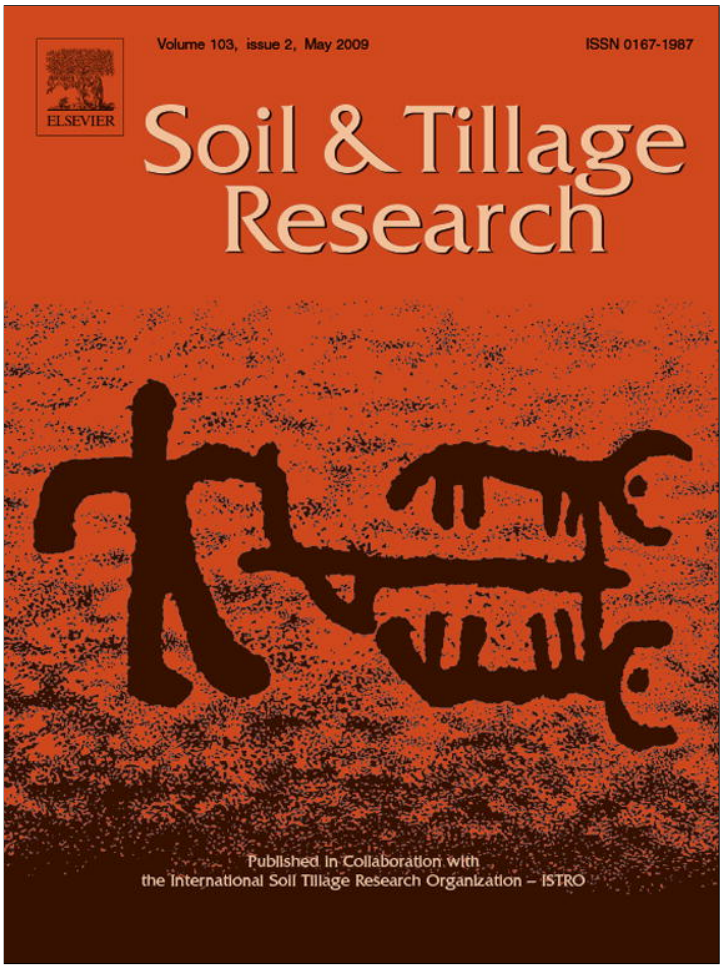

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Soil microbial communities under different soybean cropping systems: Characterization of microbial population dynamics, soil microbial activity, microbial biomass, and fatty acid profiles

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ABSTRACT

This work analyzes the direct effect of soil management practices on soil microbial communities, which may affect soil productivity and sustainability. The experimental design consisted of two tillage treatments: reduced tillage (RT) and zero tillage (ZT), and three crop rotation treatments: continuous soybean (SS), corn–soybean (CS), and soybean–corn (SC). Soil samples were taken at soybean planting and harvest. The following quantifications were performed: soil microbial populations by soil dilution plate technique on selective and semi-selective culture media; microbial respiration and microbial biomass by chloroform fumigation-extraction; microbial activity by fluorescein diacetate hydrolysis; and fatty acid methyl ester (FAME) profiles. Soil chemical parameters were also quantified. Soil organic matter content was significantly lower in RT and SS sequence crops, whereas soil pH and total N were significantly higher in CS and SC sequence crops. *Trichoderma* and *Gliocladium* populations were lower under RTSS and ZTSS treatments. Except in a few cases, soil microbial respiration, biomass and activity were higher under zero tillage than under reduced tillage, both at planting and harvest sampling times. Multivariate analyses of FAMEs clearly separated both RT and ZT management practices at each sampling time; however, separation of sequence crops was less evident. In our experiments ZT treatment had highest proportion of 10Me 16:0, an actinomycetes biomarker, and 16:1ω9 and 18:1ω7, two fatty acids associated with organic matter content and substrate availability. In contrast, RT treatment had highest content of branched biomarkers (i15:0 and i16:0) and of cy19:0, fatty acids associated with cell stasis and/or stress. As cultural practices can influence soil microbial populations, it is important to analyze the effect that they produce on biological parameters, with the aim of conserving soil richness over time. Thus, in a soybean-based cropping system, appropriate crop management is necessary for a sustainable productivity without reducing soil quality.

1. Introduction

Soybean production in Argentina has increased from 0.01 million to more than 27 million hectares in 30 years, making it the world's third largest producer (Sinclair et al., 2007). Due to the importance that the soybean crop has for Argentina, there is a great necessity to preserve soil health over time to support this huge production for the coming years, through the employment of the appropriate cultural practices.

Crop management has a direct effect on soil microbial communities, thus affecting soil productivity and sustainability. The productivity and health of agricultural systems depend greatly upon the functional processes carried out by soil microorganisms, ranging from nutrient cycling and residue decomposition to soil structural component, clearly affecting plant growth and the final crop quality (Pankhurst et al., 1996). As it is well known, ecosystem functioning is governed largely by soil microbial dynamics, being microbial communities affected by production practices such as management system (Germida and Siciliano, 2001).

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Analyzing the response of microorganisms to different cultural practices is important because soil microbiota respond quickly to environmental changes, so they are expected to be efficient bioindicators of soil conditions (Avidano et al., 2005).

The prevalence of soilborne pathogens (like *Fusarium* or *Stramenopile*) and other deleterious organisms in soybean crop can cause severe disease and/or reduce plant vigour, growth and crop yields. In contrast, an abundance of beneficial root and soil organisms can suppress soybean pathogens and diseases, improve plant nutrition, promote growth, and increase productivity (Lévesque and Rahe, 1992; Larkin, 2003). For this reason, quantification of soil microorganisms (such as *Trichoderma* spp., *Gliocladium* spp. or actinomycetes) involved in the biological control of soilborne pathogens may be useful to assess soil health (Sparling, 1997).

Numerous studies have compared microbial communities from soils subjected to different crop management practices by analyzing the influence of rhizosphere from different plant species (Miethling et al., 2000; Marschner et al., 2001; Buyer et al., 2002; Kourtev et al., 2003; Azevedo et al., 2005), different ambient conditions (Pankhurst et al., 2001; Acosta-Martínez et al., 2003), and agrochemicals and fumigants (Ibekwe et al., 2001; Reinecke et al., 2005; Meriles et al., 2006). Some authors (Lupwayi et al., 1998) reported that soil microbial diversity under wheat was affected by rotation and tillage system. Also Drijber et al. (2000) demonstrated changes in soil bacterial communities under long-term wheat-fallow management. However, as Lupwayi et al. (1998) and Larkin (2003) asserted, few experiments have documented the effects of different tillage and rotation crop management practices on soil microbial communities in a given crop production system.

Some studies have confirmed that whole-community FAME profiles constitute reproducible signature useful to characterize soil microbial communities (Schutter and Dick, 2000; Drenovsky et al., 2004). The information generated from FAME profiles, together with that obtained from the dilution-plating technique, may provide specific data about the effects of tillage and rotation management on multiple aspects of soil microbial characteristics. It is hypothesized that the use of conservation agriculture technologies based on zero tillage (ZT) and crop rotation results in a significant change in several soil chemical properties, microbial populations and microbial community structure as compared to current practices of intensive tillage and continuous crop. The objective of this paper is to examine the effects of different soybean cropping management systems on the characteristics and structure of the soil microbial community.

2. Materials and methods

The long-term tillage and rotation experiment (12 years) was conducted on a typical Haplustoll soil (clay 19.2%; loam 68.3%, silt 12.5%) at Manfredi Experimental Station – INTA, Córdoba Province, Argentina. The field experiment was divided into plots (35 m × 180 m), and planted with two crops, combined in the following three cropping sequences: continuous soybean (SS), corn–soybean (CS), and soybean–corn (SC). These two crops were planted in October, and were annually rotated, being both present in the same crop cycle. Each crop was under two different tillage systems: zero tillage and reduced tillage (RT). Reduced tillage involved disking in fall, with a minimum of one tillage operation (depending on weeds) and another tillage operation 3 days before seeding. Crops under zero tillage were planted in the stubble of the previous year. Thus, the experimental design consisted of two blocks for each one of the tillage systems (ZT and RT), each block contained six plots, with two replicate plots of each treatment. Details of the field plot managements are shown in Table 1.

Table 1
Management practices carried out on the experimental plots.

Date	Management	
September (previous year)	Zero tillage Herbicide treatment	Reduced tillage Herbicide treatment Ploughing
October (previous year)	Crop planting	Crop planting
November (previous year)	Pre-emergent herbicide treatment Soil sampling	Pre-emergent herbicide treatment Soil sampling
December (previous year)	Soil fertilization for maize	Soil fertilization for maize
January	Insecticide treatment	Insecticide treatment
February	Soil sampling	Soil sampling
May	Crop harvest	Crop harvest

Sampled plots had never been treated with inorganic fertilizer nitrogen. The soil was sampled randomly on 15 November 2005 (13 days after crops planting) and 28 February 2006 (12 days before crops harvest), at a depth of 0–5 cm. Six soil cores (2 cm diameter) were composited from each plot. Seventy-two composite soil samples per sampling time were collected from the site. Soil was transported to the laboratory in a cooler and stored at 4 °C for less than 48 h; then it was sieved (2 mm pore) to remove visible organic residues. After thorough mixing, subsamples were taken for chemical analyses, water content, microbial biomass determination, dilution-plating procedure, and extraction of lipids. Parameters were estimated from the mass of oven-dry soil collected from the six cores.

2.1. Soil chemical analyses

Soil pH was determined on 1:5 soil/0.01 M $CaCl_2$ extracts. Electrical conductivity (EC) was determined on 1:5 water extracts, by oversaturation method (Allison, 1973). Total N was measured using Kjeldahl equipment. Soil organic matter (SOM) content was determined by dry combustion using a LECO CHN-2000 analyzer (St. Joseph, MI, USA). NO_3-N and HCO_3-ext P were determined according to methods detailed by Nelson and Sommers (1982) and Kros et al. (2004).

2.2. Cultivable microbiological populations

General microbial populations were determined by soil dilution plating on selective or semi-selective media. Cultivable *Trichoderma*, *Gliocladium*, and total fungi populations were counted on PDA supplemented with rose bengal (20 mg l^{-1}), streptomycin (100 mg l^{-1}) and chloramphenicol (300 mg l^{-1}), according to Vargas Gil et al. (in press). *Stramenopile* populations were counted on maize meal agar supplemented with the following antifungal agents (per liter of distilled water): benomyl (Benlate, 50% active), 250 mg; PCNB (pentachloronitrobenzene), 100 mg; and with the following antibacterial agents: riphampicin, 10 mg and ampicillin, 250 mg. This is a modification of the medium used by Jeffers and Martin (1986). Actinomycetes colonies were counted on Küster medium modified by the addition of cycloheximide (0.15 g l^{-1}) and sodium propionate (0.4 g l^{-1}), according to previous work (Vargas Gil et al., in press). Details of media, dilution factors and incubation conditions are shown in Table 2.

Table 2
Soil populations of selected subgroups of microorganisms following cropping under various tillage and rotation crop systems, as determined by soil dilution plating on selective and semi-selective media.

Organism(s) cultured	Medium	Dilution factor[a]	Culture conditions			Reference
			Incubation (days)	Temperature (°C)	Light conditions	
Trichoderma spp.	Supplemented PDA	10^{-2}	7–10	22	Light	Vargas Gil et al. (in press)
Gliocladium spp.	Supplemented PDA	10^{-2}	7–10	22	Light	Vargas Gil et al. (in press)
Stramenopile	Corn meal agar[b]	10^{-1}	2–3	25	Dark	Jeffers and Martin (1986)
Total fungi	Supplemented PDA	10^{-2}	20–25	22	Light	Vargas Gil et al. (in press)
Actinomycetes	Küster medium[c]	10^{-4}	20	25	Light	Vargas Gil et al. (in press)

[a] Dilution factor number is the 1:10 serial dilution from each sample that was plated in duplicate.
[b] Corn meal agar with 250 mg l^{-1} benomyl, 100 mg l^{-1} PCNB; 10 mg l^{-1} riphampicin, and 250 mg l^{-1} ampicillin. This is a modification of the medium used by Jeffers and Martin (1986).
[c] Küster medium modified with cycloheximide (0.15 g l^{-1}) and sodium propionate (0.4 g l^{-1}).

2.3. Soil microbial respiration, biomass and activity

Soil respiration was measured by placing 10 g of each soil samples into 100 ml glass bottle, and then incubated with NaOH 0.2N at 28 °C during 7 days. The release of CO_2 was analyzed through the employment of HCl (0.2N). Microbial biomass C was determined according to Alef (1995). The fumigation–reinoculation technique was employed. The amount of CO_2 released was measured from soil samples (20 g) that were previously fumigated with chloroform, de-fumigated, inoculated with fresh soil, and incubated with NaOH (0.2N) during 10 days at 28 °C. Non-fumigated soil samples were incubated at the same time. The released CO_2 was measured with HCl (0.2N). For the quantification of soil microbial biomass C and microbial respiration, control treatments were included, which received the above-mentioned treatments, without containing soil.

General microbial activity was measured by hydrolysis of fluorescein diacetate (FDA) using the procedure of Adam and Duncan (2001). Briefly, 2 g of soil were placed in a 50 ml conical flask and 15 ml of 60 mM potassium phosphate buffer pH 7.6. Substrate (FDA, 1000 μg ml^{-1}) was added to start the reaction. The flasks were stoppered and the contents shaken by hand. The flasks were then placed in an orbital incubator at 30 °C for 20 min, 100 rpm. Once removed from the incubator, 15 ml of chloroform/methanol (2:1 v/v) were added immediately to terminate the reaction. Stoppers were replaced on the flasks and the contents shaken thoroughly by hand. The contents of the conical flasks were then transferred to 50 ml centrifuge tubes and centrifuged at 2000 rpm for 3 min. The supernatant from each sample was then filtered into 50 ml conical flasks and the filtrates measured at 490 nm on a spectrophotometer.

2.4. Soil microbial community structure

Soil community FAME profiles were constructed from whole soil extractions of fatty acids according to a modification of the Microbial Identification System (MIS; Microbial ID, Inc., Newark, DE 1998) standard protocol. Extractions were conducted on each of the six 10-g soil subsamples per plot. Lipids were saponified by adding 10 ml 3.25 M NaOH in methanol to each sample and heated in a 100 °C water bath for 30 min. Samples were then methylated by adding 10 ml 3.25N HCl in methanol, mixed, and placed in an 80 °C water bath for 10 min. The FAMEs were extracted from this solution by adding 5 ml of 1:1 methyl tert-butyl ether:hexane mixture. The top, organic phase was transferred to a clean culture tube and washed using 3 ml of dilute NaOH. The organic phase was then transferred to a vial for subsequent analysis by gas chromatography using a Clarus 500 PerkinElmer gas chromatograph equipped with an Elite-5 capillary column and flame ionization detector. Identification of the FAMEs was by comparison of retention time and equivalent chain length with known standards (Bacterial Acid Methyl Esters CP Mix, Supelco, USA) and confirmed by gas chromatography–mass spectrometry (GC–MS). Unsaturated and substituted FAMEs were identified by GC–MS as dimethyldisulfide and dimethyloxazoline derivatives (Drijber et al., 2000).

Standard nomenclature is used to describe FAMEs. Numbering of carbons begins at the aliphatic (ω) end of the fatty acid molecule. The number of double bonds within the molecule is given after the colon. Cis and trans conformations are designated with the suffixes "c" and "t", respectively. Other notations are "Me" for a methyl group, "OH" for hydroxyl, "cy" for cyclopropane groups, and the prefixes "i" and "a" for iso- and anteiso-branched FAMEs.

2.5. Statistical analyses

Data of soil chemical properties, cultivable microbial populations, microbial activity and biomass were analyzed through standard analyses of variance (ANOVA). In all cases, residuals were tested for normality via Shapiro-Wilks' test, and non-normal data were transformed using log scale when required. To test for differences between means an LSD-test at level of significance ($P \leq 0.05$) was used. The percent distribution of FAMEs was analyzed with INFOSTAT/Professional 2007 p.1 (F.C.A. – Universidad Nacional de Córdoba, Argentina) using principal component analyses (PCA). Two-dimensional plots of the first two principal components were made to assess the overall patterns of variation in FAMEs composition and differences due to tillage, crop sequence and sampling time. PCA was performed on combined FAMEs data of whole sampling dates as well as FAMEs data of each sampling date. Non-transformed means are reported in figures, tables, and text.

3. Results

3.1. Soil chemical properties

A summary of the chemical properties of soils under different tillage and rotation treatments is presented in Fig. 1. Soil organic matter content ranged from 2.00 to 3.18% at planting, and from 2.10 to 3.19% at harvest. There were no statistical significant differences between SOM at planting time, compared with harvest time. However, there were significant differences in SOM content between tillage treatments and crop sequence, within each sampling time. At planting, in the SC sequence, SOM was significantly higher when corn was under ZT compared with RT. The effect of previous crop on SOM content was also evident with the SOM being significantly higher under the CS sequence under RT. However, this same tendency was not observed when all the crops were under ZT because the SOM content was higher in

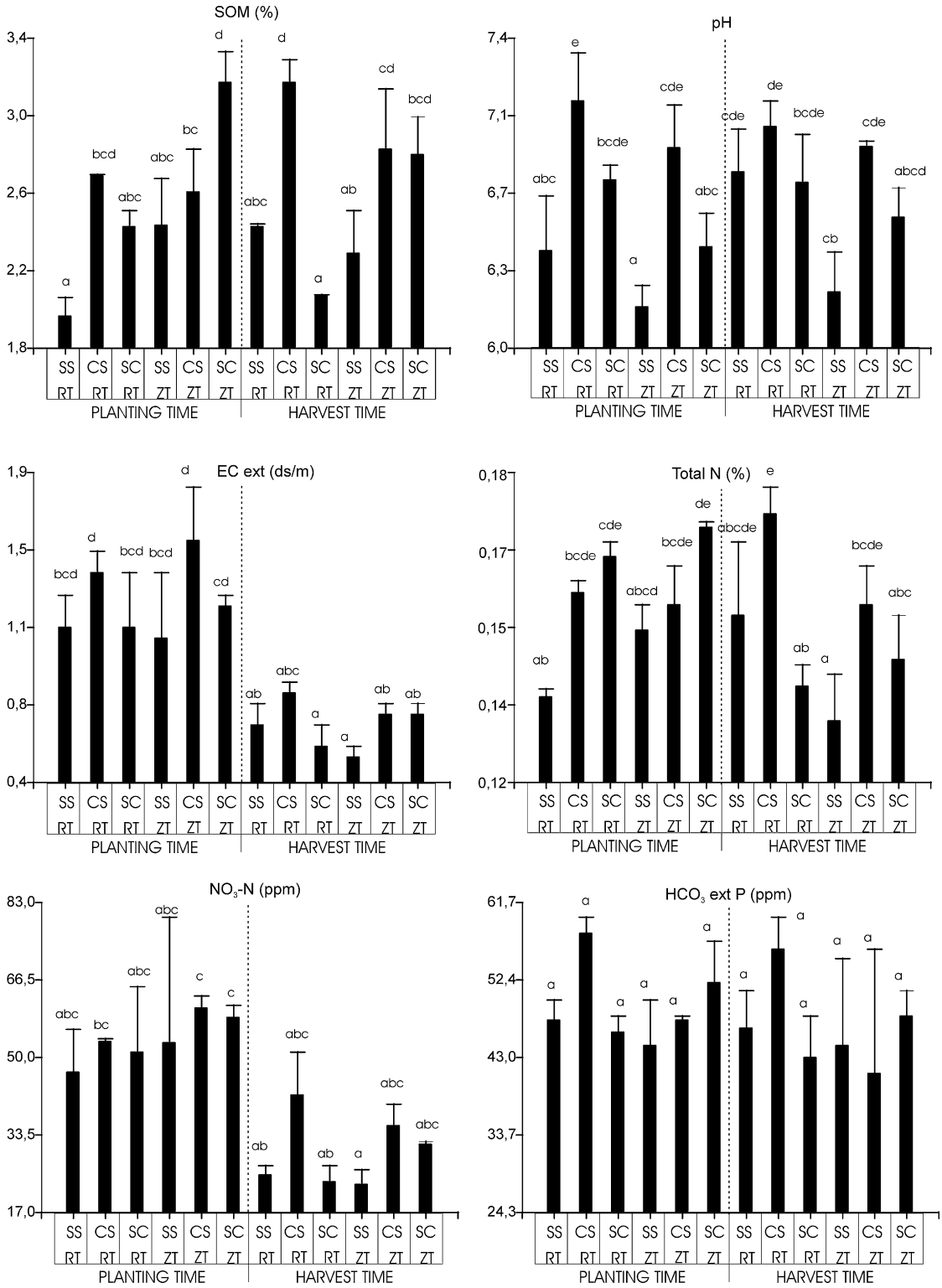

Fig. 1. Soil properties as function of tillage and crop sequence treatments at two sampling times. Error bar represents one standard error of the mean. RT, reduced tillage; ZT, zero tillage; SS, continuous soybean; CS, corn–soybean sequence; SC, soybean–corn sequence. Bars followed by the same letter are not significantly at $P \leq 0.05$.

the SC sequence. At harvest, in the SC sequence, SOM was significantly higher when corn was under ZT compared with RT, just as at the planting time. However, the effect of the previous crop on SOM was more evident at the harvest time, registering significantly higher values when corn was the previous crop, compared with soybean monoculture and soybean as a precedent crop.

There were no significant differences between pH values at planting and harvest time, except for the SS sequence under ZT. At planting time, there were no significant differences between pH values regardless the tillage system. Under continuous soybean, pH was more acidic than in the SC rotation. At harvest time, no significant differences were observed between the pH values for any crop sequence or tillage system.

Table 3
Analysis of variance significant levels for soil chemical properties from soil under different tillage and sequence crop treatments evaluated at two sampling times.

Source of variation	SOM[a]	pH	EC ext[b]	Total N	NO_3-N	HCO_3 ext P
Tillage, T	*	*	NS	NS	NS	NS
Sequence crop, S	**	**	NS	*	NS	NS
Sampling date, D	NS	NS	***	NS	**	NS
T × S	**	NS	NS	NS	NS	NS
T × D	NS	NS	NS	NS	NS	NS
S × D	*	NS	NS	*	NS	NS

NS, not significant ($P > 0.05$).
[a] Soil organic matter.
[b] Electrical conductivity.
* Significant level: $P \leq 0.05$.
** Significant level: $P \leq 0.01$.
*** Significant level: $P \leq 0.001$.

In general, EC was significantly lower at harvest time, compared with planting time. However, no differences in EC were observed among the crop sequences or tillage systems.

Total N content ranged from 0.14 to 0.17% at planting, and from 0.13 to 0.18% at harvest. During the corn crop, in the SC sequence and independently of the tillage system, total N decreased from the planting toward the harvest time. Tillage systems did not exert any effect on total N content at any sampling time. At planting time, for the SS sequence, total N was significantly lower than for the SC or CS sequences. At harvest, for the RT treatment, total N was higher in the CS than in the SC sequence.

For NO_3 and P, there were no significant differences between the values obtained at planting and at harvest time. Neither the different tillage systems nor the crop sequences influenced on the NO_3 and P levels, regardless of the sampling time. Except for SOM, no significant interaction between tillage and sequence crop was detected ($P \leq 0.05$) for the soil chemical parameters evaluated (Table 3).

3.2. Soil microbial populations

In general, tillage and sequence crop affected propagule densities of soil microorganisms of the genera *Trichoderma*, *Gliocladium*, total fungi, *Stramenopile* and Actinomycetes (Fig. 2). Propagule densities of *Trichoderma* population ranged from 0.75×10^4 to 1.25×10^4 colony forming units (CFU)/g of dry soil at planting, and from 0.51 to 0.91×10^4 CFU/g of dry soil at harvest. *Trichoderma* population at planting time was maintained at harvest, with the exception of the CS treatment under ZT, in which *Trichoderma* propagules were lower at harvest time.

With few exceptions, at both sampling times, the number of *Trichoderma* propagules was higher in plots with corn as the preceding crop than in plots with soybean as the preceding crop (SC and SS sequences). Trichoderma populations were differently affected by tillage treatments at planting time. However, no statistical differences were detected between RT and ZT treatments. There was no tillage × sequence crop interaction in propagule densities of *Trichoderma* at either sampling date (Table 4).

Propagule densities of *Gliocladium* population were slightly affected by both tillage and sequence crop, not showing a clear tendency (Fig. 2). The population of *Gliocladium* ranged from 0.25×10^4 to 1.01×10^4 CFU/g of dry soil at planting, and from 0.31×10^4 to 0.80×10^4 CFU/g of dry soil at harvest. The *Gliocladium* populations registered at planting did not show significant differences compared harvest time. There was no tillage × sequence crop interaction in propagule densities of *Gliocladium* (Table 4).

Stramenopile was affected by tillage and sequence crop (Fig. 2). Generally, propagule densities of *Stramenopile* were significantly higher in soils under ZT and SC sequence crop, ranging from 2.7×10^3 to 12.6×10^3 CFU/g of dry soil. There was a significant interaction between tillage and sequence crop at $P \leq 0.05$ (Table 4).

For total fungi, the population values at planting time generally were the same as harvest time, with the exception of some cases that showed a slight decrease toward harvest (Fig. 2). Total fungi population ranged from 49.2×10^4 to 85.6×10^4 CFU/g of dry soil. At planting, generally the highest population of total fungi was detected under RT. This tendency was no evident at harvest. Total fungi were significantly higher in the SS sequence at planting, but under the same treatment were significantly lower at harvest. This shows that although the crop sequence affected the development of total fungi in soil, no clear tendency was observed. No significant interaction was recorded between tillage and sequence crop (Table 4).

Actinomycetes population ranged from 1.6×10^6 to 5.4×10^6 CFU/g of dry soil. In general, actinomycetes population were the same at planting and at harvest time. Soil populations were significantly higher under RT, compared with ZT. There were also significantly higher in the SS sequence but only when soybean was under RT. A highly significant tillage × sequence crop interaction was detected ($P \leq 0.01$) (Table 4).

3.3. Soil microbial respiration, biomass and activity

Microbial respiration was affected by both tillage and sequence crop treatment. There was a clear decrease in soil microbial respiration in samples taken at planting, compared with those taken at harvest (Fig. 3). At both sampling times, microbial respiration was higher under ZT and SC treatment combination. At planting, SC under ZT or RT, and the ZT and SS treatment combination showed the highest values of microbial respiration. The lowest values were found under CS, even in ZT or RT. However, the highest value of microbial respiration obtained at harvest was for the ZT and CS treatment combination (Fig. 3). The values ranged

Table 4
Analysis of variance significant levels for *Trichoderma*, *Gliocladium*, *Stramenopile*, total fungi, and Actinomycetes populations from soil under different tillage and sequence crop treatments evaluated at two sampling times.

Source of variation	*Trichoderma* spp.	*Gliocladium* spp.	*Stramenopile*	Total fungi	Actinomycetes
Tillage, T	NS	NS	***	NS	**
Sequence crop, S	*	*	***	*	NS
Sampling date, D	*	NS	NE	***	NS
T × S	NS	NS	*	NS	**
T × D	NS	*	NE	NS	**
S × D	NS	NS	NE	***	**

NE, no estimated; NS, not significant ($P > 0.05$).
* Significant level: $P \leq 0.05$.
** Significant level: $P \leq 0.01$.
*** Significant level: $P \leq 0.001$.

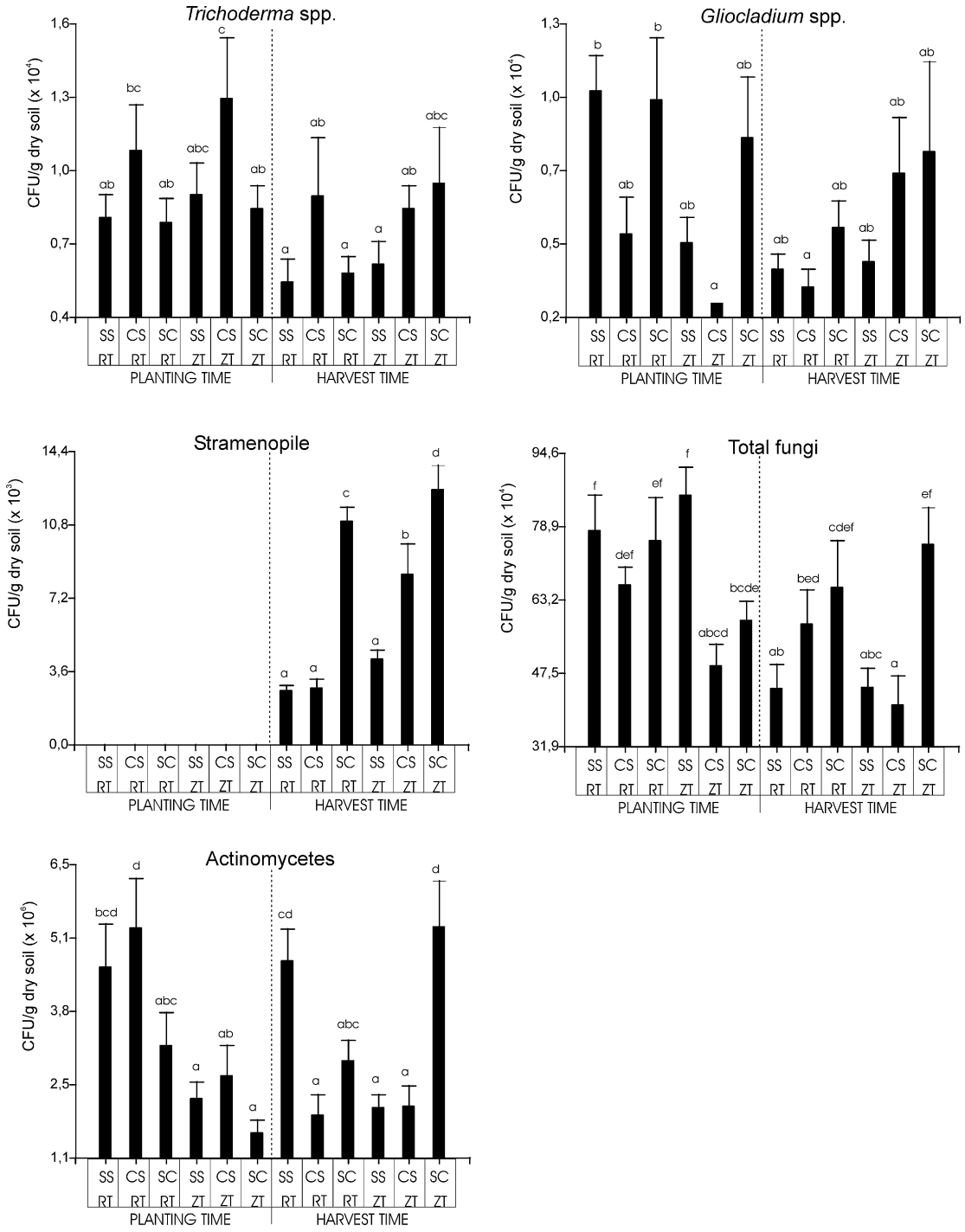

Fig. 2. Selected soilborne microorganisms as function of tillage and crop sequence treatments at two sampling times. Error bar represents one standard error of the mean. RT, reduced tillage; ZT, zero tillage; SS, continuous soybean; CS, corn–soybean sequence; SC, soybean–corn sequence. Bars followed by the same letter are not significantly at $P \leq 0.05$.

between 0.3 and 0.6 mg CO_2 g^{-1} $week^{-1}$ at planting, and between 0.2 and 0.5 mg CO_2 g^{-1} at harvest. A significant tillage × sequence crop interaction was observed (Table 5). Microbial biomass and activity were greatly affected by both tillage and crop sequence. Also microbial biomass showed a decrease in harvest time, compared with planting time. The values ranged between 0.5–0.8 and 0.3–0.8 mg CO_2 g^{-1} at planting and harvest, respectively. At both sampling times, soil microbial biomass was higher under ZT treatment (Fig. 3). No significant interaction between tillage and crop sequence was observed (Table 5).

Soil microbial activity, as determined by FDA hydrolysis, varied depending on the sampling time (Fig. 3). FDA hydrolysis showed no significant differences between sampling times. At planting, the highest value of microbial activity was observed under the ZT and SS treatment combination, ranging between 57.1 and 111.1 μg fluorescein g^{-1} soil. At harvest, microbial activity was significantly higher under ZT treatment than under RT treatment. The values ranged between 47.9 and 111.7 μg fluorescein g^{-1} soil. There was no significant tillage × sequence crop interaction was found (Table 5).

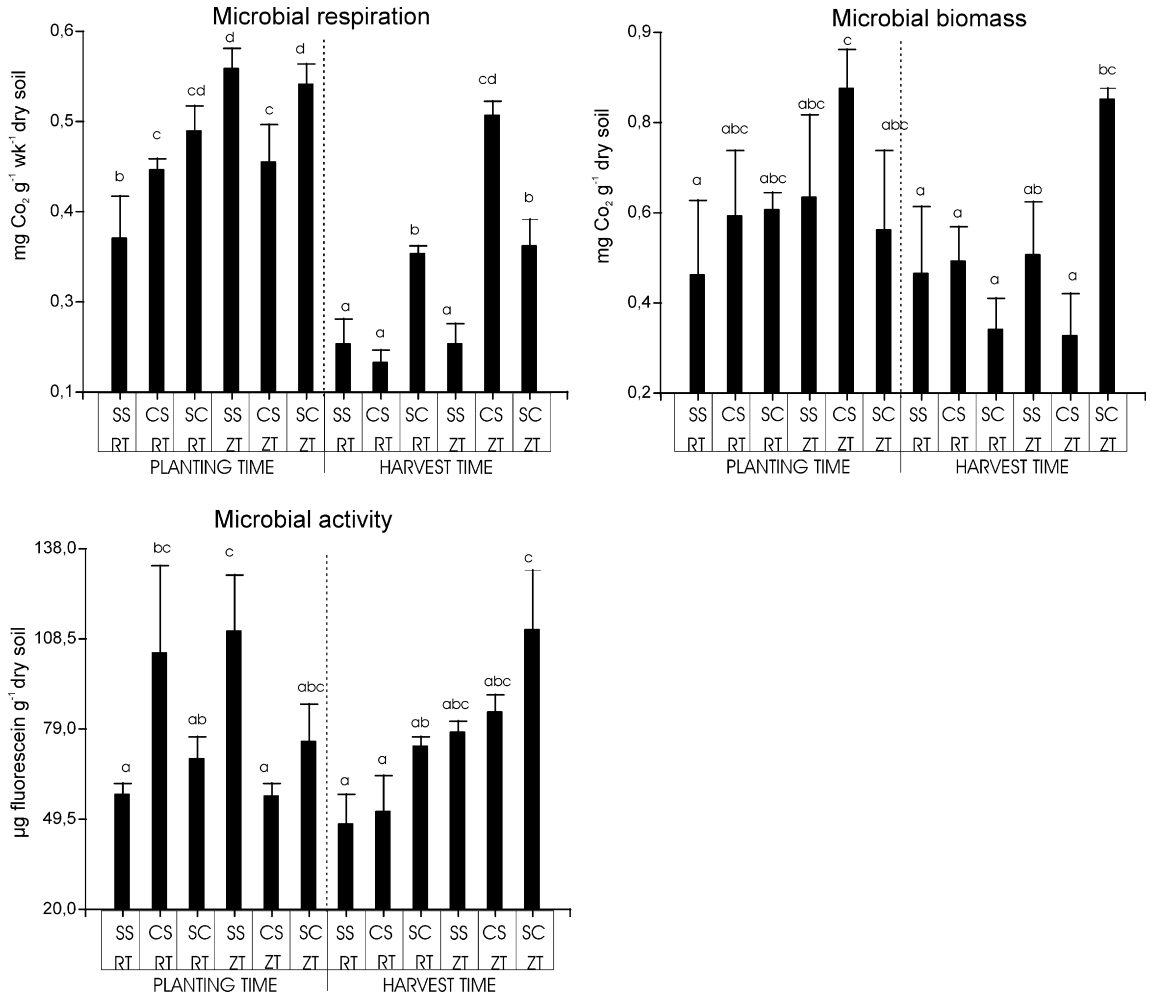

Fig. 3. Microbial respiration, microbial biomass, and microbial activity as function of tillage and crop sequence treatments at two sampling times. Error bar represents one standard error of the mean. RT, reduced tillage; ZT, zero tillage; SS, continuous soybean; CS, corn–soybean sequence; SC, soybean–corn sequence. Bars followed by the same letter are not significantly at $P \leq 0.05$.

3.4. Soil community structure

The response of the soil microbial community to tillage and sequence crop was determined from all the FAMEs identified in the extract (Table 6). In general, the soil samples studied were dominated by eubacterial fatty acids (Gram-negative: 12:0 2OH, 12:0 3OH, 14:0 2OH, 14:0 3OH, cy 17:0, 16:0 2OH, cy 19:0; Gram positive: i15:0, a15:0, i16:0, i17:0). In most cases, ANOVA of individual fatty acids for planting and harvest soil samples showed statistical differences between tillage and sequence crop treatments. The SC and CS crop sequences had high percentages of the unsaturated fatty acid 18:1ω7 and 18:1ω9, and of the fatty acids 13: 0, a15:0, and 10Me 16:0. The fatty acid 18:2ω6.9 is considered an indicator of fungal biomass. In our study, this fatty acid accounted for 0.38–3.85 of the total FAMEs, and was negatively correlated with ZT tillage treatment. In addition, the bacterial/fungal ratio was lower in plots under RT, due to an increase in bacterial fatty acids in this treatment (Table 6).

Table 5
Analysis of variance significant levels for microbial respiration, microbial biomass, and microbial activity from soil under different tillage and sequence crop treatments evaluated at two sampling times.

Source of variation	Microbial respiration	Microbial biomass	Microbial activity
Tillage, T	***	NS	*
Sequence crop, S	***	NS	NS
Sampling date, D	***	NS	NS
T × S	*	NS	*
T × D	NS	NS	NS
S × D	**	NS	NS

NS, not significant ($P > 0.05$).
* Significant level: $P \leq 0.05$.
** Significant level: $P \leq 0.01$.
*** Significant level: $P \leq 0.001$.

Principal component analyses of FAMEs data demonstrated distinct differences in soil fatty acid profiles among cropping systems (Fig. 4). PCA clearly separated the two tillage management systems. The first two axes of PCA explained 61 and 58% of the data variation at soybean planting and harvest, respectively. The analysis of individual fatty acid for whole sampling dates also revealed a separation between tillage treatments (Fig. 5a). However, the separation between sequence crop treatments was less evident. PC1 was positively correlated with RT and negatively correlated with ZT treatment. PC1 was negatively correlated with straight chain fatty acid (12:0, 14:0, 20:0) and monounsaturated (16:1ω9, 18:1ω7), whereas 18:1ω9, 16:0 2OH, cy19:0, and some branched fatty acids (i15:0, i16:0) were positively correlated with PC1. PC2 was positively correlated with hydroxyl fatty acids (12:0 2OH, 12:0 3OH, 14:0 3OH) (Fig. 5b).

Table 6
Phospholipid fatty acids from soil under different tillage and sequence crop treatments evaluated at two sampling times.

Fatty acid (% dry soil)	Planting time						Harvest time						LSD	Significance					
	RTSS	RTCS	RTSC	ZTSS	ZTCS	ZTSC	RTSS	RTCS	RTSC	ZTSS	ZTCS	ZTSC		T	S	D	T × S	T × D	S × D
12:0	1.07	1.32	1.13	1.54	1.30	1.71	1.05	1.29	1.11	1.16	1.52	1.33	0.27	***	NS	NS	NS	NS	NS
13:0	0.34	0.16	0.10	0.30	0.12	0.18	0.47	0.14	0.10	0.16	0.15	0.25	0.16	NS	***	NS	**	NS	NS
12:0 2OH	0.06	0.43	0.17	0.22	0.15	0.08	0.09	0.44	0.20	0.20	0.21	0.11	0.25	NS	*	NS	*	NS	NS
12:0 3OH	0.02	0.20	0.09	0.22	0.15	0.31	0.04	1.02	0.13	0.18	0.21	0.03	0.30	NS	***	NS	***	**	**
14:0	1.36	1.38	2.12	2.47	2.02	3.60	1.40	1.37	2.07	2.24	2.68	1.96	0.45	***	***	*	NS	*	***
i15:0	2.41	3.78	3.17	1.03	2.00	1.67	2.18	2.01	3.71	1.15	1.94	2.66	1.48	***	*	NS	NS	NS	NS
a15:0	4.66	3.24	3.16	5.16	2.64	4.82	4.77	3.10	3.43	3.14	4.32	4.40	0.86	*	***	NS	NS	NS	***
15:0	0.75	1.69	0.89	0.93	3.06	0.69	1.05	1.72	0.76	0.12	0.02	0.91	2.18	NS	NS	NS	NS	NS	NS
br16:0	1.46	1.49	1.41	6.47	2.11	1.98	2.50	3.62	0.98	3.69	2.43	0.56	1.28	***	***	NS	***	***	**
14:0 2OH	1.31	0.74	1.26	1.56	1.16	0.67	1.49	1.41	1.30	0.96	1.55	1.63	0.57	NS	NS	*	NS	NS	*
14:0 3OH	0.38	0.71	0.63	0.68	0.75	0.63	0.47	0.91	0.64	0.55	1.00	0.70	0.33	NS	**	NS	NS	NS	NS
i16:0	1.20	2.56	1.02	0.39	2.14	0.42	1.54	2.57	0.31	0.27	0.53	2.19	0.92	**	***	NS	***	NS	*
16:1ω9c	1.22	1.90	2.40	4.81	3.04	1.58	1.48	2.88	3.21	1.27	1.40	3.38	1.24	NS	NS	NS	**	***	***
16:0	24.01	24.18	23.40	22.95	17.86	24.28	23.27	25.02	21.68	25.18	32.43	23.00	5.18	NS	NS	*	NS	**	**
10Me 16:0	2.51	2.38	2.60	5.33	7.61	1.91	2.65	2.41	3.18	2.97	4.72	7.91	2.38	***	NS	NS	NS	NS	***
i17:0	1.61	1.25	1.32	1.91	1.42	1.36	1.67	1.32	1.74	1.32	1.44	1.52	0.58	NS	NS	NS	NS	NS	NS
cy17:0	2.39	1.85	1.39	1.82	1.17	2.15	4.94	1.98	1.70	1.17	1.64	2.28	1.18	**	**	*	***	*	NS
17:0	1.91	1.96	1.74	1.82	1.33	1.68	1.75	0.95	1.96	1.72	1.92	0.85	0.65	NS	NS	NS	NS	NS	NS
16:0 2OH	2.01	1.96	0.77	0.75	0.47	0.36	0.97	1.50	0.53	0.76	0.70	0.23	0.55	***	***	*	*	**	NS
18:2ω6,9c	3.85	1.98	0.90	0.69	0.88	0.68	3.65	1.89	0.60	0.38	0.73	0.59	0.88	***	***	NS	***	NS	NS
18:1ω9c	8.80	12.33	9.49	0.02	1.59	0.03	6.83	12.98	10.40	0.00	0.00	0.02	2.69	***	***	NS	*	NS	NS
18:1ω7c	23.25	15.70	20.28	26.24	29.84	32.88	22.89	13.72	20.49	30.10	22.11	28.18	5.48	***	***	NS	NS	NS	NS
18:0	5.65	6.45	12.18	7.44	7.41	4.40	5.44	5.62	11.52	13.87	8.41	6.59	3.39	NS	NS	NS	***	**	NS
cy19:0	1.28	2.16	1.64	0.71	0.52	0.54	1.47	2.07	1.40	0.45	0.54	0.71	0.57	***	NS	NS	*	NS	NS
20:0	1.66	2.98	3.18	3.65	2.96	3.73	2.21	2.72	2.98	3.48	3.89	2.72	0.80	***	NS	NS	**	NS	*
22:0	4.88	5.39	3.69	1.02	5.55	7.41	2.48	4.35	3.95	3.42	3.06	4.65	2.11	NS	***	*	**	NS	NS
Bact./fungal	4.59	13.34	22.43	28.67	15.23	13.78	5.64	14.35	18.36	26.62	22.50	18.88	9.93	***	NS	NS	***	NS	NS

Abbreviations: RT, reduced tillage; ZT, zero tillage; SS, continuous soybean; CS, corn–soybean sequence; SC, soybean–corn sequence; T, tillage treatment; S, sequence crop; D, sampling time; LSD, least significant difference ($P \leq 0.05$). NS, not significant ($P > 0.05$)

* Significant level: $P \leq 0.05$.

** Significant level: $P \leq 0.01$.

*** Significant level: $P \leq 0.001$.

4. Discussion

The effect of soybean management was analyzed in an attempt to select those practices that favour the development of soil microbial populations, due to the important role that microorganisms have in the preservation of soil health.

It is well known that agricultural management practices can affect soil chemical properties and the number and activity of numerous soil microorganisms (Lawlor et al., 2000; Balota et al., 2004). In this study, we observed a significant increase in SOM with corn preceding soybean, compared with monoculture soybean. Our results further agree with those of Martens (2000), who reported that maize residues doubled organic C content and the C to N ratio, compared with soybean, which may result in a great increase of soil OM content in presence of corn residues. The net gain or loss of soil organic C depends on the relative rates of C additions as plant biomass versus that lost through crop removal by tillage systems (Spargo et al., 2008). In this work, the inclusion of maize in the rotation sequence maximized the biomass inputs, minimizing C losses. This was due not only to the great amount of residues that corn produces, but also to the high C content of these residues compared with soybean, resulting in an increase of SOM. Total N content was not influenced by the tillage systems, but was markedly affected by the crop sequence. In the SC sequence, total N decreased from the planting toward the harvest time. The amount of total N that was left by soybean the year before, as a precedent crop, could not be recovered by the presence of maize the following year. Total N was significantly lower in SS than in the SC or CS sequences. This was also noted by other authors who found that continuous soybean had significantly lower soil organic C and total N than other cropping sequences (Dou et al., 2005).

Tillage and crop sequences also affected some soil microbial populations. In general, dilution plating and microbial activity measurements showed significant differences between soybean–corn sequence compared with continuous soybean. Our results indicate that *Trichoderma* populations increased when soybean was combined with another crop in a rotation system. These data are supported by previous findings from various long-term rotation and tillage studies. Meriles et al. (2006) observed an increase of *Trichoderma* populations with corn residues from a peanut–corn–soybean rotation. Larkin (2003) also reported that *Trichoderma* population tended to be higher in rotation systems. Wright et al. (2005) studied the impact of tillage practices on soil microbial biomass and activity, suggesting a strong influence of plant species and crop residue on soil microbial diversity and processes. If diversity of the soil microbial community mainly depends on heterogeneity of certain groups (Giller, 1996), habitats with varied litter and subjected to conservation crop management are expected to result in high diversity. Accordingly, our results also report a significant increase in *Stramenopile* populations in a plot sowed with corn and treated with zero tillage. However, we did not find consistent changes in *Gliocladium*, total fungi and actinomycetes populations. This differed from Carpenter-Boggs et al. (2003) who stated that biotic soil quality factors in surface soil may be inversely related to tillage intensity, and that in no till-systems there is an increase in microbial activity and abundance.

Microbial biomass responds rapidly to conditions that eventually result in changes of SOM content. Consequently, microbial biomass has been used as a sensitive and early indicator of soil quality (Franzluebbers et al., 1994; Motta et al., 2001). Microbial respiration and activity are other important parameters that can be quantified to evaluate the impact of different agricultural practices

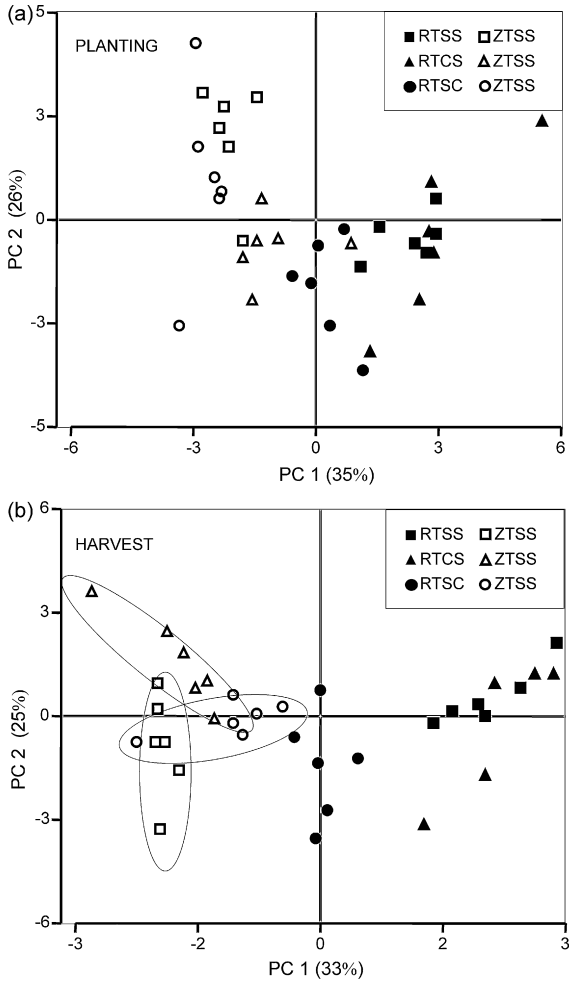

Fig. 4. PCA of all FAMEs detected in each sample of the treatment plots at planting (a) and harvest (b). RT, reduced tillage; ZT, zero tillage; SS, continuous soybean; CS, corn–soybean sequence; SC, soybean–corn sequence.

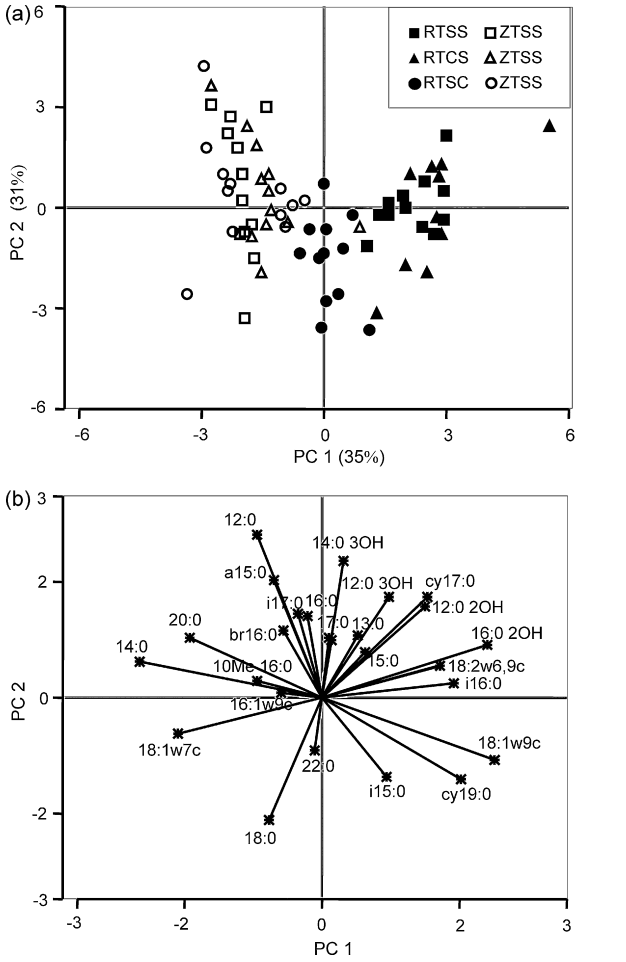

Fig. 5. (a) PCA of all FAMEs detected in each sample of the treatment plots at the two sampling times together (planting and harvest). RT, reduced tillage; ZT, zero tillage; SS, continuous soybean; CS, corn–soybean sequence; SC, soybean–corn sequence. (b) PCA showing loading values for individual FAMEs in the soils of the treatment plots given in (a).

on soil microbial communities (Aon et al., 2001) and varies with tillage system and crop sequence. Except in some cases, soil microbial respiration, biomass and activity were higher under ZT than under RT at both planting and harvest times. This result suggests that soil biological parameters responded to the management treatments mainly due to the differences in OM that tillage systems and crop rotation produced in soil. Considering that SOM is involved in the physical and chemical properties of soil and in the nutrient availability for microbial and plant growth, the changing in the SOM status directly affects the soil microbial diversity and the soil stabilization, leading to the appearance of runoff and erosion (Plassart et al., 2008). Our observation is consistent with previous reports of significant relation between tillage practices and several genera of soil microorganisms. Giller (1996) studied the effect of different management practices on microbial diversity and reported that soil was markedly affected by tillage practices. According to Angers et al. (1993) there is a growing belief that no till-systems benefit crop-enhancing soil biota. Although our results show a more clear effect of tillage on microbial respiration and activity, according to Pankhurst et al. (2002) the effect of tillage on soil microorganisms is sometimes not measurable due to the imperceptible changes that tillage produces on total SOM content during short periods of time.

In general, neither of the evaluated cultivable microbial populations, nor microbial biomass and activity showed any clear modification in their values, at planting or at harvest time, with the exception of microbial respiration which decreased at harvest time. Some authors found that the size and composition of soil microflora is crop-dependent, and that the amount and quality of different soil nutrients may vary according to crop age (Butler et al., 2003). This was not evident in our study.

In the present research, not only the amount of microbial respiration, biomass and activity was affected by soybean cropping management, but also the composition of that biomass was considerably altered, as demonstrated by the analysis of FAME profiles. These differences were identified using PCA from FAMEs presented under each crop system. Because all fatty acids are extracted (including additional fatty acids from non microbial sources) precautions were taken to minimize this impact. It is well accepted that soil management has an important effect on total mass of viable microbial community. However, there are relatively few studies that document the effect of specific crop sequence and tillage on changes in microbial community structure. Jackson et al. (2003) studied the response of soil microbial processes to tillage events and concluded that tillage causes immediate changes in microbial community and a decrease in soil quality. Similarly, multivariate analyses of our data clearly separated zero tillage plots from reduced tillage plots both at planting and harvest samplings. Thus, microbial response and the ability of FAMEs to discriminate among tillage treatments were uniform during the entire soybean growing season.

However, the separation of the three sequence crops was less evident. The relationship of crop management with the fungal biomarker 18:2ω6.9 (Klamer and Baath, 2004) and with the bacterial/fungal ratio was unclear. The ratio was higher in the RT and SS treatment combination. This result may be due, in part, to the fact that plants were affected by unidentified fungal diseases in the RT and SS treatment combination. The fatty acid 10Me 16:0 (as every methyl branching on 10th C atom) is an actinomycetes biomarker (Zelles, 1999). The monounsaturated fatty acids, especially 16:1ω9 and 18:1ω7, are associated with organic matter content and high substrate availability, and have been used as indicators of aerobic and high substrate conditions. The fatty acid 16:1ω9 has also been associated with arbuscular mycorrhizal (AM) fungi in less disturbed systems. Several authors have reported that tillage decreases hyphal lengths of all fungi, including AM fungi in soils (Larkin, 2003). In our experiments ZT treatment had the highest proportion of these fatty acids, suggesting that RT decreased AM fungi populations as compared with ZT. In contrast, RT was closely associated with branched biomarkers (i15:0 and i16:0) and with the fatty acid cy19:0, associated with cell stasis and/or stress (Drijber et al., 2000). This concomitant increase in fatty acid cy19:0 was observed under different stress conditions, such as increase in temperature, starvation, low pH and heavy metal toxicity (Kaur et al., 2005). Similarly, Calderón et al. (2000) reported an increase of cy19:0 in response to simulated tillage. Other works indicate that this fatty acid helps to maintain a functional living membrane by minimizing lipid losses during stress conditions (Guckert et al., 1986; Kaur et al., 2005). In our study, higher stress biomarker of the microbial community associated with RT may be related to more disturbed system as compared to ZT. The higher cyclopropyl FAME cy19:0 founded in soils under RT may indicate unbalance of soil microbial communities as results of RT management (Petersen et al., 2002; Macdonald et al., 2004). However, further work is required to characterize the role of this stress in regulating development of soil microbial community structure and function.

Consequently, the implementation of ZT for soybean sowing, together with the incorporation of maize in the crop sequence, demonstrated to improve the agro-ecosystem conditions based on the increase of soil biological and chemical richness. With the employment of these practices, the soybean crop can be benefited for long-term productivity and sustainability of the system.

5. Conclusions

This study represents an evaluation of the long-term effect of different soybean management practices on soil microbial community. Through the combination of tillage systems and rotation regimes, it is possible to increase some groups of microorganisms in soil that may play a role in the biocontrol of soilborne pathogens. These results confirm that tillage systems clearly affect soil chemical parameters like SOM and pH, and also crop sequences determine SOM content, pH, and total N. However, soil microbial populations and activity were more clearly affected by tillage than by sequence crop. Reduced tillage resulted in a significant decrease in total microbial biomass and activity. In addition, higher level of stress biomarker fatty acid cy19:0 was observed under RT and SS treatments, suggesting that this difference in FAME profiles are due to altered microbial community structure and function. It was clear that ZT helped to increase the size, biodiversity and activity of microbial populations in soil, and also crop rotation provided specific benefits building soil quality, especially if maize is included in the rotation scheme. Thus, in a soybean-based cropping system, appropriate crop management (i.e. specifically rotation of soybean with other crops) is necessary for a sustainable productivity while minimizing any reduction in soil quality.

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