World J Microbiol Biotechnol (2009) 25:439–448
DOI 10.1007/s11274-008-9908-y

ORIGINAL PAPER

Field assessment of soil biological and chemical quality in response to crop management practices

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Received: 29 August 2008 / Accepted: 3 November 2008 / Published online: 19 November 2008

Abstract Soil microbiological and chemical aspects were evaluated to determine the effects of conservation tillage and crop rotation on soil fertility over a 16-year period. A field trial was established to compare two cropping systems (continuous soybean and maize/soybean, soybean/maize rotation). In addition, maize (*Zea mays* L.) and soybean (*Glycine max* L., Merr) were grown in two different tillage systems: no tillage and reduced tillage. Soil populations of *Trichoderma* spp., *Gliocladium* spp. and total fungi were more abundant when maize or soybean were under conservation tillage and in the maize/soybean and soybean/maize rotation, than in continuous soybean. Furthermore, higher levels of microbial respiration and fluorescein diacetate hydrolysis (FDA), were recorded under no tillage systems. However, soil counts of Actinomycetes and *Pythium* spp., and *Pythium* diversity together with soil microbial biomass were not affected by the field treatments. To establish a correlation with soil biological factors, soil chemical parameters, such as pH, organic matter content, total N, electrical conductivity, $N–NO_3^-$ and P were also quantified, most of the correlations being significantly positive. Under no tillage there was a clear increase of the amount of crop residues and the C and N soil content due to the presence of residues. Also the distribution of crop residues in surface soil due to zero tillage and the quality of these residues, depending on the crop rotation employed, improved on soil biological and chemical characteristics. Crop yield was also enhanced by zero tillage through the management of residues. Although yield values were not directly associated with the development of microorganisms, both yield and microorganisms were influenced by crop management. These results suggest that measuring soil properties over a long period helps to define effective management strategies in order to preserve soil conditions.

Keywords Microbial communities · Crop rotation · Chemical parameters · Soil health · Tillage systems

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Introduction

Management strategies can change the nutritional status of a soil but may require many years to achieve a new equilibrium level following a modification in land use or management. However, soil microbial populations respond rapidly to such changes (Jenkinson 1988) and may therefore warn of a potential transition to another state, i.e., they may indicate that under present land management strategies the system is not sustainable (Holt 1997).

To preserve soil quality in agroecosystems over time, sustainable management practices should be appropriately applied. Combinations of cultural practices, such as tillage systems and crop rotation, were found to influence several soil quality attributes (Carter 2002). Changes in residue management as a response of tillage and crop rotation induce major shifts in the number and composition of soil flora, including both pests and beneficial microorganisms (Bockus and Shroyer 1998; Meriles et al. 2008). Microbial community dynamics changes as a result of interactions

among tillage, substrate availability, soil moisture, temperature, and aeration (Feng et al. 2003). Soil chemical nutrients are also influenced by crop management practices; changes in frequency and intensity of tillage practices alter soil chemical properties, distribution of nutrients, pH, and cation exchange capacity in the soil profile. Also, crop rotation is known to beneficially influence many soil chemical parameters, including organic C content, N supply and transformation, pH, and amount and availability of P, K, Ca, and Mg (Power 1990).

Maintenance and enhancement of soil quality are basically dependent upon improvement of biological and chemical properties of the soil. As these properties are affected by any alteration in soil quality, such as different crop management practices, they are also useful indicators of quality change (Doran and Parkin 1996). The main objective of this work was to identify soil parameters potentially useful for soil quality evaluation under different tillage and crop rotation systems. The appropriate combination of tillage systems and crop rotation is important not only in enhancing crop yields at low cost but also in maintaining the natural resource base.

Materials and methods

The experimental site

This field research was conducted at the experimental area of the EEA INTA Manfredi (Agricultural and Livestock Technology National Institute-INTA), located in Córdoba province, in the semiarid Pampa of Argentina (31°49′ S, 63°46′ W).

The climate of the region is semiarid, with little or no water deficit in January and February and important water excess from October to April. Mean annual precipitation is 750 mm, concentrated in spring-summer (81%) with highly eroding and maximum intensity events of up to 100 mm h^{-1}. This area has a probability of more than 50% of water deficit occurrence, during the year. The temperature regime is mesothermal, with annual mean values of 16.5°C. The dominant soil type of the region is a silty loam (14.6% sand, 68.7% silt, 16.7% clay) Entic Haplustoll (USDA Soil Taxonomy) serie Oncativo with A, AC and C horizons; soil chemical and physical properties make it suitable for farming. The major limitations are periodic droughts, occasional excessive rainfall, and wind and water erosion. The relief in the area is moderate with slope of up to 3%.

The field trial

The experiment was established in 1992 and consisted in 18 plots (35 × 180 m each) in a randomized complete block design with a split-plot arrangement of treatments. The main plot corresponded to the rotation treatment, including continuous soybean (CS), soybean with maize as previous crop (M/S: maize/soybean) and maize with soybean as previous crop (S/M: soybean/maize); thus, each phase of all crop sequences was present each year, crops being rotated annually. The subplot corresponded to the tillage systems, including disk harrow (reduced tillage) and no tillage or no pre-plant tillage (zero tillage), for both maize and soybean. Each treatment had three repetitions randomly distributed throughout the field plot. Crops were planted using a planter with a single coulter to cut through crop residue and loosen the soil, planting being the only soil disturbance. The zero tillage treatment was conducted with a machine with straight shanks and twisted sweeps. Tillage depth in reduced tillage treatment was 18–20 cm.

Crops were seeded on December 25, 2006 and harvested on April 23, 2007. Seeding rates were 3 and 25 seeds m^{-1} for maize and soybean, respectively. Row width was 52 cm for both crops. All plots were treated with glyphosate (48% i.a., 3 l ha^{-1}) before planting, being maize plots also treated with atrazine (50% i.a., 3 l ha^{-1}) and metolachlor (1 l ha^{-1}), besides the glyphosate application. When necessary, soybean and maize were treated with herbicides after planting and just before or after crop emergence. The type of herbicides employed depended on the type of weeds present. After maize and soybean crops reached physiological maturity, they were harvested with appropriate equipment.

Soil sampling

Soil samples were collected at a depth of 0–5 cm during the growing season of the field crops. Composite soil samples of six random sub-samples per plot were collected after planting (20 days) and before harvest (10 days) of each crop, totalling 12 samples per treatment for microbiological and chemical determinations. Samples were taken near the crop roots, with a probe (5-cm core diameter), and stored at 4°C until required for analysis. Soil dry weight was determined by oven drying 1 g of soil at 105°C for 24 h.

Quantification of soil biological parameters

Soil dilution plate technique

Populations of selected components of soil microbial community were measured with the dilution plate method (Table 1).

For the determination of Actinomycetes, a subsample (1 g) of soil was weighed, transferred into flasks containing 100 ml of sterile distilled water, and mixed on an orbital shaker. From each flask, 1 ml of soil suspension was taken to make the corresponding dilutions. An aliquot of 100 µl was taken from the last dilution ($1 \cdot 10^{-2}$) and poured on

Table 1 Microorganisms isolated from soil by plate count technique

Soil microorganisms	Method employed			
	Selective medium	Dilution	Days of incubation	References
Actinomycetes	Modified Küster medium	10^{-1}	10	Vargas Gil et al. (2007)
Trichoderma spp.	Modified PDA	10^{-2}	7	Vargas Gil et al. (2007)
Gliocladium spp.	Modified PDA	10^{-2}	7	Vargas Gil et al. (2007)
Total fungi	Modified PDA	10^{-2}	7	Vargas Gil et al. (2007)
Pythium spp.	Modified corn meal agar medium	10^{-1}	10	Jeffers and Martin (1986)

PDA Potato Dextrose Agar

culture medium. Colonies were counted on Küster medium, modified by the addition of cycloheximide (0.15 g/l) and sodium propionate (0.4 g/l) (Table 1). Plates were incubated at 25°C in the dark.

For the quantification of *Trichoderma* spp., *Gliocladium* spp. and total fungi, the procedure was the same as for actinomycetes quantification, except that 10 g of soil were employed (Table 1). An aliquot of 400 µl was taken from the last dilution ($1 \cdot 10^{-3}$) and poured on modified PDA supplemented with rose bengal (20 mg/l), streptomycin (100 mg/l), and chloramphenicol (300 mg/l). Plates were incubated at 25°C, with 8 h of light.

Data was expressed as the number of colony forming units (cfu g^{-1} of dry soil, bacteria was expressed as $\times 10^4$ cfu g^{-1} and fungi as $\times 10^2$). The cfu values were a mean of soil samples taken after planting and before each crop harvest.

*Trichoderm*a spp., *Gliocladium* spp. and total fungi were distinguished in the agar plates through the observation of morphological features under the optical microscope. The identification of isolates was made according to Barnett and Hunter (1998).

Pythium spp. populations were counted on corn meal agar supplemented with the following antifungal agents (per liter of distillated water): benomyl (Benlate, 50% active), 250 mg; pentacloronitrobenzene (PCNB), 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) (Table 1). Plates were incubated at 25°C, in the dark. *Pythium* isolates were identified using the morphological keys of Van der Plaats-Niternik (1981). Presence of *Pythium* spp. was expressed as colony forming units in each of the six soil samples taken from each plot of the field trial. Moreover, the presence of different *Pythium* species was expressed as percentages.

Microbial respiration

For the quantification of soil microbial respiration, potentially mineralizable C (CO_2–C respiration) was determined according to Alef (1995). Soil samples (10 g) were air dried, sieved, and incubated with Na (OH) 0.2 N at 28°C during 7 days. CO_2 released was analyzed using HCl 0.2 N. Control flasks without containing soil were also incubated with Na (OH) 0.2 N and at 28°C during 7 days.

Microbial biomass C

Microbial biomass C was determined employing the chloroform fumigation-inoculation technique of Jenkinson and Powlson (1976). The amount of CO_2 released from chloroform-treated and untreated soil samples (20 g) was measured. Treated samples were previously fumigated with chloroform, de-fumigated, inoculated with fresh soil, and incubated with Na (OH) 0.2 N at 28°C during 10 days. The CO_2 released was measured with HCl 0.2 N. Control flasks without containing soil were incubated with Na (OH) 0.2 N at 28°C during 10 days.

Hydrolysis of fluorescein diacetate (FDA)

General microbial activity was measured by hydrolysis of fluorescein diacetate (FDA) using the procedure of Adam and Duncan (2001). Briefly, 2 g of soil and 15 ml of 60 mM potassium phosphate buffer pH 7.6 were placed in a 50 ml conical flask. Substrate (FDA, 1,000 µg ml^{-1}) was added to start the reaction, and the flask contents were 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) was 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 2,000 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.

Quantification of soil chemical parameters

Soil samples were air-dried and sieved (2 mm) to determine organic C by wet oxidation following the Walkley and Black procedure (Black 1965), and total N by

semi-micro-Kjeldhal method (Bremner 1996). Moreover, available Phosphorus (P) was quantified by Bray-Kurtz I method (Bray and Kurtz 1945), pH with the employment of a potentiometer in a 1:2.5 soil:water suspension and electrical conductivity (EC) with a conductivity meter in a 1:2.5 soil:water suspension.

Soil organic C and N concentrations were expressed as g kg^{-1} of dry soil mass, and as mg ha^{-1}, based on the sample bulk density and thickness of the sampled layer (0.05 m).

Quantification of crop residues and crop yield

Surface residues of maize and soybean were collected from field plots after harvest, according to Steiner and Shomber (1994). Residues were placed in mesh bags, weighed at the laboratory and then converted to kg ha^{-1}. Furthermore, C content of crop residues was determined according to Nelson and Sommers (1982); N content was quantified following the method of Apostolatos (1984). Crop yield was corrected to a 12% H_2O of grain humidity. Each crop was harvested with the corresponding machinery.

Statistical analyses

Data of cultivable microbial populations, microbial activity and biomass, and soil chemical properties were analyzed through standard analyses of variance (ANOVA). In all cases, residuals were tested for normality with the Shapiro-Wilks' test. To test for differences between means, LSD-test at level of significance of $P \leq 0.05$ was used.

The diversity of *Pythium* populations was assessed by estimating species richness (i.e. number of species) and Shannon's diversity index, calculated as: $H' = -\sum pi \ln (pi)$ (pi: frequency of the ith species). Similarity of the *Pythium* communities in the different treatments was evaluated with the Jaccard's similarity coefficient: $S_J = n_{JK}/(n_{JK} + u)$ n_{JK}: number of *Pythium* species shared by the two communities (J and K); u is the sum of the *Pythium* spp. unique to each community (Sokal and Sneath 1963). S_J takes on a maximum value of 1 where all species are shared by the same treatments, and a value of 0 where the same treatments have no species in common (Mihail et al. 2002).

Moreover, Pearson analyses were performed in order to establish correlations between soil biological and chemical parameters, with crop residues and yield.

Results

Soil microbial counts and activity

The number of culturable microorganisms varied with the crop management practice used (Fig. 1). Populations of *Trichoderma* spp. were significantly higher (50, 48, and 150% in M/S, S/M, CS, respectively) when maize or soybean were under zero tillage, compared with reduced tillage, independently of the crop sequence. *Gliocladium* spp. counts were also significantly greater (40, 73 and 150% in M/S, S/M, CS, respectively) when maize or soybean were under zero tillage, under any crop rotation scheme. When maize and soybean were under zero tillage, total fungi were also more abundant (13, 82, and 51% in M/S, S/M, CS, respectively) than under reduced tillage. However, actinomycetes were not influenced by the tillage system employed.

Considering the crop sequence, a higher amount of *Trichoderma* spp. (27%), *Gliocladium* spp. (30%) and total fungi populations (18%) was recorded in the M/S rotation scheme than in soybean monoculture, with statistical significant differences between treatments. The values mentioned were obtained in soybean under zero tillage. The same trend was observed in soybean under reduced tillage. However, soil populations of Actinomycetes were not influenced by the crop sequence.

The analysis of variance showed a significant effect of tillage systems and crop rotation interactions on CFU (Table 2).

The amount of soil populations of *Pythium* was not affected by the tillage system or crop sequence employed and there was no interaction between treatments (data not shown). The effect of tillage systems and crop rotation on *Pythium* diversity was analyzed by two separate analyses. Diversity of *Pythium* communities was not influenced by crop practices, as indicated by the Shannon's diversity index, with H′ values of 1.75 and 1.12 for zero tillage and reduced tillage, respectively; and H′ values of 1.39, 1.49, and 1.01 of CS, S/M and M/S, respectively. The overlapping confidence intervals indicated that there was no statistical difference between treatments. While diversity in treatments was similar, similarity between *Pythium* communities in both treatments was low ($S_J = 0.50$ for tillage systems and $S_J = 0.40$ for crop rotation).

Nine species of *Pythium* were identified in the field trial. The most frequent species was *P. ultimum* (30% of the total *Pythium* isolates). Other frequent species were: *P. irregulare* (17.5%); *P. rostratum* and *P. polimorphom* (7.5% each); *P. ultimum* (5%); *P. spinosum*, *P. graminicola* and *P. aphanidermatum* (2.5% each); and some *Pythium* spp. (25%) that could not be identified.

Microbial respiration was also significantly influenced by tillage system, being 5% higher in both crops under zero tillage than under reduced tillage (Table 3). Crop sequence, however, did not affect this biological parameter. Likewise, microbial activity as determined by FDA hydrolysis, was 81% significantly higher under zero tillage, but was not affected by the crop sequence. Soil microbial biomass was

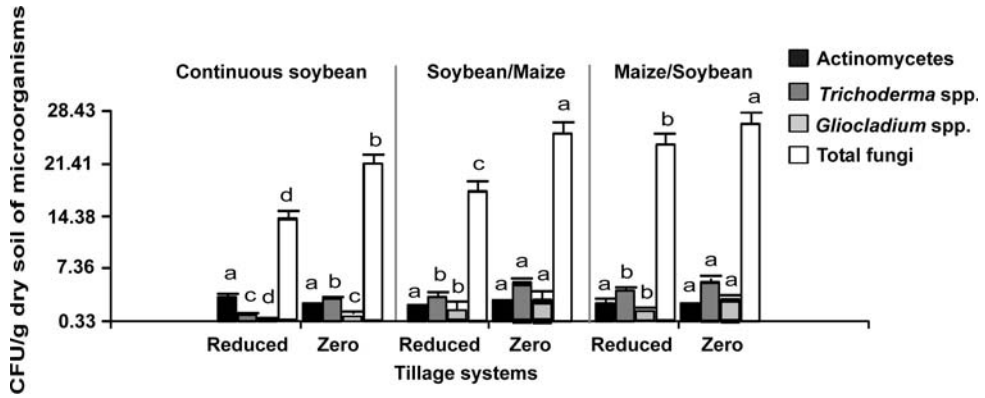

Fig. 1 Populations of Actinomycetes, *Trichoderma* spp., *Gliocladium* spp., and total fungi under different tillage systems and crop rotation schemes. *Bars* of the same colour and the same letter are not significantly different according to LSD test at $P \leq 0.05$. Each bar represents the average of values obtained from plots (3) under the same treatment. CFU: colony forming units (actinomycetes expressed as $\times 10^{-4}$, fungi as $\times 10^{-2}$)

Table 2 Results of ANOVA for Actinomycetes, *Trichoderma* spp., *Gliocladium* spp., total fungi and *Pythium* spp. under different tillage systems and crop rotation schemes

Source of variation	Soil microorganisms (CFU/g soil)									
	Actinomycetes		*Trichoderma* spp.		*Gliocladium* spp.		Total fungi		*Pythium* spp.	
	MS	P	MS	P	MS	P	MS	P	MS	P
Tillage	24.95	0.0642	77.44	0.0001	14.41	0.0001	14.28	0.0052	1.80	0.47
Crop rotation	19.64	0.0667	60.34	0.0001	26.81	0.0025	47.65	0.0012	1.40	0.30
Tillage × Crop rotation	21.00	0.0210	18.05	0.0151	14.51	0.0481	261.66	0.0066	2.63	0.65

MS mean square, *CFU* colony forming units (fungi expressed as $\times 10^2$, actinomycetes as $\times 10^4$)

P significant level observed at $P \leq 0.05$

not influenced either by tillage system or crop rotation treatment. Tillage × crop rotation interaction was not significant (Table 3).

Crop residues and crop yield

The amount of soybean residues was greater under zero tillage (64%) than under reduced tillage. Moreover, the amount of maize residues under zero tillage was 32% higher than under reduced tillage (Fig. 2).

The amount of maize residues was double the amount of soybean residues in the field plots evaluated, independently of the tillage system employed (Fig. 2). The same trend was observed the previous year (data not shown).

Crop yield was also notably influenced by the tillage system and crop rotation sequence employed (Fig. 3).

Under zero tillage and in the maize/soybean rotation scheme, soybean yield was 5% higher than under reduced tillage. In continuous soybean yield was 7% higher under zero tillage than under reduced tillage.

The effect of yield improvement with zero tillage was also evident for maize, with 12% higher yield, compared with reduced tillage.

Soil chemical analysis

Some of the soil chemical properties were affected by tillage systems and crop sequence (Table 4). Soil pH tended to be acidic under continuous soybean, but was not affected by the tillage system. Soil OM content was 18% higher when soybean was under zero tillage, compared with reduced tillage; and OM was greater in soybean with maize as previous crop, compared with continuous soybean or soybean as preceding crop. The same tendency was observed with total N: with soybean under zero tillage, total N was 13% higher than under reduced tillage. Total N was lower in soybean monoculture, and the highest amount was recorded in soybean with maize as preceding crop. Moreover, electrical conductivity was slightly affected by crop sequence, being lower under continuous soybean. $N–NO_3$, HCO_3 and P were not affected by any of the treatments.

There was no significant interaction between tillage and crop rotation (Table 4).

According to Pearson's coefficients, the amount of maize and soybean residues, together with C and N content of these residues were positively correlated with crop yield,

Table 3 Soil microbial respiration, biomass, and fluorescein diacetate (FDA) hydrolysis under different tillage systems and crop rotation schemes

Crop practice	Microbial respiration (mg CO_2 g^{-1} $week^{-1}$)	Microbial biomass (mg CO_2 g^{-1})	FDA hydrolysis (μg fluorescein g^{-1})
Zero tillage	1.85 a	0.51 a	0.35 a
Reduced tillage	1.77 b	0.39 a	0.19 b
Maize/soybean	1.78 a	0.39 a	0.31 a
Soybean/maize	1.82 a	0.43 a	0.27 a
Continuous soybean	1.80 a	0.47 a	0.35 a
Tillage	*	NS	*
Crop rotation	NS	NS	NS
Tillage × Crop rotation	NS	NS	NS

NS not significant

* Significant at $P \leq 0.05$

Different letters indicate significant differences (LSD) at $P \leq 0.05$

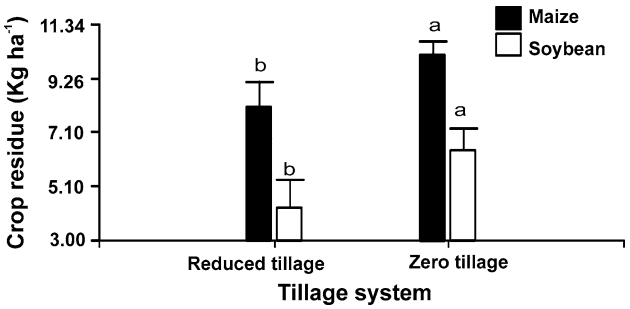

Fig. 2 Crop residues produced by maize and soybean under different tillage systems. *Bars* of the same colour followed by the same letter are not significantly different according to LSD test at $P \leq 0.05$. Each *bar* is the average of values obtained from plots (3) under the same treatment

and also with soil biological and chemical properties (Table 5).

Actinomycetes, *Trichoderma* spp., *Gliocladium* spp. and total fungi were positively correlated with soybean and maize amount of residues, and with C and N content of both crops residues. *Pythium* populations, microbial biomass and microbial respiration and activity were only correlated with C and N content of residues. However, soil biological parameters were not directly correlated with crop yield.

All the chemical parameters evaluated in this work (pH, OM, total N, EC, and extractable P) were significantly correlated with crop yield, crop residues, and amount of C and N of these residues; most of these correlations were positive (Table 5).

Although no positive correlation between soil microbial communities and maize and soybean yield was observed, chemical parameters affected crop yield as well as soil microorganisms. Therefore, soil biological aspects indirectly influenced crop yield. The correlation analysis showed that there was a significant positive interaction between most the soil biological parameters and soil chemical properties (Table 6).

Discussion

Management practices had a significant effect on some of the soil characteristics evaluated in this work. It is widely known that soil microbial populations exhibit natural seasonal dynamics, and that their biodiversity and functions are influenced by several factors, including vegetation and agricultural management (Vargas Gil et al. 2008).

We measured Actinomycetes, *Trichoderma* spp., *Gliocladium* spp., and total fungi because some of these groups are considered beneficial microorganisms, involving active biological control agents, which can suppress plant pathogens in healthy soils (Janvier et al. 2007; Govaerts et al. 2008). We also measured *Pythium* spp. because this genus includes many pathogenic species.

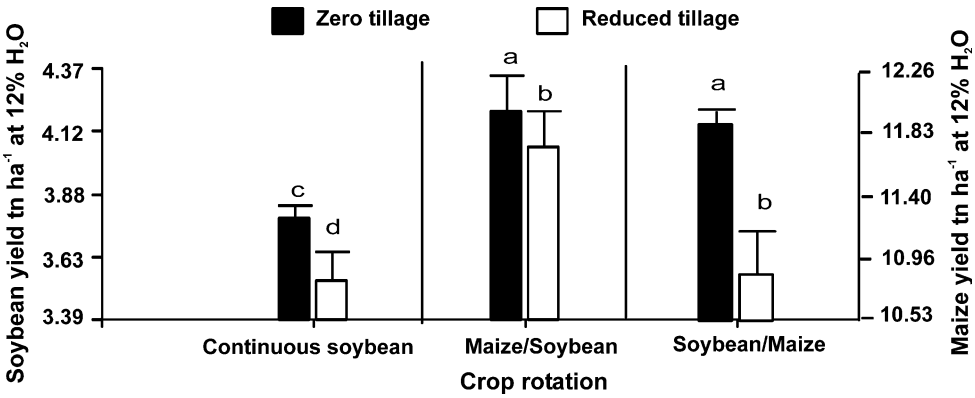

Fig. 3 Average yield of soybean and maize as influenced by tillage systems and crop rotation in a field trial. Different letters indicate significant differences (LSD) at $P \leq 0.05$

Table 4 Effect of tillage systems and crop rotation on soil chemical properties

Management practices	pH (H_2O)	OM (%)	Total N (%)	EC ext (ds/m)	$N–NO_3^-$ (ppm)	HCO_3-ext *P* (ppm)
Zero tillage	6.55 a	3.08 a	0.18 a	1.18 a	23.06 a	30.33 a
Reduced tillage	6.47 a	2.60 b	0.16 b	1.01 a	18.84 a	29.89 a
Maize/soybean	6.36 b	3.34 a	0.19 a	1.26 a	24.02 a	31.20 a
Soybean/maize	6.35 b	2.77 b	0.17 a	1.10 ab	22.38 a	27.40 a
Continuous soybean	6.84 a	2.58 b	0.15 b	0.98 b	17.98 a	31.63 a
Tillage	NS	*	*	NS	NS	NS
Crop rotation	*	*	*	NS	NS	NS
Tillage × Crop rotation	NS	NS	NS	NS	NS	NS

OM organic matter, *EC* electrical conductivity

Different letters indicate significant differences (LSD) at $P < 0.05$

* Significant at $P < 0.05$

Table 5 Correlation analysis of yield of maize and soybean and crop residue with soil biological and chemical properties

	Pearson coefficients ($P < 0.05$)					
	Soybean yield	Maize yield	Crop residue			
			Soybean (ton/ha)	Maize (ton/ha)	C content (%)	N content (%)
Actinomycetes	0.12	0.25	0.25*	0.32*	0.69*	0.82*
Trichoderma spp.	0.23	0.32	0.24*	0.36*	0.55*	0.75*
Glicoladium spp.	0.42	0.51	0.40*	0.35*	0.84*	0.78*
Total fungi	0.15	0.28	0.31*	0.27*	0.45*	0.66*
Pythium spp.	0.17	0.47	0.23	0.39	0.49*	0.54*
Microbial respiration	0.31	0.25	0.39	0.27	0.24*	0.59*
Microbial biomass	0.17	0.45	0.22	0.49	0.12*	0.10*
FDA hydrolysis	0.52	0.26	0.22	0.25	0.34*	0.69*
pH	0.59*	0.56*	0.74*	0.63*	−0.47*	−0.53*
OM	0.16*	0.34*	0.49*	0.56*	0.47*	0.89*
Total N	0.10*	0.52*	0.36*	0.52*	0.44*	0.60*
EC ext	0.15*	−0.19*	0.59*	0.62*	0.74*	0.52*
$N–NO_3^-$	0.18*	0.26*	0.38*	0.57*	0.20*	0.48*
$P–HCO_3^-$	0.66*	0.35*	0.13*	0.32*	0.19*	0.14*

FDA fluorescein diacetate, *OM* organic matter, *EC* electrical conductivity

* Significant at $P \leq 0.05$

Tillage systems

The number of culturable microorganisms quantified in this study was generally higher when maize or soybean was under zero tillage than under reduced tillage. Only the Actinomycetes and *Pythium* numbers and diversity of *Pythium* species were not influenced by tillage systems. Moreover, this beneficial effect of conservation tillage was evident in increased soil microbial respiration and greater activity of microbial communities, as revealed by the FDA hydrolysis. It has been reported that conservation tillage practices that leave crop residues on the soil surface tend to support higher densities of total fungi and bacteria populations (Pankhurst et al. 1995; Beare et al. 1997). The accumulation of residues in the topsoil generates a microhabitat with less marked fluctuations of moisture and temperature, providing soil microorganisms with a cooler and wetter habitat, with the resulting increase in their activities (Kladivko 2001). This was evidenced by the amount of crop residues left by maize or soybean, which were significantly higher under conservation tillage. Furthermore, zero tillage not only produced retention of crop residues, but also an accumulation of organic C and N in surface soil, as shown by the amounts of soil OM and N recorded as a consequence of decomposition of crop residues. Accordingly, some authors stated that the accumulation of high quality residues improve the growth and reproduction of microorganisms in soil (Salinas-Garcia

Table 6 Correlation analysis between soil biological and chemical properties

Soil biological parameters	Soil chemical parameters					
	pH (H_2O)	OM (%)	Total N (%)	EC ext (ds/m)	N–NO_3 (ppm)	HCO_3 ext *P* (ppm)
Actinomycetes	0.29*	0.21*	0.21*	0.28*	0.12	0.24
Trichoderma spp.	0.38*	0.42*	0.22*	0.19*	0.23	0.14
Glicoladium spp.	0.77*	0.28*	0.27*	0.17*	0.10	0.11
Total fungi	0.70*	0.46*	0.39*	0.22*	0.09	0.15
Pythium spp.	0.41*	0.39*	0.31*	0.21*	0.13	0.21
Microbial respiration	0.12	0.23	0.11	0.23	0.25	−0.26*
Microbial biomass	0.09	0.14	0.18	0.12	0.09	−0.46*
FDA hydrolysis	0.24*	0.26*	0.29*	0.31*	0.19*	−0.26*

FDA fluorescein diacetate, *OM* organic matter, *EC* electrical conductivity

* Significant at $P \leq 0.05$

et al. 2002). In agreement with those results, some authors (Ogle et al. 2005; Muñoz et al. 2007) mentioned that changes in frequency and intensity of tillage practices alter soil properties, distribution of nutrients, and soil organic matter in the soil profile, and consequently, the abundance and activities of microbial populations.

Furthermore, chemical soil parameters were also influenced by tillage systems. When soybean or maize where under zero tillage, soil OM and total N were higher than under reduced tillage. Our results agree with findings of Dumontet et al. (2001), in that conservation tillage can reduce erosion, increase organic matter content and improve physical, chemical and biological soil properties. OM and total N are higher under conservation tillage because the incorporation of crop residues into the soil profile is reduced and humified organic matter is much less exposed to biotic and abiotic degrading processes.

Crop rotation

Crop rotation also showed modifications in microbial counts. Indeed, the number of *Trichoderma* spp., *Gliocladium* spp. and total fungi in the maize/soybean and soybean/maize rotation sequences increased compared with soybean monoculture. However, unlike what we expected, crop rotation did not seem to affect Actinomycetes, *Pythium* spp. abundance and diversity, soil microbial respiration, biomass or activity. Several studies (Doran 1980; Gupta and Germida 1988; Vargas Gil et al. 2008) stress that, unlike monoculture, crop rotation can stimulate soil biodiversity and biological activity through changes in the soil habitat, favouring the development of specific microbial communities (Meriles et al. 2006). Accordingly, because of the different quality and amount of exudates and organic components from root systems and crop residues, some groups of microorganisms can be differently influenced by crop sequence, supporting the diverse results obtained in this work.

As mentioned above, crops can indicate soil microbial processes through the quantity and quality of residues they produce. Some crops, such as maize, leave much more residues than other crops, like soybean. Moreover, because of its greater amount of C and N, maize residue is of particular higher quality than that of soybean. Consequently, in a crop sequence, maize as previous crop increases soil organic matter content, supporting greater levels of microbial activity because of higher supplies of energy and nutrients.

Crop rotation also affected soil chemical properties. Indeed, OM and total N were significantly higher when maize preceded soybean, compared with soybean as previous crop or continuous soybean. Moreover, soil pH tended to be acidic under crop rotation compared with soybean monoculture. Precisely, Edwards et al. (1992) found that rotations with a higher frequency of maize appeared to positively affect OM, total N and pH, and that lower soil pH values are found in this kind of rotation. These results are related to the decomposition rate and amount of crop residues in soils, such as those of maize that, because of their high quality and quantity, directly increased organic matter content and nutrient cycling (Rochette et al. 1999). In this work, we found that crop residues directly affected soil biological and chemical characteristics, because of the positive correlation between C and N content of these residues.

As the result of the different combinations of crop practices, yield was differentially affected by system management. The mentioned changes of soil biological and chemical properties induced by cultural practices are known to become stable over time and might affect availability of nutrients for plant growth, crop production, and soil productivity, as it was demonstrated in this work. Through the study of modification of soil parameters, Pankhurst et al. (2003) also found that agricultural management had a greater influence on crop yields. This shows

a need for proper plant residue management in order to avoid nutrient loss and reduction in crop yield.

In conclusion, quantitative and qualitative changes in the population of soil microorganisms reflect changes in soil quality. These changes are potentially useful as responsive indicators of the effects of crop and residue management to increase systems productivity conserving soil health.

Acknowledgements We are grateful to Proyecto Regional INTA Gestion Ambiental en la provincia de Córdoba (INTA CORDO 04), coordinated by Ing. Geo. Hugo Marelli, for providing funds for the development of the study. We would like to stress Engineer Pedro Salas' encourage to begin this study. The researcher Salas belonged to the Natural Resources Department of EEA INTA Manfredi (Córdoba, Argentina). *In memoriam* of his work trajectory.

References

Adam G, Duncan H (2001) Development of a sensitive and rapid method for measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. Soil Biol Biochem 33:943–951

Alef K (1995) Soil respiration. In: Alef K, Nanninpieri P (eds) Methods in applied soil microbiology and biochemistry. Academic Press, Harcourt Brace & Company Publishers, London, pp 214–219

Apostolatos G (1984) A rapid and inexpensive procedure for determination of Nitrogen in plant materials. J Food Technol 1:639–642

Barnett HL, Hunter BB (1998) Illustrated genera of imperfect fungi, 4th edn. APS Press, St. Paul, p 218

Beare MH, Hu S, Coleman DC, Hendrix PF (1997) Influences of mycelial on soil aggregation and organic matter storage in conventional and no-tillage soils. Appl Soil Ecol 5:211–219

Black CA (1965) Methods of soil analysis, part 2. ASA Publications, Madison, p 1372

Bockus WW, Shroyer JP (1998) The impact of reduced tillage on soilborne plant pathogens. Annu Rev Phytopathol 36:485–500

Bray RH, Kurtz LT (1945) Determination of total, organic, and available forms of phosphorus in soils. Soil Sci 59:39–45

Bremner JM (1996) Nitrogen total. In: Sparks DK (ed) Methods of soil analysis: chemical methods part 3. American Society of Agronomy, Madison, pp 1085–1122

Carter MR (2002) Soil quality for sustainable land management: organic matter and aggregation, interactions that maintain soil functions. Agron J 94:38–47

Doran JW (1980) Soil microbial and biochemical changes associated with reduced tillage. Soil Sci Soc Am J 44:765–771

Doran JW, Parkin TB (1996) Quantitative indicators of soil quality: a minimum data set. In: Doran JW, Jones AJ (eds) Methods for assessing soil quality. SSSA Inc, Madison, pp 25–37

Dumontet S, Mazzatura A, Casucci C, Perucci P (2001) Effectiveness of microbial indexes in discriminating interactive effects of tillage and crop rotations in a Vertic Ustorthens. Biol Fertil Soils 34:411–416

Edwards JH, Wood CW, Thurlow DL (1992) Tillage and crop rotation effects on fertility status of a hapludult soil. Soil Sci Soc Am J 56:1577–1582

Feng Y, Motta AC, Reeves DW, Burmester CH, Van Santen E, Osborne JA (2003) Soil microbial communities under conventional till and no-till continuous cotton systems. Soil Biol Biochem 35:1693–1703

Govaerts B, Mezzalama M, Sayre KD, Crossa J, Lichter K, Troch V, Vanherck K, De Corte P, Deckers J (2008) Long-term consequences of tillage, residue management, and crop rotation on selected soil micro-flora groups in the subtropical highlands. Appl Soil Ecol 38:197–210

Gupta VVSR, Germida JJ (1988) Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. Soil Biol Biochem 20:777–786

Holt JA (1997) Grazing pressure and soil carbon, microbial biomass and enzyme activities in semi-arid northeastern Australia. Appl Soil Ecol 5:143–149

Janvier C, Villeneuve F, Alabouvette C, Edel-Hermann V, Mateille T, Steinberg C (2007) Soil health through soil disease suppression: which strategy from descriptors to indicators? Soil Biol Biochem 39:1–23

Jeffers SN, Martin SB (1986) Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis 70:1038–1043

Jenkinson DS (1988) Determination of microbial biomass carbon and nitrogen in soil. In: Wilson JR (ed) Advances in nitrogen cycling in agricultural ecosystems. C.A.B. International, Wallingford, pp 368–386

Jenkinson D, Powlson D (1976) The effects of biocidal treatments on metabolism in soil. Methods for measuring soil biomass. Soil Biol Biochem 8:209–213

Kladivko EJ (2001) Tillage systems and soil ecology. Soil Tillage Res 61:61–76

Meriles JM, Vargas Gil S, Haro R, March GJ, Guzman CA (2006) Glyphosate and previous crop residue effect on deleterious and beneficial soil-borne fungi from a peanut-corn-soybean rotations. J Phytopathol 154:309–316

Meriles JM, Vargas Gil S, Haro R, March GJ, Guzman CA (2008) Selected soil-borne fungi under glyphosate application and crop residues from a long-term field experiment. Biol Agric Hortic (in press)

Mihail JD, Hung LF, Beuhn JN (2002) Diversity of the *Pythium* community infecting roots of the annual legume *Kummerowia stipulacea*. Soil Biol Biochem 34:585–592

Muñoz A, Lopez-Piñeiro A, Ramirez M (2007) Soil quality attributes of conservation management regimes in a semi-arid region of south western Spain. Soil Tillage Res 95:255–265

Nelson DW, Sommers LE (1982) Total carbon, organic carbon and organic matter. In: Page AL (ed) Methods of soil analysis part 2: chemical and microbiological properties, vol 2. American Society of Agronomy and Soil Science Society of America, Madison, pp 570–571

Ogle SM, Breidt FJ, Paustian K (2005) Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. Biogeochemistry 72:87–121

Pankhurst CE, Hawke BG, McDonald HJ, Kirby CA, Buckerfield JC (1995) Evaluation of soil biological properties as potential bioindicators of soil health. Aust J Exp Agric 35:1015–1028

Pankhurst CE, Magarey RC, Stirling GR, Blair BL, Bell MJ, Garside AL (2003) Management practices to improve soil health and reduce the effects of detrimental soil biota associated with yield decline of sugarcane in Queensland, Australia. Soil Tillage Res 72:125–137

Power JF (1990) Legumes and crop rotations. In: Francis CA, Flora CB, King LD (eds) Sustainable agriculture in temperate zones. Wiley, NY, pp 178–204

Rochette P, Angers DA, Flanagan LB (1999) Maize residue decomposition measurement using soil surface carbon dioxide fluxes and natural abundance of carbon-13. Soil Sci Soc Am J 63:1385–1396

Salinas-Garcia JR, Velazquez-Garcia JD, Gallardo-Valdez A, Diaz-Mederos P, Caballero-Hernandez F, Tapia-Vargas LM, Rosales-Robles E (2002) Tillage effects on microbial biomass and nutrient distribution in soils under rain-fed corn production in central-western Mexico. Soil Tillage Res 66:143–152

Sokal RR, Sneath PHA (1963) Principles of numerical taxonomy. W.J. Freeman, San Francisco, p 359

Steiner JL, Shomber HH, Morrison JE Jr (1994) Measuring surface residue and calculating losses from decomposition and redistribution. In: Stewart BA, Moldenhauer WC (eds) Crop residue management to reduce erosion and improve soil quality. Conservation research report No. 37. US Department of Agriculture, pp 21–29

Van der Plaats-Niternik AJ (1981) Monograph of the Genus *Pythium*. In: Studies in mycology No. 21, Centraalburaeu voor Schimelcultures, Baarn, p 263

Vargas Gil S, Pastor S, March GJ (2007) Quantitative isolation of biocontrol agents *Trichoderma* spp., *Gliocladium* spp. and Actinomycetes form soil with culture media. Microbiol Res (in press)

Vargas Gil S, Pedelini R, Oddino C, Zuza M, Marinelli A, March GJ (2008) The role of potential biocontrol agents in the management of peanut root rot in Argentina. J Plant Pathol 90:35–41