

# The effect of crop sequences on soil microbial, chemical and physical indicators and its relationship with soybean sudden death syndrome (complex of *Fusarium* species)

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## Abstract

The effect of crop sequences on soil quality indicators and its relationship with sudden death syndrome (SDS, a complex of *Fusarium* species) was evaluated by physical, chemical, biochemical and molecular techniques. Regarding physical aspects, soybean/maize and maize monoculture exhibited the highest stable aggregate level, with values 41% and 43% higher than in soybean monoculture, respectively, and 133% higher than in bean monoculture. Bulk density (BD) was higher in soybean monoculture, being 4% higher than in bean monoculture. The chemical parameters organic matter, total N, P, K, Mg, Ca, and water holding capacity also indicated that soybean/maize and maize monoculture improved soil quality. Fungal and bacterial community fingerprints generated using Terminal Restriction Fragment Length Polymorphism analysis of intergenic transcribed spacer regions of rRNA genes and 16S rRNA genes, respectively, indicated a clear separation between the rotations. Fatty acid profiles evaluated by FAME showed that bean monoculture had higher biomass of Gram (+) bacteria and stress indicators than maize monoculture, while the soybean/maize system showed a significant increase in total microbial biomass (total FAMEs content) in comparison with soybean and bean monoculture. The incidence of SDS (*Fusarium crassistipitatum*) was markedly higher (15%) under soybean monoculture than when soybean was grown in rotation with maize. In the present work, soil microbial properties were improved under soybean/maize relative to continuous soybean. The improvement of soil health was one of the main causes for the reduction of disease pressure and crop yield improvement due to the benefits that crop rotation produces for soil quality.

**Additional key words:** soil microbial populations; T-RFLP; FAME; chemical parameters; physical variables; soybean sudden death syndrome.

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## Introduction

In an attempt to increase yields and competitiveness, conservation management practices are being disregarded, the predominant practices being crop monoculture, intensive tillage, and excessive use of pesticides

(Sainju *et al.*, 2011). Therefore, agroecosystems are becoming highly vulnerable and dependent on high chemical inputs for improving soil fertility (Vargas-Gil *et al.*, 2009a). Accordingly, evidence indicates that crops grown in short rotations or monoculture often suffer from yield decline compared to crops grown in

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Abbreviations used: ANOVA (analysis of variance); AS (aggregate stability); BD (bulk density); CFU (colony forming units); EC (electrical conductivity); FAME (fatty acid methyl ester); FDA (fluorescein diacetate); FID (flame ionization detector); GRSP (glomalin-related soil protein); INTF (iodonitrotetrazolium formazan); ITS (internal transcribed spacer); OM (organic matter); PCA (principal component analysis); PCR (polymerase chain reaction); PET (photo electron transfer); SDS (sudden death syndrome); TRF (terminal restriction fragment); T-RFLP (terminal restriction fragment length polymorphism).

longer rotations or for the first time (Bennett *et al.*, 2012). Numerous factors have been hypothesised as contributing to yield decline, including biotic factors such as plant pathogens, deleterious rhizosphere microorganisms, unusual mycorrhizal behaviour, and allelopathy or autotoxicity of the crop, as well as abiotic factors such as land management practices and nutrient availability. Moreover, in monocultural systems, the uniformity caused by the continuous growing of a single crop produces several detrimental effects on soil properties (Dijkstra *et al.*, 2010). Because plants stimulate soil microbial activity by root exudation of energy-rich-compounds, the presence of a single plant species reduces the variety of root exudates, and consequently the diversity of microbial communities (Stephan *et al.*, 2000). Moreover, the type of residues that return to soil also determines the abundance and diversity of microorganisms and their activities, which are lower in such simple systems than in those where different plant species are combined (Wang *et al.*, 2009). Indeed, plants are a primary driver of changes in soil microbial communities, and biological disease suppression is precisely a result of complex changes in soil microbial community characteristics. Because crop rotation can dramatically affect soil microbial communities, the use of crops that have specific effects on soil microbial communities and the development of disease-suppressive soils is a viable approach to disease management, sometimes referred to as active management of soil microorganisms (Larkin *et al.*, 2010). Moreover, it has been frequently reported that soil under monoculture is prone to nutrient deficiencies, because high-yielding varieties direct more growth to grain. These nutrients are harvested from the land along with the grain and less plant material is being left nutrient recycling back to the soil. Part of the instability of monocultural agroecosystems can be also associated with a soil structure weakened by increased susceptibility to compaction, reduced water infiltration, and increased erosion (Arshad *et al.*, 2011). These characteristics make monoculture systems less resilient to stress (Zuo & Zhang, 2009). To identify the effects of different monoculture crops on soil properties and crop health, the magnitude of stress, the degree of exposure to stresses or the ecological response to exposure can be quantified using ecological indicators because they provide an efficient means to characterize composition, structure, and function of complex ecological systems (Karr, 1991). A challenge in selecting ecological indicators is determining those measures

that appropriately characterize the system and yet are simple enough to be effectively and efficiently monitored (Dale *et al.*, 2008).

Different crops grown in monoculture have diverse effects on soil properties; the combined use of legume and non-legume plants plays an important role in agricultural ecosystems. Yield decline is the loss of productive capacity of soil caused by biotic and abiotic factors when crops are grown repeatedly on the same land, in short rotation or monoculture, resulting in poor plant growth and development. This work implies an important contribution to give light to the concept of yield decline, because the objective is to explore the effect of the most common crop sequences employed in north-western Argentina, such as soybean/maize and the monocropping of maize, soybean and bean, on soil quality and soybean health. Quantitative data on the relationships between on-site soil quality and long-term site productivity of crops in north-western Argentina is necessary for sustainable crop production.

## Material and methods

### Field experimental design and soil sampling

Field research was conducted at the EEA INTA Salta (Agricultural and Livestock Technology National Institute-INTA), located in Salta province, Argentina. The trial was established in 1990. The study area lies in the subtropical region of Argentina (24° 53' 43.6" W 65° 27' 58.6" S, 1,100 m asl). The climate is temperate with little or no water deficit in January and February. Mean annual precipitation is 900 mm, concentrated in spring-summer, with a prolonged dry season in winter. The temperature regime is temperate/mesothermal, with an annual mean of 16°C.

The dominant soil type is loam with 2.91% of organic matter (32% sand, 44% silt, 24% clay) Ustocrepte Udico (Soil Survey Staff, 1999) Cerrillos series with A, AC and C horizons, according to Vargas-Gil (1990). The experimental design of the field trial followed a randomized complete block with three replications, with 1-ha plots seeded with soybean in rotation with maize and monocultures of maize, soybean and bean, each of these crops have been grown for 21 years. 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. Soybean and maize

were seeded on December 15, 2008 and harvested on May 30, 2009; bean was seeded on February 15, 2009 and harvested on May 30, 2009. All plots were treated with glyphosate (48% a.i., 3 L ha<sup>-1</sup>) before planting. Seeding rates were 25, 3, and 15 seeds m<sup>-1</sup> for soybean, maize and bean, respectively, with 52-cm row width for all crops. All crops were managed using recommended production practices, including measures of fertilizer rates, pesticide application, and weed control specific for each crop. Crops were harvested with appropriate equipment at physiological maturity.

Sampling was performed at crop flowering. For biochemical, chemical and physical analyses, the same soil samples were employed. Six soil samples near the plant roots were collected from 0-15 cm in depth. Soil samples were sieved at field moisture (2-mm), homogenized, air dried and stored at room temperature for further analysis.

### Physical and chemical soil analyses

Aggregate stability (AS) was determined following the method of the micro-sieves (1-2 mm) according to Corvalán *et al.* (2000). Bulk density (BD) was measured by the core method (Blake & Hartge, 1986), using cores 3 cm in diameter, 10 cm in length, and 70.65 cm<sup>3</sup> in volume.

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 the micro-Kjeldhal method (Bremner, 1996). Moreover, extractable phosphorus (P) was quantified by Bray-Kurtz method (Bray & Kurtz, 1945), pH with 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. Na, K, Ca and Mg were extracted with 1 N ammonium acetate at pH 7, and quantified using a spectrometer with atomic bulbs (Perkin Elmer 5100 PC). Water holding capacity was measured by gravimetric method (Allen, 1989).

### Biological soil analyses

Microbial biomass C was determined employing the chloroform fumigation incubation technique of Jenkinson & Powlson (1976). To quantify soil microbial respiration, potentially mineralizable C was determined according to Alef (1995). General microbial activi-

ty was measured by hydrolysis of fluorescein diacetate (FDA) using the procedure of Adam & Duncan (2001). Dehydrogenase activity was determined according to García *et al.* (1997). Microbial populations were determined by soil dilution plating on various agar media (Vargas-Gil *et al.*, 2009b). Glomalin-related soil protein (GRSP) was determined in the easily extractable glomalin form, according to Wright & Upadhyaya (1996).

### Biochemical soil analyses

A direct DNA fingerprinting method was used to assess rhizosphere communities of fungi and bacteria. A relative dominance of each terminal restriction fragment (TRF) within a profile was obtained, as described by Singh *et al.* (2006). Community DNA was extracted with the FastDNA<sup>®</sup> SPIN Kit for soil (MP Biomedicals), using the manufacturers' protocol. Optimized PCR reactions were performed in triplicate for each sample. The DNA extracted was PCR-amplified using universal fungal and bacterial primers. For fungi, 25 pmol of photo electron transfer (PET)-labelled ITS1f (5'-CTT GGT CAT TTA GAG GAA GTA A-3') (Gardes & Bruns, 1993) and ITS 4r (5'-TCC TCC GCT TATTGA TAT GC-3') (White *et al.*, 1990) were employed. These primers amplify the intergenic transcribed spacer region (ITS) of ribosomal DNA and have been successfully used to amplify ascomycete and basidiomycete (White *et al.*, 1990; Gardes & Bruns, 1993). For bacteria, a 5 pmol of PET-labelled 1087r (5'-CTC GTT GCG GGA CTT ACC CC-3') (Hauben *et al.*, 1997), and 63f (5'-AGG CCT AAC ACA TGC AAG TC-3') (Marchesi *et al.*, 1998) were used. These primers amplify the 16S ribosomal DNA and are widely used to characterize bacterial community structure. For PCR amplification, a master mix containing 47 µL Megamix (Microzone), 10 ng of DNA, and 1 µL of a forward and reverse primer each was used. Reactions were performed in a thermocycler (Gene Amp 9600) using the temperature cycling protocol of 95°C (3 min) and 30 cycles of 95°C (30 s), 55°C (60 s), 72°C (60 s), and 72°C (30 s). To obtain sufficient DNA for T-RFLP analysis and to minimize PCR bias, amplicons from three PCR runs for each root sample were combined (Clement *et al.*, 1998) and then purified using a Quiagen PCR purification kit. Purified PCR products of 250 ng were separately digested with restriction endonucleases *HhaI* (GCG<sup>°</sup>C) or *MspI* (C<sup>°</sup>CGG)

(BioLabs, New England) at 37°C (4 h); the reaction was terminated with another incubation at 95°C (15 min). T-RFLP analysis was carried out on an automated sequencer ABI PRISM1 3130xl Genetic Analyzer on a 36 cm capillary array (Appl. Biosyst. Instrum., Warrington, UK).

Analysis of whole-soil fatty acid methyl ester (FAME) profiles were used to detect changes in microbial communities in the different crop management practices (Cavigelli *et al.*, 1995). Ten gram sub-samples were weighed in ashed glass test tubes. Lipid extraction and saponification was performed by adding 5 mL of 3.25 M NaOH dissolved in methanol and heating at 80°C for 1 h. Extraction mixtures were neutralized by adding 10 mL of 3.25 N HCl in methanol and 3 mL of hexane. Extracts were centrifuged at 1,000 rpm for 20 min. Hexane was evaporated almost to dryness under nitrogen gas and then transferred to labelled vials for injection in a gas chromatography (Clarus 500 Perkin Elmer) equipped with a flame ionization detector (FID) and Elite-5 capillary column. Methyl nonadecanoate was used as a quantitative internal standard. The separated FAMES were identified and quantified by chromatography retention time, using standard bacterial acid methyl ester mix (Supelco, Supelco UK, Poole, Dorset, UK). FAMES described here use the standard nomenclature for lipid markers, A:B $\omega$ C, where A is the number of carbon atoms, B is the number of double bonds, and  $\omega$ C indicates the number of carbon atoms from the aliphatic end of the molecule and the first unsaturated bond. The ratio between cy19:0 and their metabolic precursor 18:1 $\omega$ 9 was used as an indicator of physiological stress (Kourtev *et al.*, 2003).

### Disease incidence

The method employed for the evaluation of disease incidence was conducted according to previous work (Vargas-Gil *et al.*, 2008). The incidence of sudden death syndrome (SDS) was evaluated before harvest by establishing 10 sample stations (50 plants each) in each plot, regularly distributed in a V-shaped design (systematic sampling procedure). SDS was detected by the presence of typical foliar symptoms (interveinal chlorosis and necrosis) and root rot (Aoki *et al.*, 2005), and confirmed by isolations of the pathogens from roots. The disease incidence was determined as a percentage of plants infected.

### Statistical analyses

Statistical analyses were performed using InfoStat Professional version 2009 (Universidad Nacional de Córdoba, Argentina). Data for culturable microbial populations, microbial activity and biomass, and soil chemical and physical 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, an LSD test at a significance level of  $p \leq 0.05$  was used. Fungal and bacterial T-RFLP profiles were characterised using Gene Marker software (version 1.6 Soft Scientifics LLC). All TRFs with less than 50 fluorescence units were discarded from the data analysis to minimize the effect of artefacts. For community analysis, TRFs that were separated from one another by  $> 1$  bp were considered as distinct TRFs. The relative abundance of a TRF in a profile was calculated as a proportion of the total peak height of all the TRFs in a profile. Any peak that was less than 0.5% of the total fluorescence units was removed from the data before statistical analysis. A principal component analysis (PCA) was performed to detect differences in microbial community structure for molecular profiles of fungi and bacteria. For each data set, an ANOVA was performed on the principal component (PC) scores for the first five dimensions to examine the effect of crop rotation on soil microbial community structure. Individual FAMES and FAME clusters were analyzed by ANOVA to determine treatment effects on FAME composition in the soil. Correlation analysis was performed on FAMES data, PC 1 and PC 2 ( $p \leq 0.05$ ) to identify those fatty acids whose gradients were represented by PCs 1 and 2. Another PCA was performed to determine separation among treatments, but also to analyze which of the microbiological, chemical and physical variables best contributed to the separation of treatments. Finally, a correlation analysis was performed to establish correlations between soil biological, chemical and physical properties with the detected soilborne disease of soybean and with crop yield.

## Results

### Soil physical and chemical analyses

Aggregate stability (AS) and bulk density (BD) were clearly influenced by cropping practices (Table 1). Soybean/maize and maize monoculture exhibited the highest AS level, with values 6 % and 6.33% higher than in soy-

**Table 1.** Effect of field treatments on soil chemical and physical parameters

Soil chemical properties <sup>1</sup>	Field treatments			
	Soybean/ Maize	Maize monoculture	Soybean monoculture	Bean monoculture
OM (%)	2.13 <sup>a</sup>	1.93 <sup>a</sup>	1.36 <sup>b</sup>	0.87 <sup>c</sup>
Total N (%)	0.12 <sup>a</sup>	0.11 <sup>a</sup>	0.08 <sup>b</sup>	0.07 <sup>c</sup>
C/N (%)	10.33 <sup>a</sup>	10.00 <sup>a</sup>	9.83 <sup>a</sup>	9.17 <sup>b</sup>
P (ppm)	18.00 <sup>a</sup>	20.33 <sup>a</sup>	5.67 <sup>c</sup>	11.50 <sup>b</sup>
pH (H <sub>2</sub> O)	6.00 <sup>a</sup>	5.87 <sup>a</sup>	6.05 <sup>a</sup>	5.92 <sup>a</sup>
EC (mmhos cm <sup>-1</sup> )	0.46 <sup>b</sup>	0.40 <sup>b</sup>	0.38 <sup>b</sup>	0.61 <sup>a</sup>
Na (meq/100 g)	0.15 <sup>b</sup>	0.18 <sup>a</sup>	0.13 <sup>c</sup>	0.17 <sup>ab</sup>
K (meq/100 g)	1.17 <sup>a</sup>	1.28 <sup>a</sup>	0.59 <sup>b</sup>	0.42 <sup>c</sup>
Ca (meq/100 g)	6.98 <sup>a</sup>	6.95 <sup>a</sup>	4.63 <sup>b</sup>	5.18 <sup>b</sup>
Mg (meq/100 g)	2.57 <sup>a</sup>	2.70 <sup>a</sup>	1.85 <sup>b</sup>	1.52 <sup>c</sup>
Water holding capacity (%)	32.50 <sup>a</sup>	32.50 <sup>a</sup>	25.33 <sup>b</sup>	23.83 <sup>b</sup>
AS (%)	20.67 <sup>a</sup>	21.00 <sup>a</sup>	14.67 <sup>b</sup>	9 <sup>c</sup>
BD (g cm <sup>-3</sup> )	1.71 <sup>c</sup>	1.69 <sup>c</sup>	1.86 <sup>a</sup>	1.79 <sup>b</sup>

<sup>1</sup> OM: organic matter; P: extractable phosphorus; EC: electrical conductivity; AS: aggregate stability; BD: bulk density. Within rows, values followed by the same letter do not differ significantly ( $p < 0.001$ ), as determined by the LSD test.

bean monoculture, respectively, and 11.67% higher than in bean monoculture. BD was significantly higher in soybean monoculture, with 4% higher values than in bean monoculture. The lowest BD values were recorded in the soybean/maize and maize monoculture, without statistical difference between those treatments. BD was 9% higher in soybean monoculture than in soybean/maize.

Soil chemical properties were also affected by soybean rotation treatments (Table 1). Soil organic matter (OM) content was higher in the soybean/maize system (144%) and maize monoculture (121%) than in bean monoculture. As with OM, total N was highest in the soybean/maize system and maize monoculture, being 71% and 57% higher, respectively, than in bean monoculture. P was also 56% and 76% higher in soybean/maize and maize monoculture treatments, respectively, than in bean monoculture. K and Mg presented higher values in soybean/maize (K, 178%; Mg, 69%) and maize monoculture (K, 204%; Mg, 77%) treatments than in bean monoculture. Ca and water holding capacity showed higher soil content in soybean/maize and maize monoculture than in soybean and bean monoculture. The lowest Na content was recorded in the soybean monoculture system. The only chemical variable that was not affected by the different field treatments was the pH.

### Soil biochemical and biological analyses

Crop rotation and maize monoculture clearly influenced microbial populations and their activities

(Table 2). In general, microbial biomass and activity was low in the soybean monoculture system, and the lowest values were recorded in the bean monoculture system. Microbial biomass C was 117% higher in maize monoculture than in bean monoculture. Total fungi were more (1,476 times) abundant in soybean/maize treatment than in the bean monoculture system. The bean monoculture exhibited the lowest value of total bacteria. Moreover, the highest GRSP values were recorded in the soybean/maize treatment and maize monoculture, with no statistical difference between treatments; the lowest values corresponded to the bean monoculture treatment (122%).

The potential biocontrol agents *Trichoderma* spp. and *Gliocladium* spp. exhibited highest values in the soybean/maize treatment, being higher than in the bean and soybean monoculture systems. Microbial activities were also markedly influenced by the different treatments. In soybean/maize, microbial respiration, FDA hydrolysis, and dehydrogenase activity were markedly higher (148, 479, and 34 times, respectively), than in bean monoculture.

### Terminal restriction fragment length polymorphism (T-RFLP)

Crop rotation had influence on the number of fungal and bacterial T-RFs (*HhaI* and *MspI* combined). The total number of fungal T-RFs ( $56 \pm 1.3$ ) was significantly higher ( $p < 0.05$ ) in maize monoculture, com-

**Table 2.** Effect of field treatments on soil biological parameters

	Field treatments			
	Soybean/ Maize	Maize monoculture	Soybean monoculture	Bean monoculture
Soil biological properties				
Microbial biomass C (mg CO <sub>2</sub> g <sup>-1</sup> )	0.54 ± 0.32 <sup>b</sup>	0.76 ± 0.32 <sup>a</sup>	0.42 ± 0.28 <sup>b</sup>	0.35 ± 0.25 <sup>b</sup>
Total fungi <sup>w</sup> (CFU g <sup>-1</sup> dry soil)	26.3 ± 1.60 <sup>a</sup>	13.33 ± 5.18 <sup>b</sup>	2.50 ± 1.79 <sup>c</sup>	1.67 ± 1.46 <sup>c</sup>
Total bacteria <sup>w</sup> (CFU g <sup>-1</sup> dry soil)	1.82 ± 0.90 <sup>a</sup>	2.17 ± 0.70 <sup>a</sup>	0.2 ± 1.10 <sup>a</sup>	0.3 ± 3.12 <sup>b</sup>
GRSP <sup>x</sup> (mg g <sup>-1</sup> soil)	0.20 ± 0.02 <sup>a</sup>	0.20 ± 0.03 <sup>a</sup>	0.13 ± 0.11 <sup>b</sup>	0.09 ± 0.04 <sup>c</sup>
Potential biocontrol agents (CFU g <sup>-1</sup> dry soil)				
<i>Trichoderma</i> spp.	0.42 ± 0.67 <sup>a</sup>	0.42 ± 0.50 <sup>a</sup>	0.08 ± 0.28 <sup>b</sup>	0.08 ± 0.28 <sup>b</sup>
<i>Gliocladium</i> spp.	0.83 ± 2.33 <sup>a</sup>	0.25 ± 0.44 <sup>ab</sup>	0.08 ± 0.28 <sup>b</sup>	0.00 ± 0.03 <sup>b</sup>
Actinomycetes	1.58 ± 1.62 <sup>a</sup>	1.75 ± 1.45 <sup>a</sup>	0.08 ± 0.28 <sup>b</sup>	1.33 ± 1.46 <sup>a</sup>
Fluorescent pseudomonads	0.25 ± 0.45 <sup>a</sup>	0.33 ± 0.87 <sup>a</sup>	0.29 ± 0.38 <sup>a</sup>	0.17 ± 0.23 <sup>a</sup>
Microbial respiration (mg CO <sub>2</sub> g <sup>-1</sup> week <sup>-1</sup> )	0.77 ± 0.14 <sup>a</sup>	0.77 ± 0.21 <sup>a</sup>	0.50 ± 0.18 <sup>b</sup>	0.31 ± 0.20 <sup>c</sup>
FDA <sup>y</sup> hydrolysis (µg fluorescein g <sup>-1</sup> h <sup>-1</sup> )	4.17 ± 0.61 <sup>a</sup>	3.17 ± 0.52 <sup>b</sup>	2.68 ± 0.46 <sup>c</sup>	0.72 ± 0.35 <sup>d</sup>
Dehydrogenase activity (mg INTF <sup>z</sup> g <sup>-1</sup> soil h <sup>-1</sup> )	7.31 ± 1.89 <sup>a</sup>	8.81 ± 3.96 <sup>a</sup>	4.95 ± 3.87 <sup>b</sup>	5.45 ± 1.58 <sup>b</sup>

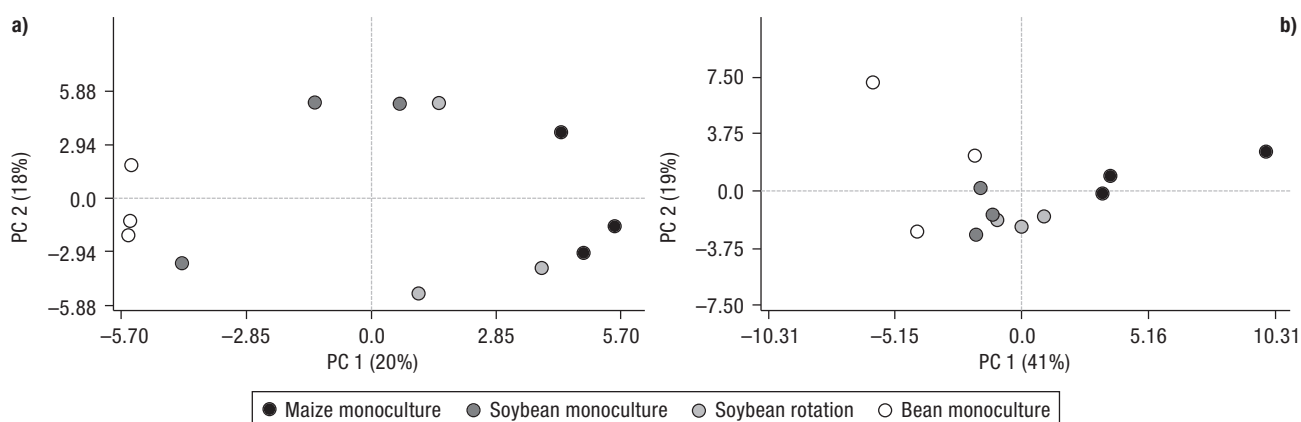
<sup>w</sup> CFU: colony forming units (Fungi expressed as ×10<sup>5</sup>, bacteria as ×10<sup>7</sup>). <sup>x</sup> GRSP: glomalin-related soil protein. <sup>y</sup> FDA: fluorescein diacetate. <sup>z</sup> INTF: iodonitrotetrazolium formazan. Within rows, values followed by the same letter do not differ significantly ( $p < 0.001$ ), as determined by the LSD test.

pared with bean monoculture ( $27 \pm 1.6$ ). Moreover, maize monoculture had significantly more ( $p < 0.05$ ) bacterial TRFs ( $57 \pm 1.7$ ) than bean ( $31 \pm 2.6$ ) and soybean ( $34 \pm 3.1$ ) monoculture and soybean rotation ( $31 \pm 2.4$ ). Regarding the fungal molecular profiles generated by *MspI*, there was a strong influence of soybean rotation on PC 2 ( $p \leq 0.001$ ). PC 1 accounted for 20% of variation and PC 2, for 18% (Fig. 1a). Samples from maize monoculture and soybean clustered together, and were separated from the crop monoculture (bean, soybean) treatments along PC1. With respect to the bacterial molecular profiles generated

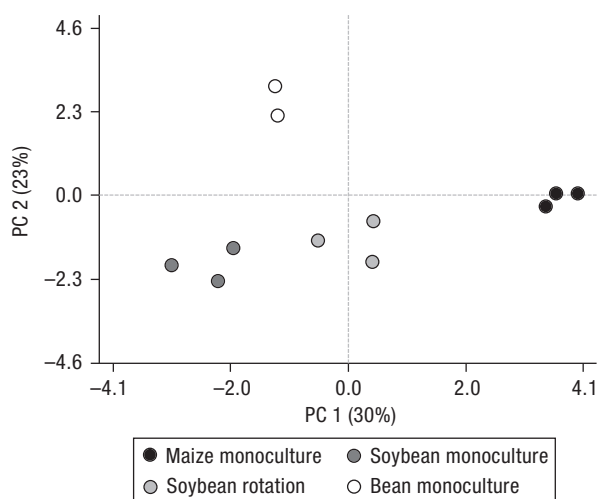
by *MspI*, the first two PCs accounted for 60%, (41 and 19% of the variation explained by PC 1 and 2, respectively) (Fig. 1b). The bacterial profiles revealed a clear treatment separation between maize monoculture and leguminous crops, the latter being under rotation (soybean/maize) or under monoculture (soybean, bean).

### Microbial community structure

PCA of the FAME profiles revealed differences in soil microbial community structure (Fig. 2). PC1



**Figure 1.** Ordination plots of principal component analysis of soil fungal (a) and bacterial (b) T-RFLP profiles of four crop treatments. Numbers in parenthesis are variance percentage explained by each principal component (PC).



**Figure 2.** Ordination plots of principal component analysis of individual FAMES.

explained 30% of variance, whereas PC2 explained 23%, for a cumulative total of 53%. The score plot indicated that bean monoculture was separated from the other treatments along axis 2; whereas soybean monoculture, maize monoculture and maize/soybean systems were separated along axis 1. Fatty acids with high correlation coefficient (cc) for PC1 and PC2 included a15:0 (cc: -0.79), i16:0 (cc: -0.77), cy17:0 (cc: -0.74), 15:0 (cc: -0.65), 16:0 (cc: 0.88), 16:1 $\omega$ 9 (cc: 0.81), 18:1 $\omega$ 9c (cc: 0.59) and i15:0 (cc: -0.80), 18:2 $\omega$ 6,9 (cc: -0.72), 18:1 $\omega$ 9c (cc: -0.71), 15:0 (cc: -0.61), 16:1 $\omega$ 11 (cc: 0.72), cy19:0 (cc: 0.69), i17:0 (cc: 0.59) (Table 3, Fig. 2). Comparison of the means of fatty acids showed that bean monoculture had higher values of Gram (+) bacteria and stress indicator than maize monoculture, with no statistical difference with soybean either under monoculture or in rotation (Table 3). The analysis also indicated a significant in-

**Table 3.** Effect of field treatments on individual fatty acid methyl esters (FAMES) and microbial FAME indicators

	Treatments			
	Maize monoculture	Bean monoculture	Soybean monoculture	Soybean/Maize
<i>Individual FAMES (nmol %)</i>				
12:0	0.76 <sup>a</sup>	2.15 <sup>b</sup>	0.84 <sup>a</sup>	1.08 <sup>ab</sup>
14:0	1.97 <sup>a</sup>	2.50 <sup>a</sup>	3.31 <sup>a</sup>	2.47 <sup>a</sup>
i15:0	2.70 <sup>a</sup>	2.50 <sup>a</sup>	3.15 <sup>b</sup>	3.31 <sup>b</sup>
a15:0	1.39 <sup>a</sup>	1.85 <sup>b</sup>	1.84 <sup>b</sup>	1.49 <sup>a</sup>
15:0	0.64 <sup>a</sup>	0.65 <sup>a</sup>	0.93 <sup>a</sup>	0.71 <sup>b</sup>
i16:0	1.86 <sup>a</sup>	2.57 <sup>a</sup>	3.74 <sup>b</sup>	2.51 <sup>a</sup>
16:0	33.40 <sup>c</sup>	23.27 <sup>a</sup>	25.4 <sup>b</sup>	26.41 <sup>b</sup>
16:1 $\omega$ 9	6.06 <sup>c</sup>	3.03 <sup>a</sup>	4.03 <sup>b</sup>	3.81 <sup>b</sup>
16:1 $\omega$ 11	7.20 <sup>b</sup>	6.91 <sup>b</sup>	3.83 <sup>a</sup>	4.34 <sup>a</sup>
i17:0	0.94 <sup>a</sup>	3.86 <sup>a</sup>	1.22 <sup>a</sup>	1.89 <sup>a</sup>
a17:0	0.65 <sup>a</sup>	1.52 <sup>a</sup>	0.98 <sup>a</sup>	1.93 <sup>a</sup>
17:0	0.65 <sup>a</sup>	0.97 <sup>a</sup>	1.09 <sup>a</sup>	0.79 <sup>a</sup>
cy17:0	0.72 <sup>a</sup>	1.52 <sup>ab</sup>	2.34 <sup>b</sup>	1.01 <sup>a</sup>
18:0	10.83 <sup>a</sup>	12.82 <sup>ab</sup>	15.00 <sup>b</sup>	14.76 <sup>b</sup>
18:1 $\omega$ 9c	10.84 <sup>b</sup>	6.45 <sup>a</sup>	9.40 <sup>b</sup>	9.32 <sup>b</sup>
18:1 $\omega$ 9t	6.58 <sup>a</sup>	5.39 <sup>a</sup>	6.63 <sup>a</sup>	6.42 <sup>a</sup>
18:2 $\omega$ 6,9c	5.32 <sup>ab</sup>	4.88 <sup>a</sup>	6.50 <sup>bc</sup>	6.95 <sup>c</sup>
cy19:0	3.24 <sup>a</sup>	10.14 <sup>b</sup>	6.06 <sup>ab</sup>	4.78 <sup>a</sup>
20:0	4.25 <sup>a</sup>	7.01 <sup>a</sup>	3.70 <sup>a</sup>	6.03 <sup>a</sup>
<i>Microbial FAME indicators</i>				
Gram (+) (nmol %)	7.55 <sup>a</sup>	12.31 <sup>b</sup>	10.93 <sup>ab</sup>	11.12 <sup>ab</sup>
Gram (-) (nmol %)	34.63 <sup>a</sup>	33.44 <sup>a</sup>	32.28 <sup>a</sup>	29.68 <sup>a</sup>
Fungi (nmol %)	5.32 <sup>ab</sup>	4.88 <sup>a</sup>	6.50 <sup>bc</sup>	6.95 <sup>c</sup>
Total biomass (nmol g <sup>-1</sup> soil)	830.68 <sup>ab</sup>	531.59 <sup>a</sup>	512.41 <sup>a</sup>	1,221.80 <sup>b</sup>
Stress indicator (dimensionless)	0.19 <sup>a</sup>	0.98 <sup>b</sup>	0.37 <sup>ab</sup>	0.31 <sup>ab</sup>

crease in total microbial biomass (estimated as total FAME content) for the soybean/maize system with respect to soybean and bean monoculture (Table 3).

### Disease incidence

Soybean was tested for the presence of symptoms of diseases caused by soilborne fungi, with the aim of quantifying disease incidence and of determining the association with soil indicators. The occurrence of SDS caused by *Fusarium crassistipitatum* Scandiani, T. Aoki et O'Donnell (the predominant species in this region), was detected in soybean monoculture, with an incidence of 15%, whereas in soybean/maize it was not detected. With the purpose of establishing the relationship between soil parameters and the incidence of SDS, a correlation analysis was performed (Table 4). A significant negative correlation of disease incidence with some biological parameters was found, such as with microbial biomass C (−0.36), GRSP (−0.24) and the potential biocontrol agents *Trichoderma* spp. (−0.11), actinomycetes (−0.69), fluorescent pseudomonads (−0.53), and FDA hydrolysis (−0.86). Moreover, a significant negative correlation was observed between disease incidence and some chemical parameters, such as K (−0.66), Ca (−0.80), Mg (−0.53). There was a positive significant correlation between disease incidence and bulk density (0.82). Finally, soybean yield was 74% higher in the soybean/maize rotation (3.46 t ha<sup>−1</sup>), compared with monocropping (1.98 t ha<sup>−1</sup>). The correlation of soybean yield with disease incidence was significantly negative (−0.90).

### Integrated multivariate analysis

Another PCA was performed with data on soil microbial, chemical and physical properties to select the soil quality indicators that best contributed to the differentiation of treatments, (Fig. 3). The first and second principal components (PC 1 and PC 2) accounted for 38% and 11% of the total variance, respectively. Based on the PC 1 axis, the patterns of soybean rotation and maize monoculture treatments were markedly different from those of bean and soybean monoculture. These separations were mainly associated with increases in the following biological parameters: GSRP content, microbial respiration, FDA hydrolysis and total fungi and bacteria counts.

**Table 4.** Correlation analysis between soil properties and sudden death syndrome (SDS) incidence (*Fusarium crassistipitatum* Scandiani, T. Aoki et O'Donnell)

Soil parameters	Pearson coefficients of SDS
Microbial biomass C (mg CO <sub>2</sub> g <sup>−1</sup> )	−0.36*
Total fungi (CFU g <sup>−1</sup> dry soil)	−0.86
Total bacteria (CFU g <sup>−1</sup> dry soil)	−0.38
GRSP (mg g <sup>−1</sup> soil)	−0.24*
Potential biocontrol agents (CFU g <sup>−1</sup> dry soil)	
<i>Trichoderma</i> spp.	−0.11*
<i>Gliocladium</i> spp.	−0.12
Actinomycetes	−0.69*
Fluorescent pseudomonads	−0.53*
Microbial respiration (mg CO <sub>2</sub> g <sup>−1</sup> week <sup>−1</sup> )	−0.51
FDA hydrolysis (µg fluorescein g <sup>−1</sup> h <sup>−1</sup> )	−0.86*
Dehydrogenase activity (mg INTF g <sup>−1</sup> soil h <sup>−1</sup> )	0.64
OM (%)	−0.73
Total N (%)	−0.68
P extractable (ppm)	0.14
pH (H <sub>2</sub> O)	0.07
EC (mmhos cm <sup>−1</sup> )	−0.23
Na (meq/100 g)	−0.14
K (meq/100 g)	−0.66*
Ca (meq/100 g)	−0.80*
Mg (meq/100 g)	−0.53*
Water holding capacity (%)	−0.72
AS	0.17
BD	0.82*
Crop yield	−0.90*

EC: electrical conductivity. GRSP: glomalin-related soil protein. FDA: fluorescein diacetate. INTF: idonitrotetrazolium formazan. OM: organic matter. AS: aggregate stability. BD: bulk density. \* Significant at  $p < 0.05$ .

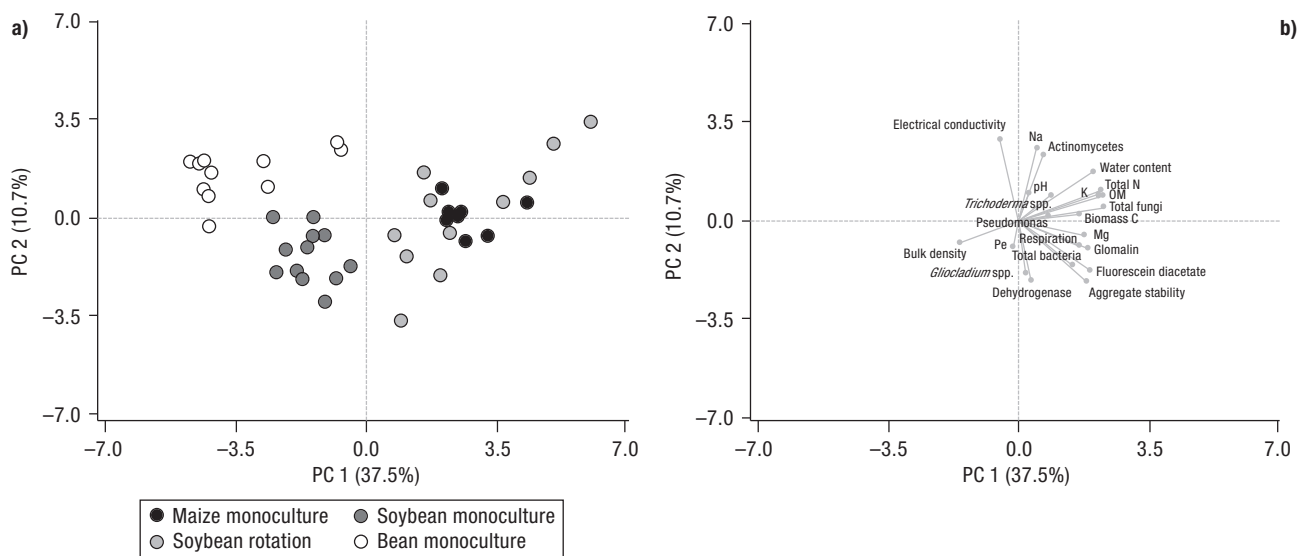
Among the chemical properties, soil organic matter, total N, K, Ca were important in separating the field treatments mentioned. Finally, the physical parameters BD and AS also contributed to that separation of treatments.

## Discussion

### Soil physical analyses

Aggregate stability (AS) significantly increased in soybean rotation and maize monoculture, as compared to soybean monoculture, with the lowest values in bean monoculture. This is in part due to the biochemical composition and amount of crop residues returned to soil which are directly related to OM affecting aggre-





**Figure 3.** Ordination (a) and loading (b) plots of principal component analysis of crop sequences on soil microbial, chemical and physical parameters. PC: principal component. OM: organic matter.

gate formation and the rate of aggregate turnover. Continuous soybean planting often increases micro-aggregates and decreases macro-aggregates compared to maize planting because of the lower concentration of phenols and lower amount of residues (Bronick & Lal, 2005). Furthermore, the higher microbial diversity in soybean/maize and maize monocropping observed also favoured AS increase, because of the presence of polysaccharide mucilages that derive from bacteria and fungi, which are believed to be effective glues binding soil particles (Oades, 1984). Accordingly, we also found a positive correlation between GRSP content and AS; according to Wright & Upadhyaya (1996), GRSP also contributes to soil agglutination. Moreover, it has also been confirmed that soil OM accumulation at surface soil increases AS, decreases erosion processes and increases the amount of nutrients available to plants. Arshad *et al.* (2011) found that indicators of soil structure, such as AS and soil OM quantity and quality, were positively influenced by perennial grasses and legume-based crop rotations. BD was higher in soybean and bean monoculture, with the lowest values in the soybean/maize and maize monoculture. In the present work, BD was negatively associated with soil OM. It is well known that OM itself enhances soil resistance to compaction through several mechanisms (strengthening of binding forces between particles and aggregates and higher elasticity of aggregates under compression) and promotes higher porosity, thus maintaining soil BD at low levels (Vargas-Gil *et al.*, 2009a).

### Soil chemical analyses

Soybean/maize and maize monoculture exhibited the highest amounts of OM, total N, P, K, Mg, Ca, and water holding capacity. The reason for the higher amounts of nutrients in soybean/maize treatment than in soybean monoculture may be that crop rotation increases the input of C to the soil through root exudates and residues; rotation schemes including legumes also increase the amount of N in the soil, thereby increasing crop production (Donnison *et al.*, 2000). However, the amounts of nutrients in maize monocropping were as high as in the rotation treatment. Fuentes *et al.* (2010) also found a greater proportion of C in soils with maize monoculture and residue retention than in soils with other treatments, regardless of tillage. The great benefits that maize produces to soil quality are attributed to the residues from C4 plants, as explained above. However, it has been documented that continuous maize systems generally contain significantly lower concentrations and qualities of soil OM (Karlen *et al.*, 2006). Likewise, Drinkwater *et al.* (1998) stated that the qualitative differences from legume-based cropping systems were important for maintaining soil C and N levels. Those authors suggested that legume-based sequences, with their narrow C-to-N organic residues, would significantly increase soil C and N retention. A higher organic C accumulation in clay minerals favours more vigorous biological activity, leading to the formation of more

microbial metabolites and residues, which are relatively less susceptible to decomposition (Kaiser *et al.*, 1998). This evidence supports the correlation between the higher OM in the treatments under soybean rotation and maize monocropping used in this work.

### Soil biological and biochemical analyses

Maize monoculture and soybean/maize exhibited more abundance and higher activity of soil microbial populations than soybean and bean monocropping, the latter system having the lowest quality from the microbial view point. Microbial biomass C, total fungi and bacteria, GRSP, and potential biocontrol agents (*Trichoderma* spp. and *Gliocladium* spp.) were some of the microbial parameters evaluated that best indicated the beneficial effect of maize on the agroecosystem. In addition, microbial activities were increased in the presence of maize, as shown by the quantification of microbial respiration, FDA hydrolysis, and dehydrogenase activity. Fungal and bacterial community fingerprints, generated by T-RFLP, also showed the same tendency. PCA revealed a clear separation between maize and bean monoculture, for both fungal and bacterial populations. The TRFs generated a transition in which maize monoculture is contrastingly different from bean monoculture, soybean rotation and soybean monoculture being in the intermediate section of the transition. FAME profiles also revealed a similar tendency, maize monoculture being clearly different from the rest of the field treatments. Maize monocropping exhibited lower stress indicator values than bean monoculture. It is widely accepted that accumulation of cyclopropyl FAMES is indicative of bacterial stress (Leckie, 2005). A high cyclopropyl to-precursor ratio has been associated with nutrient depletion, O<sub>2</sub> status, acidic pH, smotic stress and intensive tillage (Macdonald *et al.*, 2004). This suggests that soil under bean monoculture is a *hostile environment* for soil microbial communities, compared with maize monoculture. Further studies will be needed to better characterise the source of bean-induced stress and its role in regulating the development of microbial populations. Moreover, the expected increase in total microbial biomass, as total FAME content, in maize monoculture was not found; however, such increase was only detected in soybean/maize, which exhibited higher microbial biomass than soybean or bean monoculture.

It is well known that based on differences in rhizodeposition, rhizosphere microbial communities can vary in structure and species composition, depending on plant species, plant age and root zone (Kowalchuk *et al.*, 2002). For this reason, rotation has been widely considered as one of the most promising crop practices in improving soil microbial diversity and efficiently cycling nutrients, therefore favouring the development of healthy crops, as evidenced in this work in soybean/maize vs. soybean monoculture through the parameters evaluated. Vanotti & Bundy (1995) postulated that the rotation scheme including soybean stimulates soil microbial activity, increasing mineralization and N uptake by the subsequent maize crop. However, in the present work, not only soybean/maize but also maize monocropping increased microbial biomass and activities in soil. We expected a decrease in microbial abundance in continuous maize in comparison with soybean/maize rotation. However, our results indicate that different crops grown in monoculture can vary in their effects on soil properties. Our results are consistent with the findings of Smalla *et al.* (2001), who showed that different plant species select different bacterial communities and that these plant-specific enrichments can be increased by repeated cultivation of the same plant species in the same field. Moreover, Yusuf *et al.* (2009) also found that continuous maize cultivation may favour the establishment and maintenance of fungal community, leading to greater retention of nutrients in soil.

### Disease incidence and crop yield

During the field trial, the soilborne disease SDS (*Fusarium crassispitatum*) was detected. This disease is responsible for reductions in soybean yields in North and South America (Aoki *et al.*, 2005); hence, it is necessary to define management strategies to avoid the spread of the pathogen in soil. In the present work, the disease was not detected in soybean rotated with maize; conversely, in soybean monoculture the incidence was markedly high. According to Rupe *et al.* (1997), soybean rotation with non-host crops, such as maize, sorghum or wheat, reduced population densities of *Fusarium* sp., compared with continuous soybean. However, Kolander *et al.* (2012) stated that corn, wheat, ryegrass, pigweed, and lambsquarters do not develop symptoms of root necrosis by *F. virguliforme*, however these species seem to be asymptomatic hosts because

quantities of pathogen DNA detected in inoculated roots were similar to quantities detected in inoculated soybean roots. Consequently, there is controversy about the positive effects of crop rotation on the incidence of SDS, and Xing & Westphal (2009) agree that there is a lack of information on the effects of rotational maize on disease development. Although maize is also host of *Fusarium* species, based on our results we can assume that disease incidence on soybean after maize will be reduced compared to that in soybean monoculture. It has been widely reported in the bibliography that the shifting to annual rotations of corn and soybean (compared to longer rotations that involved small grains and perhaps forages) fails to reduce the risk for SDS (e.g. Xing & Westphal, 2009). However, one of the main causes for the reduction of disease pressure is the improvement of root health as a consequence of the inclusion of maize in the crop sequence, a benefit that can obviously be obtained only through crop rotation. This was confirmed by a negative and significant correlation observed between SDS incidence and biological parameters, such as microbial biomass, GRSP, the potential biocontrol agents (*Trichoderma* spp., actinomycetes, and fluorescent pseudomonads), and FDA hydrolysis. It is well known that as the active microbial biomass increases, the capacity of microorganisms to use carbon, nutrients and energy in the soil is increased; these resources will be therefore limited for soil-borne pathogens (Sullivan, 2001; Vargas-Gil *et al.*, 2008). However, the soil chemical and physical environment also contributed to the spread of the pathogen. In the present work, the increase of K, Ca, and Mg in soil produced a decrease in disease incidence. According to Sanogo & Yang (2001), disease severity was reduced by the amendment of soil with K but was increased in the presence of Ca. Finally, higher BD values seemed to contribute to the increase of SDS. Chong *et al.* (2005) also mentioned that soil with higher macroporosity resulted in low water retention capacity and maintained a higher aeration condition, which could provide better rhizosphere for plant growth. Soybean yield was 74% higher in the soybean/maize rotation, compared with monocropping. Crop yield showed a significant negative correlation with disease incidence, which in part may explain the reduction in soybean production. The cause of yield decline with more frequent cropping is not always apparent and in many cases has not been fully elucidated. Although numerous hypotheses have been proposed, solid evidence is scarce however, and causes of yield

decline are difficult to prove in field situations due to the complex nature of cropping systems (Bennett *et al.*, 2012).

In summary, considering the negative effect of monoculture on soil quality, bean proved to be the most harmful, followed by soybean, being maize the less damaging. It was also demonstrated that there are some cultural practices such as the rotation of soybean with maize, which can improve soil quality. This beneficial effect was evidenced by the increase of microbial populations and their activities, the significant rise of some important chemical parameters such as organic matter, total N and extractable P, and the augmentation of aggregate stability. As a result, soybean health was improved in a rotation system, compared with soybean monoculture, and as a consequence, crop yield was also higher under sustainable management.

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