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Multivariate approach to characterizing soil microbial communities in pristine and agricultural sites in Northwest Argentina

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ABSTRACT

Land use effects on microbial communities may have profound impacts on agricultural productivity and ecosystem sustainability as they are critical in soil quality and health. The main aim of this study was to characterize the microbial communities of pristine and agricultural soils in the central Yungas region in Northwest Argentina. As a first step in the development of biological indicators of soil quality in this region, a comprehensive approach involving a structural and functional evaluation of microbial communities was used to detect changes in soil as consequence of land use. The sites selected included two pristine montane forest sites (MF1 and MF2), two plots under sugarcane monoculture for 40 and 100 years (SC40 and SC100), one plot under 20 years of soybean monoculture (SB20), a recently deforested and soybean cropped site (RC), and two reference sites of native forest adjacent to the sugarcane and soybean plots (PF1 and PF2). We used three microbial community profiling methods: denaturing gradient gel electrophoresis (DGGE) analysis of PCR amplified 16S rRNA genes, community-level physiological profiling (CLPP) using a BD oxygen biosensor system (BDOBS-CLPP) and phospholipid fatty acid (PLFA) analysis. Deforestation and agriculture caused expected increases in pH and decreases in organic carbon and microbial biomass. Additionally, shifts in the microbial community structure and physiology were detected with disturbance, including reduced diversity based on PLFA data. The higher respiratory response to several carbon substrates observed in agricultural soils suggested the presence of microbial communities with lower growth yield efficiency that could further reduce carbon storage in these soils.

Using an integrated multivariate analysis of all data measured in this study we propose a minimum data set of variables (organic carbon, pH, sucrose and valeric acid utilizations, *a*17:0 and *a*15:0 PLFA biomarkers and the value of impact on microbial diversity) to be used for future studies of soil quality in Northwest Argentina.

1. Introduction

The activity of soil microbial communities determines the productivity and overall quality of terrestrial ecosystems due to soil microorganisms' role in nutrient cycling, pollutant transformation/detoxification processes and soil aggregate stability, among other functions. It is known that the presence of a diverse microbial community contributes to stress resistance and resilience in soils. Thus, one of the foundations for sustainable agriculture should be to preserve this diversity (Brussaard et al., 2007). However, certain agricultural practices alter physical, chemical and biological soil characteristics which can lead to the degradation of the microbial habitat and reduce soil quality (Joergensen and Emmerling, 2006).

Tropical and subtropical agroecosystems are particularly susceptible to soil degradation and associated nutrient losses because of the higher mineralization of organic matter related to inputs. Considerable changes in land use have occurred in the subtropical region of Northwest Argentina over the last decades, including an increased rate of deforestation (Grau et al., 2005; Izquierdo and Grau, 2009). This region includes the Yungas, the southern limit of the Andean subtropical rainforests of South America, which constitutes a large biodiversity reservoir. However, significant portions of the pedemontane forest of the Yungas have been converted

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to agriculture (Brown and Malizia, 2004). Fertile soils and warm temperatures facilitated the introduction of a number of crops, including sugarcane, citrus, tobacco, common bean and, more recently, soybean. To date, the long-term effects of these changes in land use on soil microbial communities and concomitant impacts on agricultural sustainability are unknown.

The assessment of changes in the quality of these soils due to altered management or conversion of natural areas into agricultural production requires a definition of soil quality. While there is no clear consensus (Bastida et al., 2008), an adequate definition of soil quality for the purpose of analyzing changes in land use as those observed in the Yungas is that introduced by Karlen et al. (1997): "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivities, maintain or enhance water and air qualities, and support human health and habitation". However, the quality of agricultural soils should consider not only plant productivity, but also soil microbial community composition and activity since the functional stability and health of the soil depend on the microbial activities. Indeed, a recent study has found that a shift from an undisturbed forest to long-term cultivation was associated with the establishment of a less functionally stable microbial community (Chaer et al., 2009).

Maintaining soil quality is a complex issue involving the interaction of climatic, soil, plant and human factors. This complexity explains the growing interest in the identification of sensitive indicators for soil quality monitoring programs. Changes in land use that can lead to soil deterioration are usually tracked with traditional physicochemical indicators. However, biological variables usually show variations before the physicochemical characteristics. Because the composition and dynamics of the soil microbial communities are also altered during the soil deterioration process, they have been more recently used to evaluate management induced-soil changes (Acosta-Martínez et al., 2008; Chaer et al., 2009).

The main aim of this study was to characterize the microbial communities of pristine and agricultural soils in the central Yungas region using a multivariate approach. Traditional physicochemical analyses were combined with three microbial community profiling methods; denaturing gradient gel electrophoresis (DGGE), community-level physiological profiling with a BD oxygen biosensor system (BDOBS-CLPP) and phospholipid fatty acid (PLFA) analysis. DGGE is a molecular fingerprinting method that separates PCR amplification products in a chemical gradient across a polyacrylamide gel. The application of DGGE to the separation of 16S rRNA genes is a useful tool to analyze the genetic structure of soil microbial communities (Muyzer et al., 1993; Heuer et al., 1997). The BDOBS-CLPP approach detects both endogenous and substrate-induced respiration of soil microbial communities without the strong selective enrichment and associated bias of previous CLPP methods (Väisänen et al., 2005; Brown et al., 2009; Garland et al., 2010). PLFA analysis provides a profile of the microbial community using microbial groups' biomarkers based on cell membrane phospholipids (Zelles, 1999). We also analyzed which parameters are better suited for detecting changes in soil microbial communities related to deforestation and agricultural land use and their potential as biological indicators of soil quality in subtropical regions.

2. Materials and methods

2.1. Sample collection sites

Table 1 shows a description of the soils analyzed. Samples were collected in March 2007 from pristine (montane and pedemontane forest soils) and agricultural soils under two different crops such as sugarcane (*Saccharum* spp.) and soybean (*Glycine max* [L.] Merrill). Pedemontane forest soils adjacent to sugarcane or soybean plots were sampled as a baseline.

Five composite samples were taken per site, 20 m apart from each other. Each composite sample consisted of 16 soil cores (10 cm depth, 5 cm diameter) collected from the surface horizon after removing the organic litter. Samples from agricultural soils were obtained from the inter-row zone. Each sample was well homogenized and divided in two parts; one was sent to a commercial laboratory (Laboratory of Soil and Water, INTA Salta) to be processed for chemical and physical analyses using standard procedures (Sparks et al., 1996), whereas the other field-moist was sieved through a 2 mm mesh for microbial analyses. Sub-samples were stored at −80 °C for molecular (DGGE) and biochemical (PLFA profiles) analyses or at room temperature for physiological profiling analysis.

2.2. DNA isolation, PCR amplification of 16S rDNA and DGGE analysis

Total microbial community DNA was extracted from 0.25 g soil samples with the Power Soil DNA isolation kit (Mo Bio Laboratories, Inc., CA, USA) according to manufacturer's instructions. The isolated DNA was quantified in a spectrophotometer at 260 nm (GeneQuant DNA/RNA calculator, Pharmacia Biotech). The V6–V8 region of 16S rRNA gene was PCR amplified using the GC-F984 and R1378 primer set (Heuer et al., 1997). The PCR mixture consisted of 30 ng of soil DNA, 0.25 mM of each primer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 5% DMSO, and 2.5 U of Platinum *Taq* DNA polymerase (Invitrogen), and the buffer (1×) provided with the enzyme. Amplification was carried out in an MJ Research PTC-100 thermocycler with the following temperature program: 5 min at 95 °C, 35 cycles consisting of 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C, and finally 30 min at 72 °C. PCR products were checked by 1% (w/v) agarose gel electrophoresis in Tris–Borate–EDTA buffer and ethidium bromide staining. DGGE analysis of 16S rRNA gene products was carried out as described by Correa et al. (2009) with a denaturing gradient of 40–60%. The gels were run 1600 V/h in Tris–Acetate–EDTA buffer at 60 °C, stained for 30 min with SYBR

Table 1
Sampling sites, codes, and land uses of the analyzed soils.

Soil codes	Land uses	Locations (Argentina)	Coordinates	Altitude (m a.s.l.)
Pristine soils				
MF1	Pristine montane forest	Calilegua National Park, Calilegua, Jujuy	23°41.160′S, 64°52.533′W	1388
MF2	Pristine montane forest	Calilegua National Park, Calilegua, Jujuy	23°41.990′S, 64°52.025′W	1178
PF1	Pristine pedemontane forest adjacent to SC40 plot	Libertador General San Martín, Jujuy	23°54.065′S, 64°49.852′W	493
PF2	Pristine pedemontane forest adjacent to RC plot	Las Lajitas, Salta	24°54.032′S, 64°20.225′W	577
Agricultural soils				
SC40	Sugarcane monoculture for 40 years	Libertador General San Martín, Jujuy	23°53.765′S, 64°49.658′W	470
SC100	Sugarcane monoculture for 100 years	Libertador General San Martín, Jujuy	23°50.056′S, 64°46.760′W	370
RC	Recently cleared and soybean cropped	Las Lajitas, Salta	24°54.040′S, 64°20.189′W	578
SB20	Soybean monoculture for 20 years	Las Lajitas, Salta	24°53.105′S, 64°12.375′W	458

Green I nucleic acid stain (molecular probes) and photographed under 254 nm UV light with a EDAS120 (Kodak). As recommended for DGGE fingerprinting analysis, comparative analysis of normalized DGGE profiles was performed with GelCompar II v. 3.0 (Applied Maths, Belgium) using Pearson's product–moment correlation coefficient (r) to calculate pairwise similarity coefficients among pattern densitometric profiles and similarity matrices were clustered using the unweighted pair group method with averages (UPGMA) algorithm (Rademaker et al., 1999).

2.3. Physiological profiling using the BD™ oxygen biosensor system (BDOBS)

Profiles of sole carbon source utilization by soil microbial communities were performed using the BDOBS (BD Biosciences, Bedford, MA, USA). This system utilizes a 96-well plate with a ruthenium dye that fluoresces as dissolved oxygen is depleted in the overlying microbial inoculum, yielding a measurement of O_2 use and, concomitantly, substrate utilization (Väisänen et al., 2005). The carbon sources selected for the experiment were L-lysine, L-arginine, L-asparagine, D-mannose, sucrose, valeric acid, D-galacturonic acid, D-glutaric acid, p-coumaric acid, D-quinic acid and vanillic acid (4-hydroxy-3-methoxybenzoic acid) (Sigma–Aldrich Inc.). Stock solutions (0.3 g L^{-1}) were prepared in deionized water, filter-sterilized (Millipore, <0.22 μm) and stored at 4 °C. Eighty microlitres of each carbon source stock solution was separately added to individual microplate wells, and 80 μL of sterile deionized water was added to control wells to monitor the response to background of soil carbon. Five grams of each soil sample were suspended in 12.5 mL of filter-sterilized deionized water in 50 mL polypropylene tubes. Sterile glass beads were added to the soil slurries and tubes were vigorously shaken by hand for 1 min. Soil suspensions (160 μL) were loaded into the wells, and microplates were immediately transferred to the plate reader (Wallac 1420 Victor2 multi-label counter, Perkin Elmer Life Sciences). Plates were incubated at 30 °C without shaking. Fluorescence readings were obtained from the bottom of the plate every 30 min for up to 48 h using a 485 nm excitation filter and a 590 nm emission filter.

Fluorescence response data obtained from BDOBS were converted to normalized relative fluorescence units (NRFU) by dividing the readings at each time point by the baseline value. The response at 1 h was used as the baseline to ensure that temperature had equilibrated in the plates given the temperature sensitivity of the ruthenium dye. The maximum respiratory response in soils with the background of soil carbon and that induced by the eleven carbon sources, expressed as NRFU, was used to classify the studied soils on the basis of the microbial community physiological profiles. The time to minimum threshold response, or TMR, defined as the time required for the NRFU to increase by 10% (i.e., 1.1) also was calculated for each test (Garland et al., 2003).

2.4. Phospholipid fatty acid (PLFA) analysis

Microbial lipids were extracted from 1.0 g of sieved root-free freeze-dried soil. One sample, which was chosen at random, was analyzed for each soil. We used a modified Bligh and Dyer (1959) extraction procedure (White et al., 1979; Guckert et al., 1985) where a single phase solvent system (chloroform) was modified to include a phosphate buffer. This technique initially extracts lipids from only viable microorganisms captured at the time of sampling. Lipid extracts were then fractionated on silicic acid columns into neutral, glyco- and polar lipids. Only polar lipids were collected and then methylated with 0.2 M methanolic KOH to form fatty acid methyl esters (FAMEs). Purified FAMEs were then brought to volume with hexane containing an internal FAME standard ($C_{19:0}$). Chromatographic peaks were quantified by using this internal FAME standard on a Varian 3800 GC-FID. FAME identification of each peak was based on retention time data with known standards. Additional confirmation of FAME peak identities by double bond and mid-chain branching positions was obtained by GC–mass spectrometry (GC–MS) at Microbial Insights, Inc.

The polyenoic unsaturated fatty acids 18:2ω6 and 18:1ω9c were considered fungal biomarkers (Bardgett et al., 1996; Bååth, 2003). Branched, saturated Gram positive fatty acids of *i*15:0, *a*15:0, *i*16:0, *i*17:0 and *a*17:0, the monoenoic and cyclopropane unsaturated Gram negative fatty acids of cy17:0, 18:1ω7c and cy19:0, the general bacterial markers 15:0, 16:1ω7c and 16:1ω7t and also, the actinobacterial 10Me16:0 were considered part of the total bacterial make-up (Ekelund et al., 2003; Leckie et al., 2004).

2.5. Statistical analysis

Differences between physicochemical parameters from pristine (MF1, MF2, PF1 and PF2) and agricultural soils (SC40, SC100, RC and SB20) were tested with multiple analysis of variance (MANOVA). Since the global difference between land uses was statistically significant, each variable was tested with an individual analysis of variance (ANOVA). An initial pattern-searching analysis using principal component analysis (PCA) was performed on the PLFA and CLPP data. For the PLFA analysis, the % nmol g^{-1} of soil dry weight for each fatty acid was considered to compensate for differences in absolute amounts among soil samples. These exploratory analyses were followed by MANOVA and ANOVA to contrast pristine and agricultural soils. The time to minimum response (TMR) data were analyzed by one-way ANOVA ($P = 0.05$).

To analyze the DGGE fingerprinting data we used the procedure described by Aboim et al. (2008), where a value called "impact on microbial diversity" (IMD) is determined. IMD is a measure of how much the microbial community structure deviated from the original reference situations (pristine pedemontane forest soils) and is calculated as the normalized Pearson distance values between a given agricultural soil (SC40, SC100, RC or SB20) and each reference pristine soil (PF1 or PF2).

Soil parameters and microbial community function and structure data from agricultural and reference pristine soils were included in a single PCA to assess the interactions between them and to choose variables that better discriminate between agricultural and reference pristine soils. The detailed selection process was as follows: original variables that were highly correlated with PC1 factor alone and variables highly correlated with PC1 and PC2 factors were selected in the first step. Secondly, if two or more variables passed the first criteria, but they showed correlation among them, only one variable of the group was considered. Then, if a given variable did not correlate with any other of its same type (physicochemical or microbiological), it was incorporated in the final set. The centroid value of each variable was used in the biplot for a better visualization of the results.

All statistical analyses were performed using R statistical software (R Development Core Team, 2004).

3. Results

3.1. Physical and chemical soil parameters

Selected physical and chemical parameters of the loam soils analyzed are shown in Table 2. Pristine soils (MF1, MF2, PF1 and PF2) differed ($P = 0.01$) from the agricultural soils (SC40, SC100, RC and SB20) due to higher WHC and lower pH, OC and total nitrogen content.

Table 2
The main physicochemical properties of soils under different land uses in the central Yungas region of Northwest Argentina.

Soil parameters[a]	Pristine soils				Mean values	Agricultural soils				Mean values
	Montane forest		Pedemontane forest[b]			Sugarcane		Soybean		
	MF1	MF2	PF1	PF2		SC40	SC100	RC	SB20	
Sand (%)	56.2	40.2	48.5	31.3	44.25 a	42.6	37.3	48.5	46.5	44.94 a
Silt (%)	30.0	36.5	38.0	53.4	39.63 a	36.6	35.0	31.5	42.0	36.38 a
Clay (%)	13.8	23.2	13.7	13.8	16.19 a	21.0	27.5	13.4	13.2	18.63 a
Textural class	Sandy loam	Loam	Loam	Silty loam		Loam	Loam	Sandy Loam	Loam	
WHC (%)	46.2	54.5	35.0	41.0	41.56 a	32.0	31.7	37.4	30.4	28.06 b
pH	5.7	5.9	7.1	6.0	6.17 b	7.8	7.4	6.4	6.9	7.06 a
EC (mmhos cm^{-1})	0.5	0.9	0.8	0.5	0.67 a	0.6	0.4	1.2	0.5	0.68 a
OC (%)	2.6	3.5	1.8	2.8	2.65 a	1.0	1.0	2.6	1.2	1.51 b
Total N (%)	0.25	0.3	0.18	0.15	0.24 a	0.10	0.10	0.22	0.13	0.13 b
C:N	11.0	11.0	10.0	12.4	11.0 a	11.8	10.8	11.5	8.2	10.88 a
P (ppm)	4.7	7.2	35.8	5.6	24.31 a	7.9	63.4	11.6	22.5	12.44 a
K (mequiv. 100 g^{-1})	0.5	1.0	0.8	1.0	0.86 a	0.3	0.5	1.0	1.4	0.84 a

Data are means of five replicates. Variables with different letters shown statistically significant differences ($P = 0.01$) between agricultural and pristine soils. See Table 1 for soil codes.

[a] WHC, water holding capacity; EC, electrical conductivity; OC, organic carbon.

[b] Reference soils.

3.2. DGGE analysis

The relative genetic structure of the soil bacterial communities was analyzed by PCR amplification of 16S rRNA genes and its subsequent sequence specific separation by DGGE. Five analytical replicates were initially analyzed for each sampling site, but given the similarity in profiles among replicates, only triplicates are shown to ease visualization of the data. 16S rDNA PCR–DGGE analysis produced distinguishable and complex banding patterns for each sampling site reflecting the composition of the bacterial community in these soil samples (Fig. 1). Cluster analysis of DGGE profiles indicated that the structure of dominant bacterial communities was different among the soils analyzed and these differences depended on land use and geographical origin of the soil samples. Based on a similarity level of 70%, 7 clusters were defined. MF1 and MF2 soils were clearly separated from all other soils and were less than 32% similar to each other. PF2 soil clustered separately (with the exception of one replicate) from RC and SB20 soils, indicating that the bacterial communities of the recently deforested soil (RC) were more similar to those exposed to 20 years of soybean monoculture (SB20) than those of its adjacent pristine pedemontane forest soil (PF2). SC100, SC40 and PF1 soils formed three related but separate clusters. Soil bacterial communities from sugarcane fields with land use histories of 40 or 100 years monoculture (SC40 and SC100) were more similar to each other (shared at least 65% similarity) than those from the adjacent pristine pedemontane forest soil (PF1).

3.3. Community-level physiological profiling

Functional profiling was based on maximum respiratory response (i.e., peak in fluorescence corresponding to the minimum dissolved oxygen within the ruthenium dye gel layer in the BDOBS for each of the 11 added substrates as well as with the background of soil carbon). PCA of the multivariate profile of peak responses revealed a clear separation between pristine montane forest (MF1 and MF2) and agricultural soils (SC40, SC100, RC and SB20) along the PC1 axis, with intermediate values for pristine pedemontane forest soils adjacent to agricultural soils (PF1 and PF2) (Fig. 2). The SC40 and SC100 soils formed a cluster well separated from the other soils along PC2. The two montane forest soils (MF1 and MF2) did not have distinct physiological profiles.

The maximum respiratory activity (NRFU) of microbial community differed ($P < 0.0001$) between the agricultural and pristine soils. Several carbon sources (arginine, lysine, coumaric acid, quinic acid and valeric acid) showed significantly higher peak values in agricultural soils, while only sucrose was more utilized in pristine soils (Table 3). The responses observed with the other carbon sources and the background of soil carbon were not significant. We also observed high differences ($P < 0.0001$) in the time to the minimum response (TMR) between agricultural and pristine soils (Fig. 3). With the background of soil carbon, agricultural soils showed greater TMR (3.3 h) than pristine soils (1.9 h). Carbon source addition affected TMR depending on land use and the carbon source tested. In agricultural soils, a reduction in TMR was observed

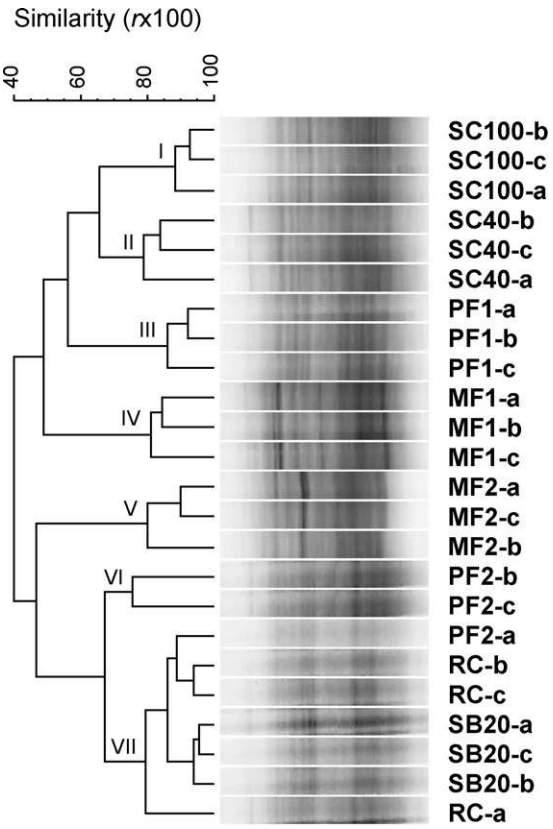

Fig. 1. Cluster analysis (Pearson/UPGMA) of 16S rDNA-DGGE fingerprints of soil bacterial communities. Lower case letters after soil codes indicate sample replicate. See Table 1 for soil codes.

Table 3
Maximum respiratory activity of the soil microbial communities expressed as normalized relative fluorescence units (NRFU).

Land uses	Carbon sources[a]											
	bc	asp	arg	lys	man	suc	van	cou	qui	gal	glu	val
Pristine soils												
MF1	2.39	4.58	3.16	5.74	4.51	4.44	5.99	7.17	3.04	4.07	7.66	5.03
MF2	2.62	4.48	4.18	6.12	4.85	3.83	5.67	6.91	3.58	4.79	6.93	6.99
PF1	1.40	5.76	7.69	7.43	5.40	4.26	7.74	8.64	5.14	2.55	8.87	8.25
PF2	3.54	6.45	7.90	7.95	6.19	4.71	7.62	8.47	4.34	6.66	7.80	7.50
Mean values	2.49 a	5.32 a	5.73 b	6.81 b	5.24 a	4.31 a	6.76 a	7.80 b	4.03 b	4.52 a	7.82 a	6.94 b
Agricultural soils												
SC40	1.63	5.92	9.61	9.35	4.20	2.23	3.51	6.70	7.53	3.72	6.76	8.44
SC100	1.61	5.46	9.76	9.21	3.61	1.84	5.96	9.55	6.81	3.97	5.59	9.44
RC	2.67	6.66	9.03	8.33	6.71	4.84	8.90	9.53	7.78	5.76	8.80	7.36
SB20	1.91	6.22	9.37	9.49	6.24	3.56	8.25	9.56	6.66	4.66	8.09	8.56
Mean values	1.96 a	6.06 a	9.44 a	9.09 a	5.19 a	3.12 b	6.65 a	8.83 a	7.20 a	4.53 a	7.31 a	8.45 a

Data are means of five replicates. Different letters indicate significant differences ($P = 0.0001$) between agricultural and pristine soils. For soil sample codes see Table 1.
[a] bc, background of soil carbon; asp, asparagine; arg, arginine; lys, lysine; man, mannose; suc, sucrose; van, vanillic acid; cou, coumaric acid; qui, quinic acid; gal, galacturonic; glu, glutaric acid; val, valeric acid.

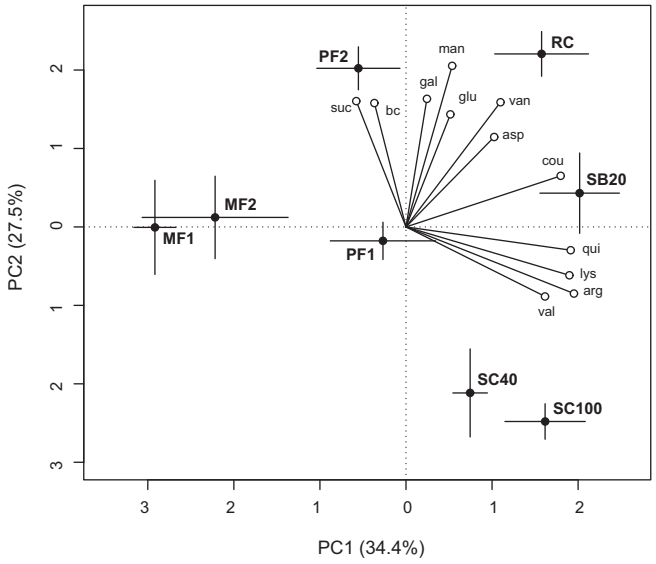

Fig. 2. Principal component analysis (PCA) of the maximum respiratory response of soil microbial communities expressed as normalized relative fluorescent units (NRFU). Data represent the average obtained from five replicates of each soil. Bars are the standard deviations along both axes. See Table 1 for soil codes.

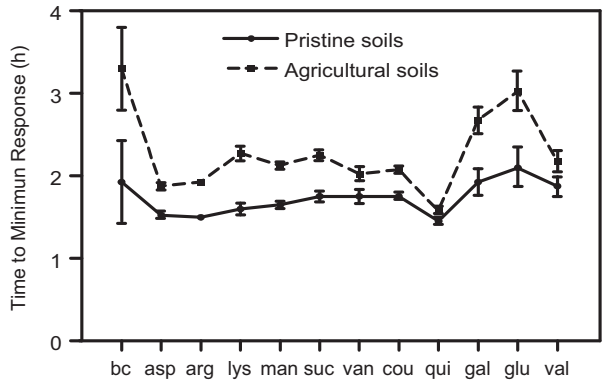

Fig. 3. The time to minimum response (TMR) of soils in the BDOBS-CLPP assay with the background of soil carbon or the addition of the different carbon sources. Data represent the mean of four replicates for each land use and the error bars are the square root of the mean square error for each carbon source's ANOVA. See Table 1 for soil codes. bc, background of soil carbon; asp, asparagine; arg, arginine; lys, lysine; man, mannose; suc, sucrose; van, vanillic acid; cou, coumaric acid; qui, quinic acid; gal, galacturonic; glu, glutaric acid; val, valeric acid.

with several carbon sources (asparagine, arginine, lysine, mannose, sucrose and vanillic acid, coumaric acid, quinic acid and valeric acid) (Fig. 3). By contrast, no effect on TMR was observed in pristine soils with any of the carbon sources tested.

3.4. Phospholipid fatty acid (PLFA) analysis

The PCA of PLFA data (nmol%) for 12 FAMEs explained 47.8% and 24.4% of the variance in PC1 and PC2, respectively (Fig. 4). Soil microbial communities from pristine soils (MF1, MF2, PF1 and PF2) showed the highest values for PC1, and were clearly separated from the agricultural soils (SC40, SC100, RC and SB20) along this axis. This separation was based on higher relative abundance of *a*15:0, 16:1ω7c, 16:1ω7t, *a*17:0 and cy17:0 biomarkers in pristine soils (to the right) and the *i*15:0 biomarker in agricultural soils (to the left).

ANOVA comparing pristine (MF1, MF2, PF1 and PF2) and agricultural (SC40, SC100, RC and SB20) soils showed that several individual fatty acids varied significantly in their relative abundance between these two land uses (Table 4). Soil microbial communities of agricultural soils showed significantly lower abundance of bacterial fatty acids (*i*15:0, *i*16:0, *i*17:0, 18:1ω7c and cy19:0), actinobacterial fatty acid (10Me16:0) and the fungal fatty acid (18:1ω9c). Microbial biomass, based on total PLFA concentra-

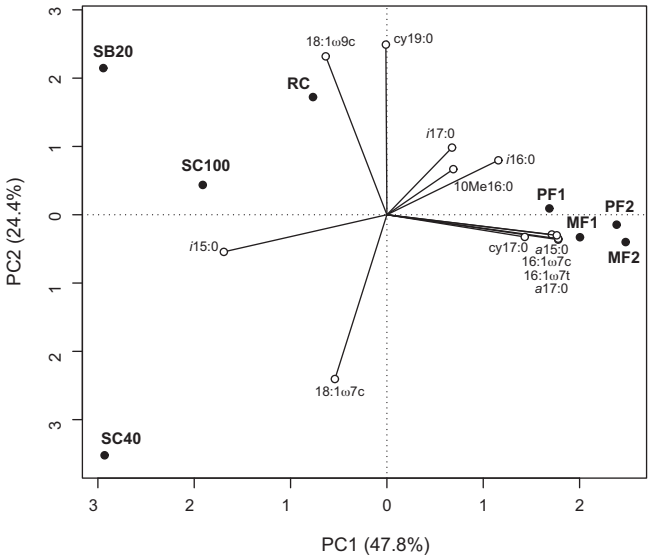

Fig. 4. Principal component analysis (PCA) of phospholipid fatty acid profiles (% nmol g^{-1} of dry soil) of the analyzed soils. See Table 1 for soil codes.

Table 4
Structure and biomass of microbial communities in pristine and agricultural soils by PLFA analysis.

Soils[a]	PLFA (nmol g^{-1} dry soil)												*F*:*B* ratio	Total biomass (nmol g^{-1} dry soil)
	Bacterial PLFA											Fungal PLFA		
	*i*15:0	*a*15:0	*i*16:0	16:1ω7c	16:1ω7t	10Me16:0	*i*17:0	*a*17:0	cy17:0	cy19:0	18:1ω7c	18:1ω9c		
Pristine	7.9 a	4.1	2.9 a	3.9	3.1	5.4 a	2.3 a	1.9	1.1	6.4 a	7.8 a	4.3 a	0.11 a	51.24 a
Agricultural	2.2 b	n.d.[b]	0.4 b	n.d.	n.d.	0.8 b	0.5 b	n.d.	n.d.	1.0 b	1.1 b	1.0 b	0.20 a	7.12 b

Data are means of four independent soil samples. Different letters indicate significant differences ($P < 0.05$) between pristine and agricultural soils.
[a] Pristine soils: MF1, MF1, PF1 and PF2; agricultural soils: SC40, SC100, RC and SB20.
[b] n.d., not detected.

tion was much higher in pristine soils (51.24 nmol g^{-1} of soil dry weight) than in agricultural soils (7.12 nmol g^{-1} of soil dry weight). Despite this important difference in microbial biomass, the fungal to bacterial PLFA ratio (*F*:*B*) did not vary between the pristine and agricultural soils.

3.5. Integrated multivariate analysis

The PCA of the integrated data set (including physicochemical parameters and microbial community function and structure data) clearly separated agricultural soils from their pristine reference soils, with the first 2 PCs capturing a significant portion (66%) of the total variance (Fig. 5). Along the PC1 axis, SC40–SC100 was separated from PF1, and SB20 differentiated from PF2, with RC occupying an intermediate position. SB20–RC was distinct from PF2 along the PC2 axis. This PCA allowed us to select a set of variables most influential in the discrimination between the agricultural soils and the pristine soils used as reference in each case (PF1 for SC40 and SC100 and PF2 for RC and SB20). This selection was based on the correlations among the measured variables showed graphically in Fig. 5 and the correlations of the variables to each axis (Table 5). The final set of variables to be considered for future studies of soil quality monitoring in this region was composed of pH, soil OC content, sucrose and valeric acid utilization, *a*15:0 and *a*17:0 fatty acids' abundances and IMD value. OC content, sucrose utilization, *a*17:0 and *a*15:0 PLFA biomarkers tend to have higher values in reference pristine soils (PF1 and PF2), while soil pH, the utilization of valeric acid and IMD value were higher in agricultural soils.

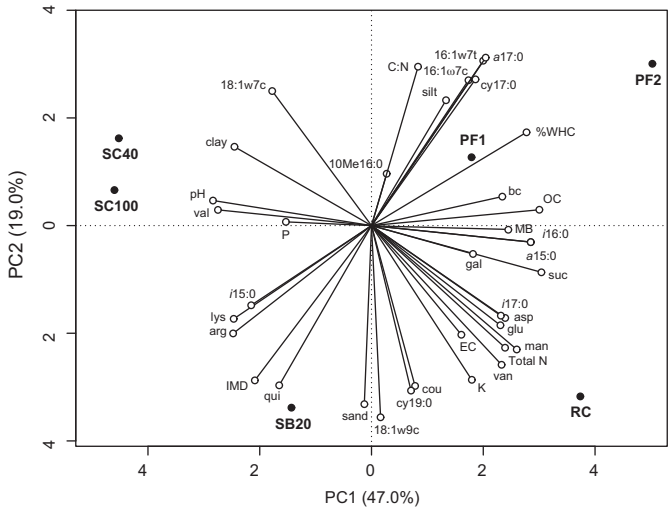

Fig. 5. Integrated multivariate analysis (PCA) of the 37 physicochemical and microbiological variables of the agricultural and the pristine reference soils. IMD, impact on microbial diversity; MB, microbial biomass; bc, background of soil carbon; asp, asparagine; arg, arginine; lys, lysine; man, mannose; suc, sucrose; van, vanillic acid; cou, coumaric acid; qui, quinic acid; gal, galacturonic; glu, glutaric acid; val, valeric acid; WHC, water holding capacity; EC, electrical conductivity; OC, organic carbon. See Table 1 for soil codes.

Table 5
Correlations of original variables to ordination axes derived from PCA of analyzed soils.

Variable[a]	*r*[b]	
	PC1	PC2
IMD	−0.67	−0.59
bc	0.75	0.11
asp	0.74	−0.38
arg	−0.79	−0.41
lys	−0.79	−0.35
man	0.83	−0.47
suc	0.97	−0.18
van	0.74	−0.53
cou	0.25	−0.61
qui	−0.53	−0.61
gal	0.58	−0.11
glu	0.76	−0.35
val	−0.88	0.06
*i*15:0	−0.69	−0.30
*a*15:0	0.91	0.06
i16:0	0.91	−0.06
16:1ω7c	0.55	0.55
16:1ω7t	0.64	0.63
10Me16:0	0.09	0.20
*i*17:0	0.74	−0.34
*a*17:0	0.65	0.64
cy17:0	0.59	0.55
18:1ω9c	0.05	−0.73
18:1ω7c	−0.57	0.51
cy19:0	0.23	−0.63
MB	0.73	−0.01
Sand	−0.04	−0.68
Silt	0.43	0.48
Clay	−0.78	0.30
WHC	0.89	0.35
pH	−0.91	0.10
EC	0.51	−0.41
OC	0.96	0.06
Total N	0.76	−0.46
C:N	0.27	0.6
P	−0.49	0.01
K	0.57	−0.59

[a] IMD, impact on microbial diversity; bc, background of soil carbon; asp, asparagine; arg, arginine; lys, lysine; man, mannose; suc, sucrose; van, vanillic acid; cou, coumaric acid; qui, quinic acid; gal, galacturonic; glu, glutaric acid; val, valeric acid; MB, microbial biomass; WHC, water holding capacity; EC, electrical conductivity; OC, organic carbon.
[b] Positive correlation indicates greater value in soils with higher coordinate scores for the axis, negative correlation indicates greater value in soils with lower coordinate scores for the axis (see Fig. 5).

4. Discussion

Our results indicated that deforestation for crop production modified the structure and function of the soil microbial communities in the studied region, as well as some soil physicochemical parameters. Microbial community profiling methods detected differences in the genetic structure and function of soil microbial communities between the agricultural soils and their adjacent pristine soils, and also between soils under sugarcane or soybean

monocultures. Furthermore, these methods allowed us to detect both historical and recent changes in land uses.

DGGE fingerprinting revealed complex banding patterns reflecting the complex bacterial community structure expected for sub-tropical soils. Although for each site a distinct cluster was observed, and also structural shifts were evident between agricultural and reference soils, we could not discern the presence or absence of dominant bands in particular positions related to changes in land use. Additionally, these soils produced very complex fingerprints and thus, it was not possible to accurately estimate the total number of bands. It is known that DGGE fingerprints describe only the predominant members in a community (representing at least ~1% of the total target pool) so it does not reflect the full complexity of the system (Muyzer et al., 1993; Gelsomino et al., 1999). In fact, in complex communities diversity indices cannot be estimated accurately from community fingerprint data (Loisel et al., 2006; Bent and Forney, 2008). Otherwise, the structural changes detected in soil bacterial community may not necessarily lead to a reduction in diversity, as it was recently observed for tropical forest soil converted to pasture and crops (da C. Jesus et al., 2009). Therefore, rather than calculating diversity indices we integrated the bacterial diversity information derived from DGGE fingerprinting using IMD values (Aboim et al., 2008). The IMD reflected the shifts in the genetic structure of soil bacterial communities as a result of deforestation for agricultural practices, which is consistent with other studies in tropical or sub-tropical areas (Bonerman and Triplett, 1997; Bossio et al., 2005; Nogueira et al., 2006; Aboim et al., 2008; da C. Jesus et al., 2009). This impact on soil microbial communities may affect the ecological and functional stabilities of these soils, and particularly the functions in specialized niches, as reported for tropical agricultural soils in Brazil (Chaer et al., 2009).

The PLFA approach discriminated pristine from agricultural soils but it was not sensitive enough to differentiate within pristine soils or between soils under different crops (sugarcane or soybean) as the DGGE analysis did. Soil cultivation induced an important decrease in several microbial fatty acids and some bacterial biomarkers were below the limit of GC–MS detection (~50 pmol), which means that the population of soil bacteria having those biomarkers was lower than $\sim 10^5$ cells (Hedrick et al., 2007). Such changes represent low evenness within the different microbial groups and ultimately, lower microbial diversity in agricultural than in pristine soils. Moreover, the PLFA profiles of agricultural soils failed to show higher relative abundance of biomarkers indicative of Gram positive bacteria, as it was reported in other agricultural tropical soils (Bossio et al., 2005). This discrepancy may be due, at least in part, to differences in the number of PLFA biomarkers analyzed in both studies.

The reduction in total viable microbial biomass (on average 86%) observed in agricultural soils is consistent with the results obtained by other researchers (Pankhurst et al., 2003; Acosta-Martínez et al., 2004; Liu et al., 2006), reflecting the lower supply of labile C and other nutrients provided by litter and crop residues.

Pristine and agricultural soils also differed in their physiological profiles using BDOBS microplates. BDOBS could not discriminate between the two pristine montane forest soils showing lower specificity than genetic profiling. Nevertheless, this result likely reflects the functional similarity of these two habitats. The lower TMR values obtained in pristine soils compared to agricultural soils with the background of soil carbon was consistent with the higher microbial biomass estimated by PLFA. The decrease in TMR observed in agricultural soils, when carbon sources were added, was not observed in the pristine soils suggesting the predominance of different ecotypes in both microbial communities. Agricultural soils may have a predominance of *r*-strategists, which are characterized by their fast respiratory response to the addition of labile carbon sources, while *K* strategists, which respond poorly to these substrates, may predominate in pristine soils (De Leij et al., 1993). Despite the reduction in TMR values with carbon source addition, the difference between agricultural and pristine soils, though smaller, remained for most of the carbon sources added.

The maximum respiratory activity showed a somewhat contradictory effect with higher values in the agricultural soils that showed lower microbial biomass. Assuming that substrates were completely utilized in all samples (a reasonable assumption given the simple and labile nature of the substrates tested), these results may reflect a shift in community physiology towards greater respiration of carbon sources relative to assimilation (i.e., lower growth yield efficiency, or GYE). Studies with ^{13}C-labeled substrates have shown that GYE is a dynamic property of soils, varying among different substrates for a given soil (Brant et al., 2006), and in response to soil management (Thiet et al., 2006). Similarly, Nogueira et al. (2006) found differences in the respiration of soil microbial community as response to soil management, which were related to the ratio between *r* and *K* strategist microbial ecotypes. Non-equilibrated environments showed a higher respiration to microbial biomass ratio as a result of the dominance of *r*-strategists (fewer species with high growth rate) over *K* strategists (more species with low growth rate). Because *r*-strategists evolve more CO_2 per unit of degradable carbon than *K* strategists it is equivalent to consider that the former have a lower GYE. Due to the central role that soil microorganisms have on C cycling, we could speculate that the activity of microbial communities with low GYE in agricultural soils might lead to a decrease in the C storage in the organic matter. However, as suggested by Garland et al. (2010) further studies combining the BDOBS approach with direct estimates of GYE using ^{13}C-labeled substrates are needed to establish if the new CLPP approach provides significant insight into GYE variations.

Deforestation for cultivation had a profound early impact on microbial communities in the studied soils. Indeed, soil microbial community in the recently cleared and cropped soybean soil was substantially different from that of its adjacent pristine reference soil, despite these two soils showed minor differences in their physicochemical parameters. This finding highlights the higher sensitivity of microbiological attributes compared to physicochemical parameters for detecting changes in land use. Similarly, in tropical soils, parameters associated with microbial activity were more responsive to soil management than the soil C and N contents, demonstrating their usefulness as indicators of soil quality in the tropics (Franchini et al., 2007).

The integrated multivariate analysis allowed us to define a minimum data set of seven soil quality indicators for discriminating agricultural soils from pristine reference soils. Five of these variables were related to attributes of soil microbial communities (sucrose and valeric acid utilizations, *a*15:0 and *a*17:0 fatty acids abundances and IMD value) and two were measures of soil chemical parameters (pH and OC). Taken together they may provide indices of soil quality. However, additional surveys and comparisons at other locations and agricultural management practices will be needed to validate this proposed set of soil quality indicators.

5. Conclusions

The multivariate approach we used to analyze soil microbial communities separated soils primarily related to land use. Our results indicated that microbial properties are sensitive indicators of changes in soil quality or functioning due to management, and also highlighted the need to consider a wide variety of both biochemical and microbiological analyses when comparing the impacts of agricultural management practices on soil quality.

This study reports, for the first time, a comparative analysis of the changes in the soil microbial community associated with deforestation for agriculture in an ecologically relevant region

of Argentina. Due to the intrinsic environmental fragility of this region, the change in properties between the cultivated soils and the non-cultivated pristine soils can be considered a measure of decreased soil quality. This work can be used as a starting point for further investigations leading to a better understanding of the impact of land use changes on soil microbial communities in Northwest Argentina.

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