USDA-ARS
Crop Production Systems Research Unit
P.O. Box 350
Stoneville, MS 38776

Conservation practices are increasingly important components of sustainable management systems, and information about their influence on soil characteristics is needed. Soil parameters were assessed in no-till (NT) or minimum tillage (MT) cotton (*Gossypium hirsutum* L.) production near Stoneville, MS, Mississippi Delta region, that included cover crop (rye [*Secale cereal* L.] or Balansa clover [*Trifolium michelianum* Savi var. *balansae* (Boiss.) Azn.]) vs. no cover crop. Soils (0–2, 2–5, and 5–15 cm) were sampled (2001–2006) before cotton planting. Independent of tillage, both cover crops accumulated more soil C than no cover, and N was greatest under clover. Soils (0–15 cm) under clover had greater aggregate stability than rye or no cover. The major factor influencing bulk density and infiltration was proximity to crop row bed and wheel traffic, but infiltration rates were sixfold greater under MT than NT ($P < 0.01$), with less effect of cover crop ($P < 0.06$, clover > rye or no cover). Moderate tillage slightly increased abundance of both reniform nematodes and earthworms, but neither was affected by cover crop. Fluorescein diacetate hydrolytic activity was higher in clover (50%) and rye (20%) in surface soil than with no cover. Soil microbial community structure (total fatty acid methyl ester analysis) (2005–2006) indicated a significant cover crop effect but no tillage effect. Mycorrhizal bioindicator (16:1 ω5c) was greater in soil with rye than clover or no cover; however, cotton mycorrhizal infection was 40% greater in fibrous roots from rye or clover plots than roots from plots with no cover. Collectively, cotton production with a cover crop and reduced tillage resulted in soil conditions indicative of soil quality.

Abbreviations: FDA, fluorescein diacetate hydrolytic activity; MT, minimum tillage; NT, no-till; TC, total carbon; TN, total nitrogen.

Concerns associated with water quality and hypoxia in the Gulf of Mexico have renewed focus on the research needed to provide the scientific basis for adapting farming practices to accommodate soil and water conservation (Mississippi River/Gulf of Mexico Watershed Nutrient Task Force, 2008). Growing awareness of the potentially positive effects of conservation practices is leading to increased adoption of these practices throughout the Mississippi Basin (NRCS, 2009, 2010). In the Lower Mississippi Basin, large areas of relatively flat alluvial land are devoted to row crop agriculture (NRCS, 2006). Historically, cotton was the staple crop in this region, but other crops such as soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.), and rice (*Oryza sativa* L.) now involve major areas of production (National Agricultural Statistics Service, 2012).

In recent years, conservation measures have been increasingly adopted in cotton production (Conservation Technology Information Center, 2012), but information regarding the environmental impacts is not sufficiently documented. Furthermore, the widespread use of transgenic herbicide-resistant crops has

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*Corresponding author (Martin.locke@ars.usda.gov).

revolutionized row crop farming by giving farmers more flexibility in weed management while reducing the need for tillage (Brookes and Barfoot, 2005). Information on the effects of transgenic crops on soil in conservation tillage systems, however, is not readily available (Locke et al., 2008).

Adopting conservation practices such as minimum tillage and cover crops may alter soil characteristics, particularly at or near the surface (Locke and Bryson, 1997; Reeves, 1997; Liu et al., 2005; Puget et al., 2005). Periodic soil disturbance, improved moisture, and increased organic C in surface soils (Halvorson et al., 2002; Reddy et al., 2003; Blanco-Canqui and Lal, 2008; Novak et al., 2009; Varvel and Wilhelm, 2010) can influence nutrient dynamics (Six et al., 2002; Zibilske and Bradford, 2007; Sainju et al., 2009; Halpern et al., 2010; Spargo et al., 2011) and microbial populations and activities (Feng et al., 2003; Lupwayi et al., 1998; Zablotowicz et al., 1998, 2000b, 2010; White and Rice, 2009), and other soil biota (Reeleder et al., 2006; Ernst and Emmerling, 2009; Umiker et al., 2009; Ahmed et al., 2012).

Increased coverage of the soil surface by plant residues may reduce the loss of soil and associated nutrients in runoff by impeding surface flow (Rhoton et al., 2002), thus potentially improving the quality of water bodies receiving runoff (Knight and Welch, 2004; Zablotowicz et al., 2006) and preserving valuable soil resources (Locke et al., 2010). Changes in physical properties such as bulk density, water infiltration, and aggregate stability may also influence water movement in soils managed with conservation tillage or cover crops. Several studies have reported increased aggregate stability and size of aggregates in these soils (Six et al., 2002; Zibilske and Bradford, 2007; Mikha et al., 2010; Blanco-Canqui et al., 2011; Raczkowski et al., 2012). Reports of the effects of conservation practices on other physical characteristics such as soil bulk density and water infiltration are variable (Blanco-Canqui and Lal, 2007; Blanco-Canqui et al., 2011; Kumar et al., 2012; Raczkowski et al., 2012).

Although the preservation and sustainability of soil and water resources are goals of conservation management, periodic minimal tillage in some soils may be beneficial in redistributing nutrients, increasing C sequestration (Halvorson et al., 2002), improving aeration, or ameliorating compaction. Furthermore, completely eliminating tillage is less acceptable to some farmers because of concerns about how NT may negatively impact yield. Because of stratification, changes in soil characteristics induced by strict NT management may be observed only at or near the soil surface (Blanco-Canqui and Lal, 2008; Novak et al., 2009; Locke et al., 2010). Positive outcomes such as soil and water conservation might be achieved by moderately reducing soil disturbance compared with many conventional tillage systems, where intensive and multiple tillage operations are performed. Therefore, if farmers used a moderate, minimum tillage approach, the benefits of conservation might be preserved while ameliorating some of the negative aspects of strict NT.

In the present study, changes in soil characteristics as influenced by conservation tillage and cover crops were evaluated in a Mississippi Delta soil cropped with transgenic herbicide-resistant cotton from 2001 to 2006. Two conservation tillage systems, NT and MT, and two cover crops, rye and Balansa clover, were components of the study. Some aspects of the soil were monitored all 6 yr, but because changes in soil due to management practices often take several years to have an effect (Zibilske and Bradford, 2007; Sainju et al., 2009; Halpern et al., 2010; Kumar et al., 2012), more comprehensive assessments were done in the last 2 yr of the study.

MATERIALS AND METHODS

Site Description and Soil Management

The study was conducted on the USDA-ARS Crop Production Systems Research Unit farm near Stoneville, MS, to evaluate the effects of tillage and cover crops on soil characteristics. The soil series was Dundee silt loam (a fine-silty, mixed, active, thermic Typic Endoaqualf). Before establishment of this study, the area was in continuous soybean and managed with conventional tillage practices for several years.

The study area was plowed and 38-cm row beds were established in October 2000 after soybean harvest. After the first cotton-growing season (2001), the row configuration was converted to 1-m widths in fall 2001. Row widths remained at 1-m widths for the duration of the study. Experimental plots were 8 m wide and 32 m long (0.026 ha) and were arranged in a split-plot design with four blocks. Tillage (NT or MT) was the main effect, with cover crop (rye, clover, or none) as the split effect. After the initial plowing in fall 2000, the NT plots were not tilled again for the duration of the study period. When row widths were reconfigured to 1 m after the 2001 growing season, the extra beds in between were simply skipped. The MT plots were disked and rows prepared each fall after cotton harvest. These were the only tillage operations.

Crop Management

Balansa clover (9 kg ha^{-1}) and Abruzzi rye (67 kg ha^{-1}) were seeded as cover crops. Rye was planted each fall after cotton harvest and soil preparation (in MT plots). When rye reached the milk stage the following spring, it was killed in April with either paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride; 1.12 kg a.i. ha^{-1}) or glyphosate [*N*-(phosphonomethyl) glycine; 1.12 kg a.i. ha^{-1}] followed by crimping the stalks with a roller. Balansa clover was selected as a legume cover crop because preliminary experiments indicated that it was successful in reseeding within the time frame needed for a cotton production system in this region. The clover was planted in the fall 2000 and allowed to reseed the following spring. Clover was not planted after 2000 because it was reestablished in subsequent years from reseeding. The clover was killed each spring approximately mid-April using glyphosate. Winter vegetation was allowed to grow undisturbed on the plots designated as no cover crop until treatment in late spring with paraquat or glyphosate to kill any existing vegetation.

Glyphosate-resistant cotton cultivars were planted in late April to early May from 2001 to 2006. Glyphosate and paraquat applications to kill the rye cover crop averaged 14 d (7–20 d)

before cotton planting and 20 d (4–33 d) before cotton planting for the clover and no-cover-crop plots. Fluometuron (*N*,*N*-dimethyl-*N*′-[3-(trifluoromethyl) phenyl]urea; 1.7 kg a.i. ha^{-1}) was applied to the experimental area immediately after planting to provide early-season weed control. Glyphosate (0.84 kg a.i. ha^{-1}) was applied post-emergence two to three times for weed control as needed. Weed counts were taken periodically in May, June, and July of each year to assess the impact of tillage and cover crops on weeds. Although there were a few differences in individual weed species density, overall control of all weeds by the herbicides applied was sufficient to support cotton production regardless of cover crop and tillage system.

Nitrogen fertilizer was applied at planting. In 2001, all cover crop treatments received the same rate of 140 kg N ha^{-1}. Based on reports for fertilizer N applications in cover crops and MT systems, variable N applications were made in subsequent years (Reeves, 1994; Reiter et al., 2008). Fertilizer N applications in Mississippi are also based on yield goals. For example, the current recommendation is approximately 160 kg N ha^{-1} for a yield goal of two bales of cotton on medium- to heavy-textured soils (Oldham and Dodds, 2010). From 2002 to 2003, the clover cover crop, no cover crop, and rye cover crop plots received 45, 112, and 134 kg N ha^{-1}, respectively, and from 2004 to 2006 received 90, 157, and 179 kg ha^{-1}, respectively. The increase in N fertilizer in 2004 was in response to increasing soil C. The rationale used for variable rates among the treatments was that management systems were being assessed, and each system would be managed in the recommended way. For example, including a cereal cover crop might require more N fertilizer or a leguminous cover crop might require less N fertilizer (Zablotowicz et al., 2011).

Soil Sampling

Soils were sampled in late spring each year before cotton planting for most characterizations. Soil samples (7.5-cm-diameter cores) were collected from the plant row at six sites within each plot and at depth increments of 0 to 2, 2 to 5, and 5 to 15 cm. Before a sample was collected, any thatch or other plant residues were removed from the soil surface. Samples were placed in plastic bags, sealed, and stored at 4°C until further processing. Soil sampled before cotton planting each year as described here was used for C, N, fluorescein diacetate hydrolytic activity (FDA), aggregate stability, and water-dispersible clay analyses. Soil sampled for other analyses is described below.

Soil Chemical Analyses and Enzyme Activity

Total microbial heterotrophic activity was estimated using FDA as an indicator using methods described elsewhere (Zablotowicz et al., 2000a). The substrate FDA is relatively nonspecific, thus it can represent hydrolytic activity for a range of natural compounds such as lipids, esters, and proteins and can reflect relative microbial biomass (Zablotowicz et al., 2000a). Field-moist soil was passed through a 2-mm sieve, and FDA was adjusted based on an oven-dried equivalent basis.

Soil total C (TC) and total N (TN) concentrations were determined using a Flash EA1112 NC elemental analyzer (CE Elantec, Inc.). Analysis was performed on duplicate air-dried (30-mg oven-dried basis) samples. The results for TC and TN are presented as concentrations in soil (g N or C kg^{-1}) for 2001 to 2006 and on an area (Mg N or C ha^{-1}) basis in 2005 and 2006 because bulk density samples were collected the last 2 yr of the study (see below).

Soil Physical Analyses

For physical analyses, soils were air dried, sieved, and ground as appropriate for individual analyses. The particle size distribution was determined using the hydrometer method (Day, 1965). Water-dispersible clay, aggregate stability, bulk density, and infiltration were evaluated in 2005 and 2006. Water-dispersible clay was measured using the pipette method according to Day (1965), except that water was used instead of sodium hexametaphosphate. Aggregate stability was determined by wet sieving 1- to 2-mm aggregates on a 0.25-mm (60-mesh) sieve for 5 min, similar to the procedure of Kemper and Rosenau (1986).

Within each plot and for each row position, bulk density and infiltration were measured at three locations ($n = 3$). Procedures described by Blake and Hartge (1986) were used to determine the bulk density in the soil surface (a single determination for the surface 15 cm, using a 7.6-cm-diameter, 15-cm-high aluminum cylinder). The infiltration rate was determined using a single-ring infiltrometer (15-cm-diameter, 12-cm-high aluminum cylinder) method according to Bouwer (1986). The infiltration rate was measured in three positions within the crop rows: on the row bed 15 to 30 cm from the crop, in the middle of the furrow between row beds where field equipment was driven (with wheel tracks), and in the middle of the furrow between row beds where no field equipment was driven (no wheel tracks).

Soil Biology

The microbial community structure of the soils as affected by tillage and cover crop was assessed on soils sampled in late spring 2005 and 2006 after cotton emergence. Soils were maintained at the moisture content at sampling, passed through a 2-mm sieve, and stored at −80°C until analyzed. Total soil fatty acids (fatty acid methyl esters [FAMEs]) were methylated and extracted using protocols described elsewhere (Zablotowicz et al., 2007, 2010). For the upper two soil depths, 2 g (fresh weight) was extracted, and for the 5- to 15-cm soil depth, three 2-g soil samples combined were extracted. Extracts from all three depths were concentrated to 0.5 mL of hexane. The FAMEs were identified and quantified with an Agilent 6890 gas chromatograph (Agilent Technologies) and MIDI EUKARYOTE protocols (MIDI FAME standards, Microbial ID, MIDI Inc.). The relative molar percentage of fatty acids as identified by the MIDI software was used in the analysis, and no attempt was made at a quantitative extraction for the estimation of biomass.

Cotton roots were harvested in 2006 for determination of mycorrhizal infection at initiation of flowering. Twenty-

five 3-cm-long fibrous root segments of about 1- to 2-mm diameter were collected from each plot. The roots were washed in water to remove soil particles, cleared in KOH, and stained with lactophenol according to the method of Silvia (1994). The roots were observed under a dissecting microscope at 20× magnification, and the number of infection clusters per root segment was counted.

Earthworms were evaluated only in 2005 by excavating a 30- by 30- by 20-cm volume of soil in duplicate from each plot. The procedures were patterned after the methods described in NRCS (2001).

Soil samples were collected from each plot (composite of six cores per plot) to a depth of 15 cm on 15 Apr. 2005 and 29 Nov. 2006. Soil samples were sent to the Mississippi State University Soil Testing Laboratory, Starkville, MS, for evaluation of nematode populations. Soil samples were screened for 17 nematode species, and the only two identified were reniform (*Rotylenchulus reniformis*) and spiral (*Helicotylenchus pseudorobustus*). The predominant species was *R. reniformis*. Nematodes were recovered from the soil by mechanical sieving with a North Carolina style semiautomatic elutriator, followed by sucrose gradient centrifugation for separation. Nematodes were identified visually by species, and the population density was determined.

Statistical Methods

Data on soil properties were subjected to analysis of variance using PROC Mixed or PROC GLM to assess the effects of tillage (MT or NT), cover crop (none, rye, or clover), and the interaction of these variables (SAS version 9.2, SAS Institute). Each sampling for the 4 yr of the study was analyzed separately. Treatment means were separated at appropriate levels of significance using Fisher's protected LSD test. Differences in microbial community structure were detected and described using principal component analysis (SAS, PROC PRINCOMP). Fatty acids that were very rare (<0.5 mol%) or absent from a majority of samples (<20% of samples) were excluded from analysis to reduce minor experimental variations (Zablotowicz et al., 2007, 2010; Locke et al., 2008). Following principal component analysis, the contributions of cover crop, tillage, and interactions between cover crop and tillage on the principal components were analyzed using SAS PROC MIXED. The relative occurrence (mol% of total FAMEs) for the basidiomycete–mycorrhizal biomarker 15′ω5c was also analyzed using SAS PROC MIXED.

RESULTS AND DISCUSSION

Soil Total Carbon and Nitrogen Concentration

In general, the TC concentration for the surface 5 cm of soil tended to be lower in the first 3 yr than in the last 3 yr of the study period (overall mean TC across treatments: 11.7 g kg^{-1} for 2001–2003 vs. 14.2 g kg^{-1} for 2004–2006). The TC concentration declined with soil depth in all years (Tables 1 and 2). Comparing the TC concentration in 2001 (the initial year) with that of 2006, averaged across all conservation management treatments, there was a slight increase in TC for each depth during the 6-yr study period. The overall mean TC concentration across treatments in 2001 and 2006 was 13.6 and 17.7 g kg^{-1} for 0 to 2 cm, 7.9 and 11.7 g kg^{-1} for 2 to 5 cm, and 7.0 and 8.1 g kg^{-1} for 5 to 15 cm, respectively.

There were gradual increases in the TC concentration in the surface 2 cm of soil in response to cover crop and tillage (Tables 1 and 2). In 2001, the first year of the study, cover crop was the only treatment that was significant ($P < 0.05$), with clover greater than rye or no cover crop across all soil depths (8.4 clover > 7.9 rye = 7.9 no cover crop). In 2002 and 2003, Balansa clover similarly had the highest TC concentrations in the 0- to 2-cm depth at planting compared with no cover crop or plots planted to rye (Table 1). In another as yet unpublished aspect of this project, plant biomass was sampled at the same time that soil samples were taken (2001–2005). Plant biomass sampled from 2003 to 2005 (3551 kg ha^{-1}) tended to be greater than for 2001 to 2002 (2492 kg ha^{-1}). Also, rye and clover cover crop treatments consistently produced more plant biomass (3953 and 3826 kg ha^{-1}, respectively, averaged across years) than the no-cover-crop treatment (1603 kg ha^{-1}). Leguminous cover crop residues often have a threefold lower C/N ratio than cereals and subsequently will degrade twice as fast as cereal crop residues (Cookson et al., 1998). Thus, under our experimental scenario with the cover crop residues remaining on the soil surface, the clover residue should have decomposed more rapidly than that of rye. In the last 3 yr of the study, TC concentration enrichment in the surface 0 to 2 cm relative to the no-cover-crop soil was similar for both rye and clover but with little or no effect of cover crop on the TC concentration in the soil from the 2- to 15-cm depths. Likewise, tillage management affected the TC concentration only in the 0- to 2-cm depth of soil (Table 2), with soils managed under NT having significantly higher TC than MT in all 6 yr of the study. For 2005 and 2006, the mean TC (for the entire surface 15 cm) was influenced by cover crop ($P < 0.05$, 19.1 Mg C ha^{-1} for rye = 18.9 Mg C ha^{-1}

Table 1. The interaction of cover crop and soil depth on the soil total C concentration during the 6-yr cotton study, Stoneville, MS.

Cover crop	Soil depth	Total C concentration					
		2001	2002	2003	2004	2005	2006
	cm	g kg^{-1}					
None	0–2	7.7 ns†	9.2 c	11.7 b	13.0 b	16.1 b	15.2 b
	2–5	8.2 ns	9.3 c	9.2 cde	10.9 bc	9.9 cd	10.6 cd
	5–15	7.8 ns	9.1 c	8.5 e	7.8 d	7.9 d	7.9 e
Clover	0–2	8.8 ns	12.4 a	15.4 a	17.1 a	20.2 a	19.1 a
	2–5	8.7 ns	10.3 b	10.2 c	11.4 b	11.5 c	12.5 c
	5–15	7.8 ns	8.9 c	8.8 de	8.6 cd	8.3 d	8.2 e
Rye	0–2	7.4 ns	9.3 c	11.9 b	18.0 a	18.2 ab	19.0 a
	2–5	8.4 ns	9.4 c	9.8 cd	10.7 d	10.8 d	12.1 de
	5–15	7.8 ns	8.6 c	8.4 e	8.0 d	7.9 d	8.3 de

† Within a column, means followed by the same letter are not significantly different ($P < 0.05$); ns, not significant.

for clover > 17.6 Mg C ha^{-1} for no cover crop) but not by tillage.

Others who have undertaken a more comprehensive assessment of the profiles of soils managed with conservation practices have observed stratification of soil TC, with the most dramatic effects due to conservation practice observed in the surface (e.g., Blanco-Canqui and Lal, 2008). Also, a greater proportion of TC in the surface of tilled soils may be comprised of more stable humic components than that of nontilled soils or soils with higher surface accumulation of plant residues such as cover crops or mulch (Bird et al., 2003), as in the present study with NT or MT.

Table 2. The interaction of tillage (no-till, NT, or minimum tillage, MT) and soil depth on the soil total C concentration during the 6-yr cotton study, Stoneville, MS.

Tillage	Soil depth	Total C concentration					
		2001	2002	2003	2004	2005	2006
	cm	g kg^{-1}					
NT	0–2	8.1 ns†	11.3 a	13.8 a	19.2 a	20.7 a	19.5 a
	2–5	8.4 ns	9.5 bc	9.5 c	11.2 bc	10.5 c	11.0 c
	5–15	8.1 ns	8.9 c	8.6 e	7.7 d	7.9 d	7.8 d
MT	0–2	7.8 ns	9.3 bc	12.2 b	12.9 b	15.6 b	16.0 b
	2–5	8.5 ns	9.8 b	9.9 cd	10.8 c	10.9 c	12.4 c
	5–15	7.5 ns	8.8 c	8.6 de	8.6 d	8.1 d	8.4 d

† Within a column, means followed by the same letter are not significantly different ($P <$ 0.05); ns, not significant.

The magnitude of the increased TC concentration observed in the uppermost 2 cm due to cover crop and MT or NT was similar to those of other studies in the Mid-South region of the United States (Locke et al., 2010): Mississippi (Zablotowicz et al., 1998, 2010; Reddy et al., 2003), Louisiana (Gaston et al., 2003), and Alabama (Feng et al., 2003). Although significant effects of both tillage and cover crop on TC concentration were observed during the course of this study, the magnitude of change may have been tempered compared with what might be expected in regions with lower humidity and lower average temperatures (e.g., Halvorson et al., 2002; Blanco-Canqui and Lal, 2008; Halpern et al., 2010), where sequestration of soil TC may be more stable. Higher temperatures and increased precipitation in some areas with warmer climates contribute to more rapid mineralization of C, resulting in more transient effects of potential gains from C sequestration. Additionally, although tillage was included as a component of MT, the MT system used in this study is considered to be moderate and may be an acceptable alternative conservation practice when periodic tillage is needed to offset any negative consequences (e.g., compaction) of long-term NT systems. This study indicated that after 6 yr of MT vs. NT, TC sequestered in the entire 15-cm plow layer (Mg ha^{-1} basis) was the same for both tillage treatments, even though there were tillage effects on the TC concentration due to stratification.

The TN concentration in the soil was reflective of both cover crop and the variable N applications applied to each cover crop treatment. In 2001, the TN concentration tended to decline with soil depth but was not influenced by cover crop or tillage (Table 3). The effects of cover crop on the soil TN concentration were similar to TC concentrations in that soils from Balansa clover plots had higher TN levels in the surface 0 to 2 cm than rye or no cover crop in the first 3 yr of the study (Table 3). In 2004 and 2005, however, TN in the 0- to 2-cm soil depth of the rye plots was comparable to that in the clover plot soils. Minor enhancement of TN in the 2- to 5-cm soil depth was observed only in 2003, 2004, and 2006, with higher levels in clover than the no-cover-crop plots. The consistently higher TN concentrations in the 0- to 2- and 2- to 5-cm depths of the clover soils is attributed to N_2 fixed by the clover because the clover always received the least quantity of N fertilizer. Although the rye plots received more N fertilizer than the no-cover-crop plots, the TN concentrations were equivalent in the surface 0- to 2- and 2- to 5-cm soil depths for most years (Table 3), indicating that a portion of the additional N added to the rye plots was immobilized in plant residues.

The TN concentration in the 0- to 2-cm depth of the NT soils was significantly greater than in the same depth of the MT soils in all 6 yr of the study (mean 1500 vs. 1100 mg N kg^{-1}), but no effect of tillage was observed in the two lower soil depths (Table 4). For both NT and MT, soil C/N ratios in the 0- to 2-cm depth tended to be in the order of rye $\geq$ no cover > clover, reflecting the average C/N ratios of the respective plant cover (rye = 37, no-cover-crop winter vegetation = 24, clover = 16). Total N in the 15-cm plow layer (averaged for 2005 and 2006) was influenced by cover crop ($P < 0.05$, 1.62 Mg ha^{-1} for clover = 1.58 Mg ha^{-1} for rye > 1.48 Mg ha^{-1} for no cover crop). On an area basis, TN for the 15-cm plow layer under NT was slightly higher than under MT for 2005 and 2006 ($\alpha = 0.11$, 1.60 Mg ha^{-1} for NT > 1.52 Mg ha^{-1} for MT).

Table 3. The interaction of cover crop and soil depth on the soil total N concentration during the 6-yr cotton study, Stoneville, MS.

Cover crop	Soil depth	Total N concentration					
		2001	2002	2003	2004	2005	2006
	cm	g kg^{-1}					
None	0–2	0.76 ns†	0.78 c	0.834 b	1.13 b	1.31 b	1.30 b
	2–5	0.78 ns	0.76 c	0.69 d	0.89 bcd	0.86 cde	0.80 de
	5–15	0.66 ns	0.74 c	0.66 d	0.65 de	0.73 e	0.61 f
Clover	0–2	0.83 ns	1.09 a	1.20 a	1.58 a	1.68 a	1.73 a
	2–5	0.79 ns	0.88 b	0.80 bc	0.98 bc	1.04 c	1.01 c
	5–15	0.69 ns	0.71 c	0.71 cd	0.71 cde	0.74 de	0.66 ef
Rye	0–2	0.78 ns	0.78 c	0.88 b	1.50 a	1.43 b	1.56 a
	2–5	0.74 ns	0.79 c	0.71 cd	0.85 cde	0.91 cd	0.90 cd
	5–15	0.68 ns	0.74 c	0.65 d	0.64 e	0.71 e	0.65 ef

† Within a column, means followed by the same letter are not significantly different ($P <$ 0.05); ns, not significant.

Table 4. The interaction of tillage (no-till, NT, or minimum tillage, MT) and soil depth on the soil total N concentration during the 6-yr cotton study, Stoneville, MS.

Tillage	Soil depth	Total N concentration					
		2001	2002	2003	2004	2005	2006
	cm	g kg^{-1}					
NT	0–2	0.81 ns†	0.97 a	1.04 a	1.66 a	1.70 a	1.69 a
	2–5	0.77 ns	0.81 b	0.73 cd	0.93 bc	0.93 c	0.85 c
	5–15	0.68 ns	0.73 cd	0.69 cd	0.64 d	0.72 d	0.62 d
MT	0–2	0.77 ns	0.79 bc	0.9 b	1.14 b	1.24 b	1.37 b
	2–5	0.77 ns	0.81 b	0.73 c	0.88 cd	0.94 c	0.96 c
	5–15	0.68 ns	0.73 d	0.66 d	0.69 d	0.73 d	0.67 d

† Within a column, means followed by the same letter are not significantly different ($P < 0.05$); ns, not significant.

Soil Enzyme Activity

Enhanced levels of FDA were observed in the last 3 yr of the study compared with the first 3 yr. The overall mean FDA across treatments in the 0- to 2-cm soil depth was 149 μmol kg^{-1} soil h^{-1} (SE = 27 μmol kg^{-1} soil h^{-1}) for 2001 to 2003 vs. 215 μmol kg^{-1} soil h^{-1} (SE = 39 μmol kg^{-1} soil h^{-1}) for 2004 to 2006. The initial plowing before plot establishment in fall 2000 homogenized the upper 15 cm of soil, thereafter demonstrating the increasing effects of conservation management practices with time. From 2003 to 2006, FDA tended to decline with depth regardless of tillage treatment (Tables 5 and 6).

Both tillage and cover crop influenced FDA, although for both management treatments, different effects were observed with soil depth (Tables 5 and 6). The 0- to 2-cm soil depth was most affected by management, with the highest activity observed in Balansa clover plots in 2003 and in both rye and clover plots from 2004 to 2006. No consistent patterns in FDA among the soil depths were observed in 2001 (Tables 5 and 6), possibly because the soil was more homogenized at that point due to the initial plowing in 2000. In 2002, FDA in the lower two depths, and particularly for the 2- to 15-cm depth, tended to be higher (Tables 5 and 6). An opposite trend was observed in other years. Little explanation can be given for the anomaly in 2002. No precipitation occurred during the 7 d before soil sampling in 2002 (Fig. 1), and the higher FDA at lower soil depths might be partially explained by lower soil moisture in the surface. There was little (0.1 cm) precipitation in 2003 during that same period (Fig. 1), however, and FDA declined with soil depth in 2003 (Tables 5 and 6). From 2004 to 2006, FDA in soils from the surface 0 to 2 cm in NT plots was significantly greater than that measured in MT plots (Table 6). Similarly, both cover crop treatments significantly increased FDA levels in the surface depth (Table 5). Under various cover crop–tillage regimes in soybean, the cereal cover crop rye and the leguminous cover crop hairy vetch (*Vicia villosa* Roth ssp. *villosa*) elicited similar increases in FDA activity (Zablotowicz et al., 2010). Enhanced FDA in the present study was attributed to both the direct (substrate for microorganisms) and indirect (greater biomass C and enzymes) effects of TC, including providing sites for binding enzymes and increased stability of the enzymes.

Table 5. The interaction of cover crop and soil depth on fluorescein diacetate hydrolytic activity (FDA) in soil during the 6-yr cotton study, Stoneville, MS.

Cover crop	Soil depth	FDA product formation					
		2001	2002	2003	2004	2005	2006
	cm	μmol kg^{-1} soil h^{-1}					
None	0–2	129 abcd†	107 c	133 b	178 b	194 b	123 b
	2–5	142 abc	137 bc	132 bc	105 cd	128 de	114 b
	5–15	114 d	213 ab	70 d	71 d	101 e	113 b
Clover	0–2	142 abc	201 ab	220 a	272 a	239 a	196 a
	2–5	154 a	202 ab	139 b	117 cd	154 cd	139 b
	5–15	134 bcd	235 a	93 cd	87 d	114 e	116 b
Rye	0–2	153 ab	113 c	146 b	290 a	235 a	202 a
	2–5	139 abcd	113 c	149 b	146 bc	173 bc	138 b
	5–15	116 cd	203 ab	96 d	83 d	111 e	124 b

† Within a column, means followed by the same letter are not significantly different ($P < 0.05$).

Table 6. The interaction of tillage (no-till, NT, or minimum tillage, MT) and soil depth on fluorescein diacetate hydrolytic activity (FDA) in soil during the 6-yr cotton study, Stoneville, MS.

Tillage	Soil depth	FDA product formation					
		2001	2002	2003	2004	2005	2006
	cm	μmol kg^{-1} soil h^{-1}					
NT	0–2	139 ab†	161 abcd	169 a	272 a	249 a	180 a
	2–5	118 b	150 cde	146 ab	121 c	155 c	128 b
	5–15	144 a	218 ab	74 d	82 d	107 d	108 b
MT	0–2	144 a	119 d	164 ab	225 b	196 b	167 a
	2–5	145 a	152 bcde	140 b	124 c	148 c	133 b
	5–15	114 b	217 abc	96 c	79 d	110 d	121 b

† Within a column, means followed by the same letter are not significantly different ($P < 0.05$).

Nematodes and Earthworms

The nematode *R. reniformis* is one of the most problematic parasites in cotton production (Robinson, 2008). Although cultural practices such as tillage and cover crops have been proposed as methods to reduce nematode numbers in soils where cotton and other susceptible crops are grown, scientific evidence is inconclusive as to their effectiveness. In the present study, *R. reniformis* nematodes in the MT soil were slightly higher than in the NT soils in spring 2006 (Table 7), but there was no difference due to tillage observed in the fall sampling in 2005 (data not shown). Soil sampling in fall 2005 occurred shortly after tillage in the MT plots, but the soil was not tilled again before the soil sampling in spring 2006. The fall tillage might have resulted in aeration of the soil and decomposition of plant residues, resulting in more favorable overwintering conditions in the MT soil. The cover crops did not have an effect on *R. reniformis* either year.

A few studies have assessed the effects of conservation practices on *R. reniformis*, but the results have generally shown

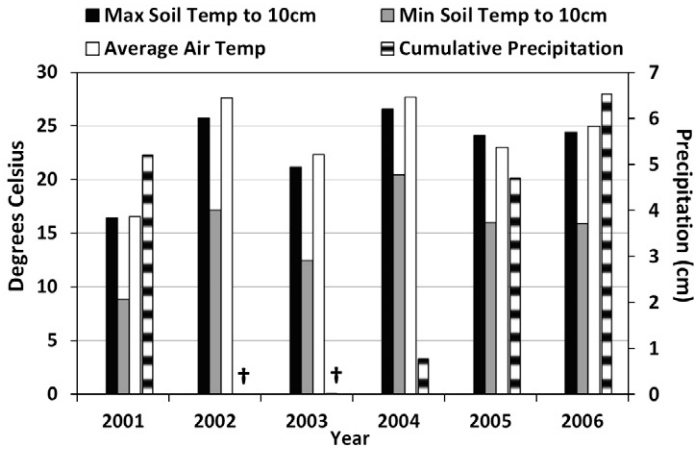

Fig. 1. Stoneville, MS, weather data for the 7 d before soil sampling. Included are maximum and minimum soil temperature to 10 cm, average air temperature, and cumulative precipitation during the 7-d period. †Precipitation measured during the period was <0.1 cm. Soil sampling dates were 19 Mar. 2001, 17 Apr. 2002, 17 Apr. 2003, 27 Apr. 2004, 18 Apr. 2005, and 13 Apr. 2006.

inconsistent or minimal effects of these practices on populations in soil (Cabanillas et al., 1999; Westphal and Smart, 2003; Timper et al., 2012). Cabanillas et al. (1999) did not observe differences in *R. reniformis* numbers between CT and NT at one site but measured higher numbers under NT and ridge tillage than CT at another site. Westphal and Smart (2003) observed that reduced tillage practices lowered *R. reniformis* populations.

According to several reports, *R. reniformis* can thrive in a wide range of soil textures (Robinson, 2008), but some reports have indicated that higher populations may be enhanced by finer textured soils. Results in the current study indicated that *R. reniformis* numbers were lowerer in soils with finer texture. The soils in Blocks 3 and 4 of the current study had higher clay (340 g kg^{-1}) than those in Blocks 1 and 2 (260 g kg^{-1}), which were positioned farther up the slope. When the 2005 and 2006 data were grouped accordingly, the numbers of *R. reniformis* were higher in the soils with lower clay: averaged across treatments and years, mean nematode numbers were 11,746 kg^{-1} soil (SE = 3642 kg^{-1} soil) for lower clay soils and 631 kg^{-1} soil (SE = 930 kg^{-1} soil) for higher clay soils. Reniform nematodes correlated negatively with soil clay (r = −0.59, P < 0.01) and positively with sand (r = 0.72, P < 0.01).

In terms of relevance to crop production, it is of interest to know what population of reniform nematodes might be considered detrimental in this region. The threshold for damage according to Patel (1999) is 1000 nematodes kg^{-1} soil for spring samples and 5000 kg^{-1} soil for fall samples. Robinson (2008) suggested similar threshold ranges. Nematode numbers in this study were higher than the threshold in both spring and fall samples.

Earthworm counts were variable (range 11–172). The number of earthworms in the MT soil was marginally higher (P < 0.12) than in the NT soil in 2005, but no effect was observed due to cover crop (Table 7). Other studies have shown that tillage can reduce earthworm populations (Hubbard et al., 1999; Reeleder et al., 2006; Smith et al., 2008; Castellanos-Navarrete et al., 2012). The earthworms were sampled in the spring before planting cotton. Although MT was tilled the previous fall, potential inhibition of earthworm numbers due to tillage may have been offset during the overwinter months.

Table 7. The interaction of tillage (no-till, NT, or minimum tillage, MT) on populations of earthworms and nematodes in soil during the 6-yr cotton study, Stoneville, MS.

Tillage	Abundance of earthworms (2005)	Abundance of nematodes (2006)
	no. m^{-2}	no. kg^{-1} soil
MT	88 a†	8916 a
NT	75 b	6553 b
P	<0.12	<0.06

† Means within a column followed by the same letter are not significantly different.

Other studies have shown that in soils managed with conservation practices, much of the earthworm activity is concentrated near the soil surface, where there is an enhanced accumulation of soil C associated with plant residues (Blanco-Canqui and Lal, 2007; Castellanos-Navarrete et al., 2012). An association of earthworm numbers with TC might therefore be expected. Interestingly, the only significant correlation for earthworms in this study was with TC at the 5- to 15-cm soil depth (r = 0.5, P < 0.05) rather than the 0- to 5-cm range, where TC was more impacted by conservation practice (Table 1).

Soil Microbial Community Structure and Mycorrhizae

Soil microbial community structure based on total FAME profiles in the upper two soil depths (0–2 and 2–5 cm) was significantly affected by cover crop (Table 8; Fig. 2a and 2b). Thirty-six of 40 FAMES were used for analysis for the 0- to 2-cm depth, 29 of 32 for the 2- to 5-cm depth, and 19 of 21 for

Table 8. Analysis of variance of principal components based on microbial community fatty acid methyl ester analysis of soil sampled in 2005. The analysis demonstrates the effects of tillage (no-till, NT, or minimum tillage, MT) and cover crop.

Parameter	Mean principle component ordinates					
	0–2-cm depth		2–5-cm depth		5–15-cm depth	
	PC1	PC2	PC1	PC2	PC1	PC2
Cover crop						
Clover	0.73 b†	1.83 a	1.12 a	−1.26 b	2.34 a	0.88 a
Rye	−3.48 c	−0.72 b	−1.31 b	1.97 a	−1.06 a	−0.94 a
None	2.72 a	1.10 b	0.19 ab	−0.71 b	−0.42 a	−0.46 a
Tillage						
NT	0.23 a	0.10 a	−0.65 a	−0.59 a	0.89 a	−0.22 a
MT	−0.23 a	0.10 a	0.65 a	0.90 a	−0.32 a	−0.11 a
			P values			
Tillage	0.591	0.815	0.591	0.814	0.389	0.877
Cover crop	<0.001	0.022	<0.001	0.0223	0.127	0.216
Tillage × cover crop	0.697	0.802	0.696	0.802	0.552	0.326

† For cover crop or tillage effects, means within a column followed by the same letter are not significantly different (P < 0.05).

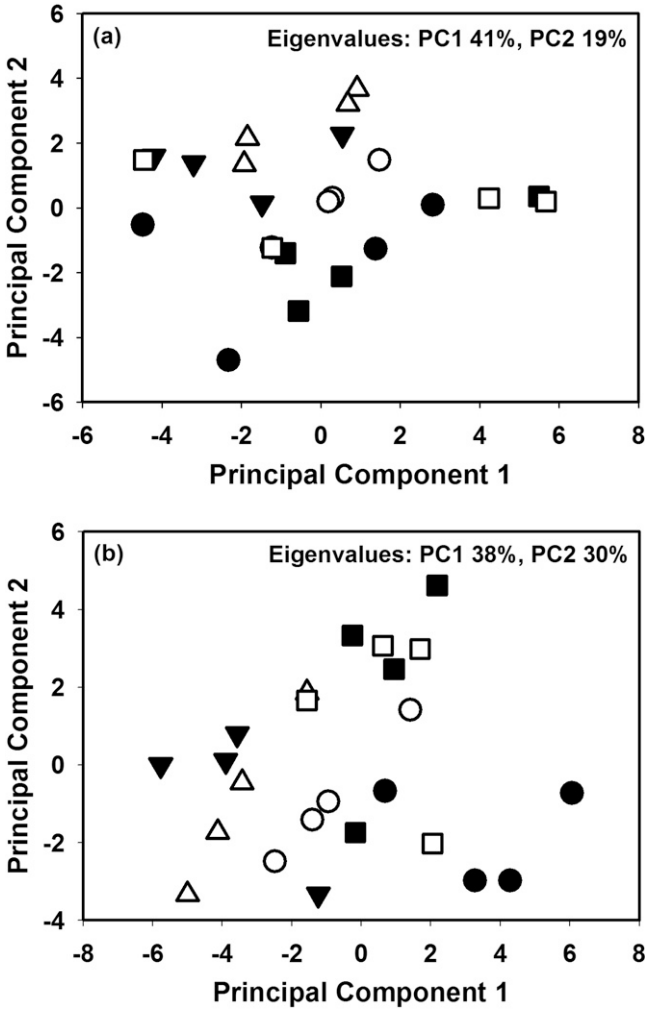

Fig. 2. Principal component ordinates (PC1 and PC2) and respective eigenvalues for tillage and cover crop treatments in 2005 for the (a) 0- to 2-cm depth, and (b) 2- to 5-cm depth, based on fatty acid methyl ester microbial community analysis. Circles represent no cover crop, squares represent Balansa clover cover crop, and triangles represent rye cover crop. Open symbols represent minimum tillage (MT) and filled symbols represent no-till (NT).

Table 9. Occurrence of mycorrhizal biomarker fatty acid methyl ester (FAME) 16ω5c in two soil depths of Dundee silt loam in 2005 and 2006 and mycorrhizal infections in 2006 as affected by cover crop and tillage (no-till, NT, or minimum tillage, MT).

Treatment	Marker 16ω5c occurrence				Mycorrhizal infections (2006)
	2005		2006		
	0–2 cm	2–5 cm	0–2 cm	2–5 cm	
	molar %				no. cm^{-1} root
Cover crop					
Clover	4.44 b†	5.69 b	3.4 ab	4.3 b	0.73 a
Rye	7.63 a	11.8 a	4.0 a	7.5 a	0.62 a
None	3.39 b	7.00 b	3.0 b	4.0 b	0.39 b
Tillage					
MT	4.69 ns	8.67 ns	3.5 ns	5.0 ns	0.59 ns
NT	5.62 ns	7.92 ns	3.5 ns	5.5 ns	0.57 ns
			P values		
Cover crop	<0.001	<0.001	0.049	<0.001	0.002
Tillage	0.117	0.329	0.878	0.350	0.708
Tillage × cover crop	0.604	0.775	0.839	0.393	0.403

† Means within a column followed by the same letter are not significantly different ($P < 0.05$); ns, not significant.

the 5- to 15-cm depth. The greater number of FAMEs found in the upper 2 cm of soil is indicative of a greater richness of the microbial community in the upper sample, which decreased with depth. Tillage did not affect FAME-based community structure, however, nor were any differences observed in the lowest 5- to 15-cm depth (data not shown). Data are presented only for 2005 (Table 8) because a similar trend was observed in 2006. In a nearby soybean field study, tillage had a greater role in altering microbial structure than either a rye or hairy vetch cover crop (Zablotowicz et al., 2010); however, the comparisons in that study were between NT and conventional tillage. It might therefore be expected that tillage differences would be more distinct in that study than the present study, where the comparison was between NT and MT (only one tillage operation). Studies in Alabama assessing community structure based on phospholipid fatty acids showed a greater abundance of certain bacterial and actinomycete biomarkers present in NT than conventional tillage soils, with temporal and spatial variation associated with soil moisture levels and stages of cotton development (Feng et al., 2003). Apparently, the minor perturbation of soil during tillage did not alter FAME-based communities in the present study.

Although there were minor differences in some bacterial FAME biomarkers, e.g., Gram-positive terminal branched fatty acids and Gram-negative monounsaturated fatty acids, a dramatic enhancement of the vesicular arbuscular mycorrhizal (VAM) FAME biomarker 16:1ω5c (Muller et al., 1994; Olsson, 1999) was observed in response to cover crop management (Table 9). This FAME was enriched in both depths of soil from the rye plots in 2005 and 2006; however, the generic fungal biomarker 18:2ω6c (Muller et al., 1994) was slightly higher in the no-cover-crop soils than either cover crop (data not shown). It should be noted that one Gram-negative unsaturated FAME 16:1ω7c was also significantly more prevalent in the rye cover crop soil (7.4 and 9.3% in the upper two soil depths, respectively) compared with <5.6% in the clover and no-cover-crop soils. In some soils extensively colonized by VAM, the hyphae can constitute up to 30% of the total microbial biomass (Read, 1991; Hamel and Strullu, 2006). To assess the implications of the elevated levels of the FAME biomarker 16:1ω5c, fibrous roots collected from cotton plants in 2006 at the initiation of flowering were stained, and mycorrhizal (VAM) infections were assessed (Table 9). A greater abundance of VAM infection was found in roots maintained under both cover crops compared with no cover crop, while there was no effect of tillage. Hamel and Strullu (2006) suggested that FAME analysis can be economically used to assess mycorrhizal potential in soils to facilitate biorational soil management of this symbiosis in crop management. Studies by Curaqueo et al. (2010) in Chile showed a greater abundance of mycorrhizae associated with soils managed under NT than those under conventional tillage management. Because only minor disturbance of the soil occurred under MT in the present study, however, the slight differences in tillage

Table 10. Effect of tillage (no-till, NT, or minimum tillage, MT) on bulk density, infiltration (middle of the furrow with, FWT, and without wheel tracks, FNoWT, and in the row), and water-dispersible clay at three soil depths assessed in 2005 and 2006.

Tillage	Bulk density†	Infiltration rate			Water-dispersible clay					
		2006			2005			2006		
		FNoWT	FWT	Row	0–2 cm	2–5 cm	5–15 cm	0–2 cm	2–5 cm	5–15 cm
	Mg m^{-3}	mm h^{-1}			%					
MT	1.23 b‡	24.4 aB	4.32 aB	87.1 aA	6.30 bB	8.22 bA	8.50 aA	5.11 bB	5.89 bB	8.24 aA
NT	1.26 a	12.3 aA	4.58 aB	33.1 bA	7.87 aB	8.89 aA	8.69 aA	6.79 aB	7.23 aB	8.44 aA
P	<0.06		<0.05			<0.05			<0.06	

† 2005 and 2006 combined.

‡ Means within a column followed by the same lowercase letter are not significantly different; means within a row followed by the same uppercase letter are not significantly different.

might not be expected to have as great of an effect on either microbial community structure or the incidence of mycorrhizae. The stimulation of mycorrhizae under cover crop management is an important benefit to sustainable agriculture because plants that are well colonized by VAM are more resistant to moisture deficits and have improved mineral nutrition, especially N and P (Read, 1991). In addition, the network of mycorrhizae provides enhanced soil structure stability (Hamel and Strullu, 2006).

Soil Physical Characteristics

Both tillage and cover crop influenced the soil bulk density in the surface 15 cm (Tables 10 and 11). No-till had a higher bulk density (Table 10), which might be expected from soil compaction after 5 yr without tillage. Surprisingly, the soil with no cover crop had the lowest bulk density (Table 11). Regardless of tillage or cover crop, bulk density in the plant row was lower than in the furrows with and without wheel tracks (Table 11). The furrow with the wheel track had the highest bulk density.

The major factor influencing water infiltration was intrarow position. Regardless of tillage or cover crop, the lowest infiltration rates (Tables 10 and 12) were measured in the furrows, where bulk density was higher, particularly in the furrows that involved wheel traffic. The opposite trend occurred with measurements taken within the plant row. Some differences occurred between years. The infiltration rate was slower in both furrow positions (with and without wheel tracks) than in the plant row position for the rye and clover plots in 2005 (Table 12). In 2006, infiltration was higher in the row position than in the furrows regardless of cover crop, but was most rapid in the clover cover crop. No significant tillage effect was observed for infiltration in 2005, but for plant-row infiltration rate measurements, higher infiltration rates occurred in 2006 under MT (Table 10) and in both years for clover plots (Table 12), corresponding to the lower bulk densities in the rows (Table 11).

Both cover crops improved aggregate stability in all three soil depths relative to no cover crop (Table 12), but no effect of tillage was observed (data not shown). Regardless of cover crop or tillage, aggregate stability was highest in the surface 0 to 2 cm and declined with each incremental depth (70.4 > 59.9 > 49.7% for 0–2-, 2–5-, and 5–15-cm depths, respectively). The decline in aggregate stability with depth may be due in part to a relationship with soil total organic C, which slightly correlated with aggregate stability ($r = 0.33$, $P < 0.01$).

Overall, water-dispersible clay increased with soil depth (Table 10), primarily due to increasing soil clay with depth. Water-dispersible clay in the surface was higher under NT than MT (Table 10), but no differences due to tillage were observed at depths below 5 cm. The slightly lower water-dispersible clay in the surface soil under MT may have resulted from mixing with the subsurface soil each year during tillage. Cover crop treatments did not influence water-dispersible clay.

SUMMARY AND CONCLUSIONS

The overall objective of this study was to evaluate changes in soil with time as a result of implementing two conservation tillage systems: (i) a complete NT system; and (ii) a system where there was one tillage operation in the fall following harvest. Under both tillage systems, surface residues from cover crops and winter vegetation were not disturbed when the cotton crop was planted in the spring. A second aspect of this study compared either a cereal (rye) or leguminous (Balansa clover) cover crop with native winter vegetation. The following general observations can be made from these studies assessing conservation tillage and cover crop management:

1. Soil TC and microbial activity were greater in the surface of NT and cover crop soils than MT and no-cover-crop soils. In particular, the Balansa clover cover crop enhanced microbial activity and TN in the surface soil. These differences declined with depth.

Table 11. Effect of cover crop and row position (middle of the furrow with, FWT, and without wheel tracks, FNoWT, and in the row) on soil bulk density in 2005 and 2006 combined.

Parameter	Bulk density
	Mg m^{-3}
Cover crop	
No cover	1.23 b†
Clover	1.24 ab
Rye	1.26 a
P	<0.06
Row position	
FWT	1.32 a
FNoWT	1.22 b
Row	1.19 b
P	<0.05

† Means followed by the same letter are not significantly different.

Table 12. Effect of cover crop and row position on soil aggregate stability at three soil depths and water infiltration into soil at three row positions (middle of the furrow with, FWT, and without wheel tracks, FNoWT, and in the row) in 2005 and 2006.

Cover crop	Aggregate stability†			Infiltration rate					
				2005 (n = 2)			2006 (n = 4)		
	0–2 cm	2–5 cm	5–15 cm	FNoWT	FWT	Row	FNoWT	FWT	Row
	%			mm h^{-1}					
No cover	64.9 b‡	48.1 b	44.2 b	4.83 aA	2.40 aA	17.6 bA	31.3 aB	6.8 aB	45.1 bA
Clover	73.7 a	67.6 a	55.6 a	7.68 aB	2.32 aB	86.6 aA	14.6 aB	4.2 aB	85.9 aA
Rye	72.7 a	64.1 a	48.9 ab	2.78 aB	1.72 aB	36.7 bA	9.14 aB	2.35 aB	49.3 bA
P	<0.01	<0.05	<0.13		<0.07			<0.11	

† 2005 and 2006 combined.

‡ Means within a column followed by the same lowercase letter are not significantly different; means within a row followed by the same uppercase letter are not significantly different.

2. The benefits of cover crops on soil quality via increasing soil TC and TN and stimulating soil microflora are well documented. Stimulation of mycorrhizal populations and infection in soil with cover crops such as rye is an additional benefit from a soil quality perspective. With the need to conserve soil moisture and soil fertility, the benefits of mycorrhizae in sustainable cropping systems may be dramatically improved using appropriate cover crop management.

3. Only minor differences in biological and physical characteristics were observed between the two tillage practices, probably because the disturbance from tillage was moderate compared with that observed with a conventional tillage system in another study on the same soil (Locke et al., 2005). Therefore, the benefits from a cover crop were equivalent for both tillage systems.

4. Overall improvement in soil quality with time was observed for both conservation tillage practices and both cover crops. The implications for farmers are that, given parity in crop production, any combination of these management practices should provide similar environmental benefits.

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