Soil Use and Management

Soil Use and Management doi: 10.1111/sum.12349

Effects of cropping systems under no-till agriculture on arbuscular mycorrhizal fungi in Argentinean Pampas

M. Noelia Cofré[1], A. E. Ferrari[2], A. Becerra[1], L. Domínguez[1], L. G. Wall[2] & C. Urcelay[1]

[1]*Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET, Universidad Nacional de Córdoba, CC 495, 5000, Córdoba, Argentina, and* [2]*Laboratorio de Bioquímica, Microbiología e Interacciones Biológicas en Suelo (LBMIBS), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, R. Sáenz Peña 352, B1876BXD Bernal, Argentina*

Abstract

Here, we compare arbuscular mycorrhizal fungi (AMF) communities and fatty acids in soils under different no-till (NT) agricultural managements over two seasons in two consecutive years. Two NT practices with different agricultural managements were compared: crop rotation (CR) and soya bean monoculture (MC). Soils of natural grasslands (NGs) were used as a reference. Treatments were tested along a regional gradient (four geographical locations) across a 400-km transect of the Argentinean Pampas. We identified 46 morphospecies. Several morphospecies occurred abundantly at all soils; others appeared to be restricted to specific situations. At the regional scale, CR maintained the same richness levels of AMF spores, whereas MC showed less richness, when compared with the NG. Although AMF spore density was clearly affected by cropping practices in the four locations, we could observe some changes in AMF species richness, and similar diversity under agricultural and natural soils. Fatty acid concentrations (whole-cell, phospholipid and neutral lipid fatty acids) revealed differences between soil managements and showed similar patterns of variation in all locations. Spore density positively correlated with all soil lipids fractions. The results suggest that AMF spore communities and fatty acids in soils are suitable indicators of soil management involving different levels of crop rotation. Spore richness measured at a regional scale proved to be sensitive to different NT agricultural managements. Moreover, certain morphospecies could be good bioindicators for NT practices based on cropping systems on the Argentinean Pampas.

Keywords: Glomeromycotina, spore community, fatty acids, cropping system, natural grassland

Introduction

Worldwide, no-tillage (NT) systems have expanded at an average rate of 6 million ha per year, from 45 to 111 million ha since 2005, showing the increased interest of farmers in this technology. In Argentina, NT practices cover almost 20 million ha, but 60% of the total cultivated area is currently dedicated to soya bean [*Glycine max* (L.) Merr.] monoculture, whereas other crops such as maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) cover 5 million hectares (Albertengo *et al*., 2013).

Crop rotation, compared with monoculture, increases soil biodiversity, nutrient cycling conditions, microclimate control and regulation, local hydrological processes; it also decreases undesirable organisms (Finckh & Wolfe, 2006). Soil microbial diversity is a crucial factor that regulates ecosystem functioning (Copley, 2000). Among soil microbes, arbuscular mycorrhizal fungi (AMF) constitute a key functional group that greatly contributes to crop productivity and ecosystem sustainability in new plant production strategies (van der Heijden *et al*., 2008), enhancing plant performance and soil quality (Jeffries *et al*., 2003). In consequence, information concerning the activity and composition of AMF communities could potentially replace other biological indicators such as bacteria, macroarthropods (millipedes, centipedes, earthworms and others) and microarthropods (mites and collembolans) (Mummey *et al*., 2002; Hartmann & Widmer, 2006) currently used to assess overall soil quality or the effect of management strategies on soils (Jansa *et al*., 2014).

Several studies have shown that agricultural practices affect AMF variables such as fungal composition in plant roots (McGonigle & Miller, 2000), spore communities (Oehl *et al*., 2010), extraradical mycelium (Boddington & Dodd, 2000) and fungal phospholipids in soil (Grigera *et al*., 2007).

Correspondence: M. Noelia Cofré. E-mail: noelicof@gmail.com
Received October 2014; accepted after revision March 2017

© 2017 British Society of Soil Science

Cropping systems that include a wider range of host plants might promote greater AMF diversity compared with monoculture, due to the presence of hosts with differential affinities for distinct AMF species (Öpik *et al.*, 2009). However, these effects are context dependent and vary across ecosystems, soil types, precipitation regimens, etc. (González-Cortés *et al.*, 2012). Accordingly, regional gradients (including differences in soil types and precipitation) constitute an ideal scenario to accurately test general ideas on the effects of agricultural practices on soil microorganisms.

Whereas AMF spores represent a measure of richness and density of AMF propagules in soil, the mono-unsaturated fatty acid *16:1ω5c*, considered a biomarker of AMF, is potentially useful for estimating AMF biomass in soils (Ferrari *et al.*, 2015). AMF spores are sensitive to certain disturbances or soil use and management systems (e.g. fire, habitat fragmentation, forest structural changes or agricultural practices) (Colombo *et al.*, 2014; Säle *et al.*, 2015; Soteras *et al.*, 2015; Longo *et al.*, 2016). Therefore, they could be useful as indicators for agricultural soil use and management.

Despite the widespread adoption of NT agriculture in many parts of the world, different practices within NT systems have been poorly studied (Wang *et al.*, 2011). In this study, we assessed AMF richness, abundance and biomass in soils from two contrasting cropping practices under NT systems: crop rotation (CR) versus soya bean monoculture (MC). We also assessed soil from adjacent natural grasslands (NGs) that had not been disturbed for at least 30 years (no-tillage or management input) as a reference containing the potential soil communities in the studied regions (Oehl *et al.*, 2010; Jansa *et al.*, 2014). These treatments were tested along a regional gradient (four geographical locations) on the Argentinean Pampas. Two complementary methodological approaches were used: AMF spore and fatty acid analyses. The analyses of spores included two seasons for 2 years, while fatty acids were analysed only in the first season.

Based on the above background information, we expected geographical variation in AMF in response to agricultural practices, but at a regional scale, we predicted that land use under no-tillage systems affects AMF communities in comparison with NG, and within no-tillage, CR would promote greater richness and biomass than MC.

Materials and methods

Soil managements and experimental design

The geographical locations and soil managements were selected between scientists and farmers as part of a multidisciplinary project (Wall, 2011). The experimental areas were established at four geographical locations along a west–east transect of 400 km with a gradient of soil texture (sandy soils in the west to clayey soils in the east) in the central part of the Argentinean Pampas (Figure 1): Bengolea, Monte Buey (Córdoba Province), Pergamino (Buenos Aires Province) and Viale (Entre Ríos Province). The minimum distance between locations was 150 km. Location, climate, texture and soil classifications at each geographical location are summarized in Table 1 (see Figuerola *et al.*, 2012 and Duval *et al.*, 2013 for more details). The standing crops present during the years of sampling and during three previous years of the soil studied at each location are summarized in Table S1 (Supporting information).

Two different cropping systems were examined as treatments in productive fields managed according to the good agricultural practices of Certified Agriculture established by the Argentine No-Till Farmers Association (AAPRESID) and by the Food and Agriculture Organization (FAO). Specifically, good agricultural practices based on crop rotation (CR) management consisted of NT wheat–soya bean–maize rotations, including a winter cover crop (*Vicia villosa* Roth.). The ‘low rotation’ close to soya bean monoculture (MC) management consisted of little, if any, crop rotation and no winter cover crops. Under this framework, the main difference between both agricultural managements was the predominance of soya bean in the crop succession of MC and a more balanced proportion of corn, wheat and soya bean in the rotation of CR management. The latter resulted in larger crop yields. Incorporation of cover crops also distinguished CR soils from MC soils. Both NT managements (CR and MC) in the four locations received different applications of fertilizers and pesticide between 2006 and 2011 (Table 2).

As reference treatment, we used soils from natural grasslands (NGs) close to the cultivated plots, where no cultivation had been practiced for at least 30 years. The dominant plants present in the grasslands were as follows: *Andropogon ternatus* (Spreng.) Nees, *Aristida adscensionis* L., *Bromus catharticus* Vahl., *Paspalum dilatatum* Poir., *P. notatum* Flüggé, *Poa ligularis* Nees ex Steud., *Setaria vaginata* Spreng. and *Stipa speciosa* Trin. & Rupr.

To cover seasonal and interannual variation, soil samples were collected in February (summer) and September (late winter) during 2010 and 2011. In each treatment plot, three samples (as repetitions) of the top 10 cm of soil were collected, each being a composite of 20 randomly selected cores (10-cm-diam. metal corer) collected within an area of 5 m^2. Each sample area was separated by least 50 m from each other. The surface area of CR, MC and NG fields at each location ranged from 2 to 4 ha.

A total of 144 soil samples [four geographical locations × three soil management treatments × three samples per soil management (repetitions) × four times (two seasons for 2 years)] were analysed. Soil samples were transported to the laboratory at 4 °C for later analysis (spores). For fatty acid analysis, soil samples were stored at 18 °C for 1 week,

© 2017 British Society of Soil Science, *Soil Use and Management*

Figure 1 Cropping systems in productive fields (●) in each geographical location: natural grassland (NG), crop rotation (CR) and soya bean monoculture (MC), on the Argentinean Pampas.

freeze-dried, milled under liquid nitrogen and stored in the freezer (20 °C) until further analysis. Each sample was analysed separately.

Soils were classified according to Soil Taxonomy and the Argentine soil map of the Instituto Nacional de Tecnología Agropecuaria (INTA). The main chemical soil properties were determined by standard methods on part of the samples collected for the analysis of the biological variables. The pH was determined on mixtures of sample:water (1:2.5). Total organic carbon (TOC, %) was measured by dry combustion using a LECO CR12 carbon analyser, total nitrogen (TN, %) being obtained by the Kjeldahl method and the available phosphorus (P, mg/kg) being determined by Bray and Kurtz (1945) method.

AMF spore isolation

Arbuscular mycorrhizal fungi spores were extracted, by wet sieving and decanting, from 100 g of dry soil, followed by centrifugation in sucrose (Walker *et al.*, 1982). A fine sieve

© 2017 British Society of Soil Science, *Soil Use and Management*

Table 1 Location, climate, texture and soil classifications at each geographical location of the Argentinean Pampas. Chemical properties of soils according to different cropping systems: natural grassland (NG), crop rotation (CR) and soya bean monoculture (MC)

	Bengolea			Monte Buey			Pergamino			Viale		
	NG	CR	MC	NG	CR	MC	NG	CR	MC	NG	CR	MC
Latitude (°S)	33°01′31″			32°58′14″			33°56′36″			31°52′59″		
Longitude (°W)	63°37′53″			62°27′06″			60°33′57″			59°40′07″		
Elevation (m a.s.l)	231			110			56			91		
Annual rainfall (mm)	880			930			1000			1156		
Mean annual temperature (°C)	17			17			16			18		
Texture	Sandy loam			Silty loam			Silty loam			Silty clay / Silty clay loam		
Soil classification	Entic Haplustoll			Typic Argiudoll			Typic Argiudoll			Vertic Argiudoll		
TOC (%)	1.69 ± 0.10	1.44 ± 0.019	1.19 ± 0.17	3.06 ± 0.13	1.83 ± 0.07	1.55 ± 0.04	3.21 ± 0.77	1.75 ± 0.16	1.73 ± 0.15	3.53 ± 0.43	3.41 ± 0.24	2.48 ± 0.19
TN (%)	0.143 ± 0.004	0.13 ± 0.006	0.11 ± 0.006	0.28 ± 0.04	0.176 ± 0.004	0.13 ± 0.005	0.246 ± 0.02	0.14 ± 0.01	0.14 ± 0.001	0.203 ± 0.01	0.23 ± 0.03	0.153 ± 0.007
P (mg/kg)	43.1 ± 3.35	37.2 ± 3.26	26.17 ± 5.43	395.07 ± 21.07	122.3 ± 37.86	17.27 ± 3.49	16.6 ± 2.62	25.23 ± 10.10	22.03 ± 2.46	24.33 ± 10.38	50.37 ± 4.65	43.17 ± 2.49
pH	6.7 ± 0.00	6.5 ± 0.00	6.6 ± 0.03	6.4 ± 0.07	6.6 ± 0.07	6.6 ± 0.00	6.6 ± 0.06	6.4 ± 0.03	6.6 ± 0.03	7.03 ± 0.12	6.9 ± 0.10	6.7 ± 0.03

TOC, total organic carbon; TN, total nitrogen; P, available phosphorus.

© 2017 British Society of Soil Science, *Soil Use and Management*

Table 2 Description of fertilizer and pesticides applied during the years of sampling and during three previous years, for the two agricultural cropping systems at geographical locations in the Argentinean Pampas

			Fertilizer[b] (kg/ha)						
Site	Year	Treatment[a]	SSP	TSP	MAP	DAP	Urea	UAN	Pesticide[c] applied
Bengolea	2006/7	CR			140		140		Glyphosate, Thiram, Carbendazim
		MC				75			Glyphosate, Thiram, Carbendazim, 2-4-D
	2007/8	CR	75	75					Thiram, Carbendazim, Atrazine
		MC				75			Glyphosate, Thiram, Carbendazim, 2-4-D
	2008/9	CR			130			135	Glyphosate, Thiram, Carbendazim, Met
		MC				75			Glyphosate, Thiram, Carbendazim, 2-4-D
	2009/10	CR			70		30		Glyphosate Thiram Carbendazim
		MC				75			Glyphosate 2,4-D Atrazine Thiram Carbendazim
	2010/11	CR			70		30		Glyphosate Met Thiram Carbendazim
		MC							Glyphosate Met Thiram Carbendazim
Monte Buey	2006/7	CR			200			310	Glyphosate, Thiram, Carbendazim, Met
		MC						230	Glyphosate, Thiram, Carbendazim, 2-4-D, Atrazine
	2007/8	CR			70				Glyphosate, Thiram, Carbendazim
		MC						230	Glyphosate, Thiram, Carbendazim, Met
	2008/9	CR			60				Glyphosate, Thiram, Carbendazim, Atrazine
		MC					140		Glyphosate, Thiram, Carbendazim, Met
	2009/10	CR		80		120			Glyphosate Met Thiram Carbendazim
		MC				75			Glyphosate Met Thiram Carbendazim
	2010/11	CR		80		120			Glyphosate Met Thiram Carbendazim
		MC				75			Glyphosate Met Thiram Carbendazim
Pergamino									Glyphosate, Thiram, Carbendazim, Met, 2,4-D, Atrazine
	2006/7	CR			60		40		Glyphosate, Thiram, Carbendazim, Atrazine
		MC							Glyphosate, Thiram, Carbendazim, Met, 2,4-D
	2007/8	CR				60			Glyphosate, Thiram, Carbendazim
		MC							Glyphosate, Thiram, Carbendazim, Met, 2,4-D
	2008/9	CR						80	Glyphosate, Thiram, Carbendazim
		MC							Glyphosate, Thiram, Carbendazim, Met, 2,4-D
	2009/10	CR				50	100		Glyphosate Thiram Carbendazim
		MC							2,4-D Glyphosate Met Thiram Carbendazim
	2010/11	CR	40	40					Glyphosate Thiram Carbendazim
		MC							2,4-D Glyphosate Met Thiram Carbendazim
Viale	2006/7	CR		120					Glyphosate, Thiram, Carbendazim, Met, Dicamba
		MC				90			Glyphosate, Thiram, Carbendazim, Met
	2007/8	CR		95	55				Glyphosate, Thiram, Carbendazim, Met, Dicamba
		MC				90			Glyphosate, Thiram, Carbendazim, Met
	2008/9	CR			80		210		Glyphosate, Thiram, Carbendazim, Met, Dicamba
		MC				90			Glyphosate, Thiram, Carbendazim, Met
	2009/10	CR		96		118	80		Glyphosate Met Dicamba Thiram Carbendazim
		MC				90			Glyphosate Met Thiram Carbendazim
	2010/11	CR		95	55				Glyphosate Met Dicamba Thiram Carbendazim
		MC		85		100	150		Thiram Carbendazim

[a]CR, Crop rotation; MC-soya bean monocropping. [b]Fertilizers applied; SSP, single superphosphate; TSP, triple superphosphate; MAP, monoammonium phosphate; DAP, diammonium phosphate; Urea; UAN, urea-ammonium nitrate. [c]Pesticides applied – glyphosate, thiram, carbendazim, Met – metsulfuron-methyl; 2-4-D – 2,4-dichlorophenoxyacetic acid; atrazine.

(38 μm) was used to collect small spores, and the top sieve (125 μm) was checked for sporocarps and larger spores. Only apparently healthy spores were isolated by direct observations using a stereomicroscope (SMZ745T, Nikon). For quantification and taxonomic identification, fungal spores were mounted onto slides using PVA with and without Melzer's reagent (Omar *et al*., 1979) and examined under compound microscope (E200, Nikon). AMF morphospecies identification was based on current species classification (Redecker *et al*., 2013) and INVAM (http://

© 2017 British Society of Soil Science, *Soil Use and Management*

invam.caf.wvu.edu/fungi/taxonomy/speciesID.htm). The data of the AMF community were expressed as spore density (total number of spores in 100 g of soil dry weight), spore density (per 100 g of soil) of each morphospecies and total species richness (the total number of different species occurring under each cropping system).

Analysis of soil lipid fatty acid 16:1ω5c

The WCFA (whole-cell fatty acid analysis) method consists of a direct soil saponification, followed by methylation and further extraction of FAMEs (fatty acid methyl esters). The chromatographic analysis of the extracted FAMEs was performed according to the TSBA 40 MIDI system (Sasser, 1990). The PLFA/NLFA (phospho- and neutral lipid fatty acid) methodology consisted of extraction (3 g of soil) with chloroform/methanol/citrate buffer (Bligh–Dyer method), fractionation with a solid-phase extraction column filled with silica, elution of the neutral fraction with chloroform and the phospholipid fraction with methanol, amendment with 33.75 μg of the standard fatty acid 19:0 methyl ester, mild alkaline methanolysis with KOH/methanol, extraction with hexane and evaporation under N_2 stream, and resuspension in 100 μL of hexane (see Frostegård *et al.*, 1993 for analytical details). The mono-unsaturated fatty acid *16:1ω5c* was used as a biomarker of AMF (see Ferrari *et al.*, 2015 for more details). The fatty acid *16:1ω5c* concentrations were expressed as nanomoles per gram (nmol/g) of soil dry weight.

Statistical analysis

To study the effects of soil managements on AMF species richness, the number of morphospecies per sample was used to calculate rarefaction curves with the program EstimateS9 (Colwell, 2013). To estimate the completeness of sampling at each cropping system, minimal species richness estimate Chao2 was calculated with 100 permutations, and sampling units (samples) were selected randomly without replacement.

To test differences in AMF diversity between cropping systems, seasons and years, Pielou's evenness and the Shannon–Weaver diversity index of the AMF community were calculated (Magurran & McGill, 2011).

All data are presented as means and standard error (±SE). AMF spore density, richness, evenness and diversity were analysed by a three-way ANOVA with cropping system, season and year as the main effects including the interaction terms. Assumptions of normality and homoscedasticity were tested using the Shapiro–Wilk's and Levene's tests, respectively, and the variables that were not normally distributed were log10-transformed. AMF biomarker taxon *16:1ω5c* from soil lipid fractions WCFA, NLFA and PLFA was analysed with the same model. We used DGC test ($P < 0.05$) to detect differences between means. We performed Spearman's correlation analyses between fatty acid *16:1ω5c* and AMF spore density to examine possible relationships. Principal component analysis (PCA) was performed to assess the relationship among AMF spore variables and chemical soil properties, using cropping systems and geographical location as the classification criteria. Statistical analyses were performed with the INFOSTAT version 2012.

Results

More than 9700 spores were isolated from all soil samples; 2803 spores were detected in Bengolea (1654 in NG, 753 in CR, 396 in MC), 726 in Monte Buey (100 in NG, 262.78 in CR, 364 in MC), 3153 in Pergamino (1975 in NG, 771 in CR, 407 in MC) and 3089 in Viale (1985 in NG, 866 in CR, 237 in MC). We identified 46 AMF morphospecies (eight families), 40 of these being attributed to known morphospecies (Table 3–S2).

AMF spore communities at regional scale

Rarefaction curves approached the asymptote, suggesting that the sampling intensity was adequate to detect most of the AMF morphospecies and to make accurate comparisons between cropping systems at a regional level (Figure 2). Total AMF species richness was greatest in NG (44 morphospecies) closely followed by CR (43 morphospecies). The lowest level of richness was found in MC (37 morphospecies), 16 and 14% less than NG and CR, respectively. A similar pattern was observed according to Chao2 estimator, but the differences were less pronounced (Figure 2).

AMF communities at each geographical location

Acaulospora scrobiculata, Archaeospora trappei, Funneliformis geosporum, Glomus brohultii and Rhizophagus intraradices were the most abundant taxa considering all samples together (whose densities were 847.38, 1167.54, 1351.25, 1980.14 and 570.62 spores/100 g of soil dry weight, respectively). *A. bireticulata, A. rehmii, A. spinosa, Claroideoglomus claroideum, C. etunicatum, C. luteum, G. margarita, G. rosea, Scutellospora biornata, F. mosseae, R. clarus* and *Septoglomus constrictum* occurred in all locations regardless of soil management. Other morphospecies apparently were more restricted to specific situations. *A. laevis, C. pellucida, G. decipiens, R. fulgida, G. aggregatum, Glomus* sp. 2, *Glomus* sp. 3, *Pacispora* sp. were shared between NG and CR management, and *S. dipapillosa* was shared between NG and MC management (Table 3–S2).

© 2017 British Society of Soil Science, *Soil Use and Management*

Table 3 Spore density (per 100 g soil) of each arbuscular mycorrhizal fungi (AMF) species identified from the spore morphotypes isolated from soil of different cropping systems: natural grassland (NG), crop rotation (CR) and soya bean monoculture (MC) in the Argentinean pampas

	Bengolea				Monte Buey				Pergamino				Viale			
Morphospecies	NG	CR	MC	P	NG	CR	MC	P	NG	CR	MC	P	NG	CR	MC	P
Acaulosporaceae																
Acaulospora alpina	0.09 ± 0.09	0	0.26 ± 0.19	ns	0	0	0	ns	0	0.10 ± 0.10	0.10 ± 0.10	ns	0	0	0.10 ± 0.10	ns
A. bireticulata	0.19 ± 0.13	0.37 ± 0.22	0.44 ± 0.27	ns	**0.84 ± 0.45 b**	**0.51 ± 0.23 b**	**2.36 ± 0.75 a**	*	**0.26 ± 0.26 b**	**1.12 ± 0.32 b**	**3.36 ± 0.10 a**	**	2.84 ± 1.12	0.76 ± 0.30	1.89 ± .189	ns
A. delicata	0	0	0	ns	0	0	0	ns	0	0.10 ± 0.10	0.30 ± 0.30	ns	0	1.57 ± 1.57	0	ns
A. denticulata	0	0	0.21 ± 0.21	ns	0	0	0	ns	0	0	0	ns	0	0	0	ns
A. excavata	0.47 ± 0.47	0.08 ± 0.08	0	ns	0	0.10 ± 0.10	0.10 ± 0.10	ns	0	0	0	ns	0	0.22 ± 0.22	0.10 ± 0.10	ns
A. laevis	0.56 ± 0.32	0.11 ± 0.11	0	ns	0.11 ± 0.11	0	0	ns	0	2.40 ± 1.71	0	ns	0.21 ± 0.21	0	0	ns
A. mellea	0.27 ± 0.19	0	0	ns	0.11 ± 0.11	0.12 ± 0.12	0	ns	0.11 ± 0.11	0	0	ns	1.96 ± 0.96	1.06 ± 1.06	0.10 ± 0.10	ns
A.rehmii	**1.93 ± 0.73 a**	**0.28 ± 0.15 b**	**0.55 ± 0.21 b**	*	0	0	0.18 ± 0.12	ns	0.83 ± 0.38	0.31 ± 0.22	0.59 ± 0.23	ns	0.43 ± 0.43	0.21 ± 0.21	0	ns
A. scrobiculata	**15.45 ± 3.2 a**	**4.12 ± 1.27 b**	**7.61 ± 1.87 b**	**	**0.31 ± 0.16 b**	**2.00 ± 0.85 b**	**4.88 ± 1.42 a**	**	**19.30 ± 3.63 a**	**9.98 ± 1.95 a**	**4.81 ± 1.02 b**	***	**2.23 ± 1.11 a**	**0.34 ± 0.25 b**	**0 b**	*
A. spinosa	**2.75 ± 1.41 a**	**0 b**	**0.09 ± 0.09 b**	*	0	0	0.09 ± 0.09	ns	0.37 ± 0.27	0.10 ± 0.10	0.68 ± 0.23	ns	0.73 ± 0.44	0.21 ± 0.14	0.10 ± 0.10	ns
Ambisporaceae																
Ambispora leptoticha	0.19 ± 0.13	0	0.09 ± 0.09	ns	0	1.09 ± 1.09	0	ns	0	0.10 ± 0.10	0	ns	0	0	0	ns
Archaeosporaceae																
Archaeospora trappei	1.68 ± 1.38	0	0.09 ± 0.61	ns	0	0.48 ± 0.26	0.77 ± 0.36	ns	2.23 ± 1.44	0.31 ± 0.22	0	ns	**74.66 ± 27.51 a**	**0 b**	**3.86 ± 2.29 b**	**
Claroideoglomeraceae																
Claroideoglomus claroideum	3.4 ± 1.83	2.13 ± 0.73	0.45 ± 0.45	ns	0.30 ± 0.22	0.69 ± 0.43	1.63 ± 0.70	ns	3.06 ± 1.42	1.93 ± 0.81	1.57 ± 0.76	ns	1.55 ± 0.73	2.47 ± 0.80	0.48 ± 0.30	ns
C. etunicatum	3.12 ± 1.26	1.62 ± 0.75	1.53 ± 0.96	ns	0.10 ± 0.10	0.83 ± 0.83	1.08 ± 0.66	ns	4.86 ± 2.57	0.31 ± 0.16	0.90 ± 0.64	ns	4.02 ± 2.21	1.81 ± 1.09	0.72±0.72	ns
C. luteum	1.49 ± 1.3	0	0.43 ± 0.43	ns	0.20 ± 0.13	0.95 ± 0.75	0	ns	4.98 ± 2.41	1.54 ± 1.03	0.60 ± 0.51	ns	4.2 ± 2.74	0.71 ± 0.48	0	ns
Entrophosporaceae																
Entrophospora infrequens	**1.27 ± 0.47 a**	**0.29 ± 0.15 b**	**0.09 ± 0.09 b**	*	**0 b**	**0 b**	**0.29 ± 0.15 a**	*	1.44 ± 0.61	0.21 ± 0.14	0.50 ± 0.23	ns	0.85 ± 0.54	0.59 ± 0.48	0.21 ± 0.21	ns
Gigasporaceae																
Cetraspora pellucida	**1.90 ± 1.11 a**	**0 b**	**0 b**	*	0	0.10 ± 0.10	0	ns	0	0.31 ± 0.31	0	ns	0.21 ± 0.21	0	0	ns
Dentiscutata heterogama	**8.21 ± 3.25 a**	**1.37 ± 1.06 b**	**0.55 ± 0.28 b**	**	0.25 ± 0.25	0	0	ns	1.19 ± 0.81	0	0.20 ± 0.20	ns	0	0	0	ns
Gigaspora decipiens	0.51 ± 0.29	0.22 ± 0.22	0	ns	0	0	0	ns	0.68 ± 0.39	0.51 ± 0.41	0	ns	0.22 ± 0.22	0.22 ± 0.15	0	ns
G. gigantea	3.56 ± 1.71	0.63 ± 0.36	1.26 ± 0.66	ns	0	0.11 ± 0.11	0	ns	0.75 ± 0.46	0.21 ± 0.14	0	ns	0	0.30 ± 0.30	0.10 ± 0.10	ns
G. margarita	10.03 ± 3.37	4.6 ± 1.44	6.59 ± 1.94	ns	0.62 ± 0.42	1.59 ± 1.48	0.29 ± 0.29	ns	1.71 ± 0.67	1.33 ± 0.53	0.40 ± 0.22	ns	0.82 ± 0.48	1.70 ± 0.63	0.39 ± 0.22	ns
G. rosea	1.03 ± 0.75	0.75 ± 0.47	0.09 ± 0.09	ns	0	0.42 ± 0.42	0.09 ± 0.09	ns	0.69 ± 0.50	0.30 ± 0.16	0.68 ± 0.37	ns	0	0	0	ns
Racocetra fulgida	0.46 ± 0.26	0.55 ± 0.55	0	ns	0.10 ± 0.10	0.10 ± 0.10	0	ns	0	0	0	ns	0	0	0	ns
Scutellospora biornata	**8.72 ± 2.16 a**	**2.98 ± 0.97 b**	**0.73 ± 0.65 b**	***	0	0	0.20 ± 0.13	ns	**4.52 ± 1.57 a**	**0.31 ± 0.22 b**	**0.88 ± 0.30 b**	**	0.71 ± 0.24	0.34 ± 0.24	0.83 ± 0.52	ns
S. calospora	3.83 ± 1.90	0.89 ± 0.53	0.09 ± 0.09	ns	0.21 ± 0.21	0	0	ns	2.59 ± 2.31	0.21 ± 0.14	0.10 ± 0.10	ns	0.1 ± 0.1	0.1 ± 0.1	0	ns
S. dipapillosa	0.57 ± 0.38	0	0.09 ± 0.09	ns	0	0	0	ns	0.42 ± 0.32	0	0	ns	0.40 ± 0.31	0	0	ns
S. rubra	**3.45 ± 0.98 a**	**0.56 ± 0.36 b**	**0.45 ± 0.36 b**	**	0.10 ± 0.10	0	0.30 ± 0.30	ns	0.21 ± 0.21	0	0.20 ± 0.13	ns	0	0	0	ns
Scutellospora sp.	0.65 ± 0.39	0.33 ± 0.33	0.66 ± 0.48	ns	0	0	0	ns	1.09 ± 0.82	0.81 ± 0.62	0.80 ± 0.52	ns	0.64 ± 0.43	0.11 ± 0.11	0.10 ± 0.10	ns
Glomeraceae																
Funneliformis coronatum	0.09 ± 0.09	1.18 ± 0.80	0	ns	0	0.41 ± 0.48	0	ns	1.68 ± 0.96	3.10 ± 2.03	0.90 ± 0.55	ns	0.51 ± 0.40	0.21 ± 0.21	0	ns
F. geosporum	9.84 ± 3.2	8.73 ± 3.75	1.82 ± 1.27	ns	1.47 ± 0.54	4.69 ± 1.40	6.49 ± 3.25	ns	**30.85 ± 9.79 a**	**10.03 ± 1.94 b**	**4.36 ± 1.53 b**	**	**19.23 ± 6.63 a**	**12.50 ± 3.63 a**	**2.58 ± 0.86 b**	*
F. mosseae	0.74 ± 0.36	4.03 ± 2.51	1.36 ± 0.62	ns	1.14 ± 0.45	1.72 ± 0.55	1.38 ± 0.86	ns	7.91 ± 2.53	6.12 ± 4.08	1.09 ± 0.50	ns	4.73 ± 1.72	3.67 ± 1.07	1.66 ± 0.92	ns
Glomus aggregatum	1.06 ± 0.95	0.55 ± 0.55	0	ns	0.43 ± 0.33	0	0	ns	0	0.31 ± 0.31	0	ns	0	0	0	ns
G. brohultii	**23.50 ± 6.3 a**	**9.76 ± 3.94 b**	**3.38 ± 0.84 b**	**	**0.39 ± 0.22 b**	**2.35 ± 0.77 b**	**4.95 ± 1.50 a**	**	**64.32 ± 18.80 a**	**6.45 ± 2.60 b**	**4.48 ± 1.49 b**	***	**25.39 ± 8.86 a**	**16.53 ± 4.19 b**	**3.53 ± 1.54 c**	*
G. fuegianum	**2.83 ± 1.57 a**	**0 b**	**0 b**	*	0	0	0	ns	0	0	0.20 ± 0.20	ns	1.80 ± 1.12	0.11 ± 0.11	0	ns
G. microaggregatum	**6.06 ± 2.02 a**	**0**	**0.35 ± 0.35 a**	***	0.11 ± 0.11	0	0	ns	0.53 ± 0.36	0.10 ± 0.10	0.10 ± 0.10	ns	1.44 ± 1.23	0.55 ± 0.55	0.51 ± 0.32	ns
Glomus sp. 1	0.11 ± 0.11	0	0	ns	0	0.12 ± 0.12	0.09 ± 0.09	ns	0	0	0.10 ± 0.10	ns	0	0	0	ns
Glomus sp. 2	0.22 ± 0.22	0	0	ns	0.11 ± 0.11	0.12 ± 0.12	0	ns	0	0	0	ns	0	0	0	ns
Glomus sp. 3	0.11 ± 0.11	0.49 ± 0.29	0	ns	0	0	0	ns	0	0	0	ns	0	0	0	ns

© 2017 British Society of Soil Science, Soil Use and Management

Table 3 (continued)

Morphospecies	Bengolea NG	Bengolea CR	Bengolea MC	Bengolea *P*	Monte Buey NG	Monte Buey CR	Monte Buey MC	Monte Buey *P*	Pergamino NG	Pergamino CR	Pergamino MC	Pergamino *P*	Viale NG	Viale CR	Viale MC	Viale *P*
Rhizophagus clarus	4.4 ± 1.89	1.39 ± 0.66	1.17 ± 0.88	ns	0.56 ± 0.47	0.94 ± 0.75	2.56 ± 0.89	ns	3.29 ± 0.80	9.23 ± 4.28	1.30 ± 0.58	ns	5.53 ± 2.74	9.31 ± 4.56	1.94 ± 0.96	ns
R. fasciculatus	2.72 ± 1.29	0	0.63 ± 0.63	ns	0	0	0	ns	0	0.10 ± 0.10	0	ns	0.10 ± 0.10	0	0	ns
R. intraradices	**4.67 ± 2.68 b**	**14.29 ± 6.2 a**	**0.67 ± 0.30 b**	*	0.19 ± 0.19	2.31 ± 1.51	2.00 ± 1.14	ns	1.68 ± 1.17	4.77 ± 2.13	3.96 ± 1.43	ns	9.06 ± 4.73	3.46 ± 2.06	0.48 ± 0.26	ns
Sclerocystis sinuosa	**2.5 ± 1.13 a**	**0.09 ± 0.09 b**	**0 b**	*	0	0.10 ± 0.10	0	ns	1.23 ± 0.67	0.42 ± 0.32	0	ns	0.64 ± 0.43	0.67 ± 0.56	0.10 ± 0.10	ns
Sclerocystis sp.	0.39 ± 0.29	0	0	ns	0.10 ± 0.10	0	0	ns	0.11 ± 0.11	0	0	ns	0	0	0	ns
Septoglomus constrictum	2.83 ± 1.64 a	0.18 ± 0.18 b	0 b	ns	0.40 ± 0.40	0.21 ± 0.14	0.99 ± 0.99	ns	0.86 ± 0.86	0.42 ± 0.24	0.40 ± 0.27	ns	0.31 ± 0.31	0.11 ± 0.11	0	ns
Pacisporaceae																
Pacispora boliviana	0.12 ± 0.12	0	0.45 ± 0.45	ns	0	0	0	ns	0.52 ± 0.52	0	0.20 ± 0.20	ns	0.21 ± 0.14	0.11 ± 0.11	0	ns
Pacispora sp.	0	0	0	ns	0	0	0	ns	0.32 ± 0.23	0.70 ± 0.52	0	ns	0.10 ± 0.10	0	0	ns

Ns, non significant. The values are mean density ± SE of each morphospecies. Fungal species differing significantly when comparing between cropping systems (DGC test $P < 0.05$) are marked in bold. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

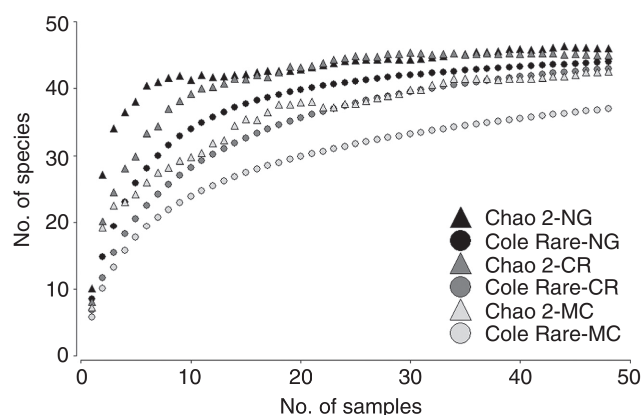

Figure 2 Coleman rarefaction curves and Chao2 minimal species richness estimates with respect to each cropping system: natural grassland (NG), crop rotation (CR) and soya bean monoculture (MC), on the Argentinean Pampas.

Sixteen of the 46 morphospecies identified showed significant differences between soil management systems (*A. bireticulata, A. rehmii, A. scrobiculata, A. spinosa, A. trappei, E. infrequens, C. pellucida, D. heterogama, S. biornata, S. rubra, F. geosporum, G. brohultii, G. fuegianum, G. microaggregatum, R. intraradices, S. sinuosa*). In general, these morphospecies showed higher levels of spore numbers in NG than no-tillage systems, albeit exceptions existed (Table 3).

Mean values of AMF spore density and morphospecies richness ranged between 6.15 ± 2.9 and 438.65 ± 360.37, and 2.00 ± 1.73 and 19.33 ± 4.51 per 100 g of dry soil, respectively. In general, NG showed larger values of AMF spore density and richness than both no-till managements (CR and MC) in both seasons (February and September) and years (2010–2011) at four locations, but Monte Buey showed the opposite trend for these variables (Table S3). Spore density differed significantly between geographical locations (Table 4). Each location showed different patterns of variation in each season (Figure 3a,b).

Species richness exhibited significant differences between cropping systems in three of the four locations. Bengolea showed differences in both seasons, while Monte Buey did only in February and Viale in September. In addition, some differences were observed between years and seasons in Monte Buey and Viale (Table 4, Figure 3c, d).

Arbuscular mycorrhizal fungi species diversity varied between cropping systems in Bengolea in both seasons, and in Monte Buey during February. Viale showed differences in diversity between seasons (Table 4, Figure 4a,b). Their mean values varied from 0.98 ± 0.86 to 3.60 ± 0.31 per 100 g of dry soil (Table S3). Evenness showed significant differences between cropping system in Pergamino (in February) and Viale. In Viale, evenness also differed between years (Table 4, Figure 4c, d).

© 2017 British Society of Soil Science, *Soil Use and Management*

Table 4 ANOVA of AMF spore density (number of spores/100 g soil dry weight), richness, diversity and evenness in soils of different cropping systems: natural grassland (NG), crop rotation (CR) and soya bean monoculture (MC) within each geographical location in the Argentinean Pampas are shown. Ns, non significant; ** $P < 0.01$; *** $P < 0.001$.

	Variable							
	Density		Richness		Diversity		Evenness	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Source of variation Bengolea								
Cropping system (C)	14.14	***	16.18	***	12.23	***	1.35	ns
Year (Y)	3.41	ns	2.65	ns	1.03	ns	1.03	ns
Season (S)	4.07	ns	1.11	ns	0.31	ns	1.96	ns
C*Y	1.1	ns	0.47	ns	0.49	ns	0.16	ns
C*S	3.72	ns	3.5	ns	2.98	ns	1.55	ns
Y*S	0.31	ns	0.09	ns	0.02	ns	0.17	ns
C*Y*S	0.71	ns	0.93	ns	1.36	ns	0.47	ns
Source of variation Monte Buey								
Cropping system (C)	17.7	***	10.52	***	8.71	***	1.00	ns
Year (Y)	2.43	ns	15.38	***	19.71	***	1.53	ns
Season (S)	0.86	ns	13.08	**	4.73	ns	0.0028	ns
C*Y	3.15	ns	4.53	ns	6.8	**	0.79	ns
C*S	3.7	ns	3.51	ns	1.33	ns	0.66	ns
Y*S	3.26	ns	0.25	ns	1.66	ns	0.1	ns
C*Y*S	1.08	ns	2.56	ns	2.32	ns	1.1	ns
Source of variation Pergamino								
Cropping system (C)	16.19	***	0.54	ns	1.12	ns	8.82	***
Year (Y)	0.43	ns	0.05	ns	0.35	ns	1.36	ns
Season (S)	2.37	ns	0.66	ns	0.37	ns	0.37	ns
C*Y	0.24	ns	1.46	ns	2.32	ns	2.1	ns
C*S	1.57	ns	1.99	ns	1.48	ns	0.47	ns
Y*S	0.31	ns	1.2	ns	1.36	ns	0.0004	ns
C*Y*S	3.21	ns	1.55	ns	0.39	ns	0.15	ns
Source of variation Viale								
Cropping system (C)	26.7	***	16.66	***	0.44	ns	8.82	***
Year (Y)	46.62	***	61.62	***	2.43	ns	21.54	***
Season (S)	0.29	ns	8.01	**	8.03	**	0.47	ns
C*Y	1.24	ns	1.68	ns	5.11	ns	2.22	ns
C*S	1.31	ns	1.03	ns	0.82	ns	0.06	ns
Y*S	10.37	ns	32.37	***	9.98	**	0.3	ns
C*Y*S	2.76	ns	1.85	ns	0.74	ns	0.39	ns

Concentration of AMF biomarker 16:1ω5c and its relation with spore density

Fatty acid concentrations revealed differences between soil managements. The WCFA and NLFA concentrations were greater in NG than NT treatments in Bengolea and Pergamino. PLFA concentrations revealed the same pattern, except in Monte Buey (Figure 5).

The NLFA/PLFA ratio for *16:1ω5c* showed mean values of 17.7 for NG, 6.5 for CR and 5.5 for MC. In each location, that ratio revealed the following mean values for NG, CR and MC: in Bengolea, 21.63, 4.82 and 6.88; in Monte Buey, 0, 2.75 and 6.17; in Pergamino, 10.06, 12.50 and 4.50; and in Viale, 6.42, 17.50 and 57.76.

Spore density was positively correlated with WCFA, NLFA and PLFA (Figure 6) in February 2010.

PCA among biotic and abiotic parameters

The PCA resulted in a clear separation of NG and no-till plots for all locations (Figure 7). The variation along the PC1 axis (43.1%) was mainly explained by diversity index, TN, richness, P and TOC, while the variation along the PC2 axis (39.2%) was mainly explained by evenness, TOC, pH and TN. Eigenvalues are presented in Table S4.

Discussion

In this study, the total number of morphospecies (46) was similar to that reported recently in agroecosystems of Central Europe (Säle *et al*., 2015), but greater than that reported in previous research from the studied region (Colombo *et al*., 2014) and arable soils in Europe (Wetzel *et al*., 2014). These differences might be related to the wider scale of the present study in comparison with others (three soil management approaches based on two levels of crop rotation and a natural grassland, regionally replicated at four geographical locations) and the sampling intensity (two seasons in two consecutive years). When compared with the NG, CR treatment maintained similar richness levels, while the MC management showed a smaller AMF spore richness (Figure 2).

Several morphospecies (17) occurred abundantly at all soils, independent of location and cropping system, whereas others were more or less restricted to specific situations. These morphospecies could be defined as generalists and specialists, respectively, according to Oehl *et al*. (2010). Generalist species had been commonly found in previous studies from Central Europe (Wetzel *et al*., 2014; Säle *et al*., 2015) such as *A. trappei*, *C. etunicatum* and *R. intraradices*. Furthermore, some AMF species could be used as indicators of land use (Jansa *et al*., 2014; Säle *et al*., 2015). In our study, *A. laevis*, *Cetraspora pellucida, G. decipiens, Racocetra fulgida, G. aggregatum, Glomus* sp. 2, *Glomus* sp. 3 and *Pacispora* sp. were present in NG and CR, but absent in MC (Table 3). We suggest that these morphospecies could be good candidates as bioindicators for NT practices based on CR management in the Argentinean Pampas.

The intensity of agricultural management differently affects AM fungal spore communities (Oehl *et al*., 2010). In

© 2017 British Society of Soil Science, *Soil Use and Management*

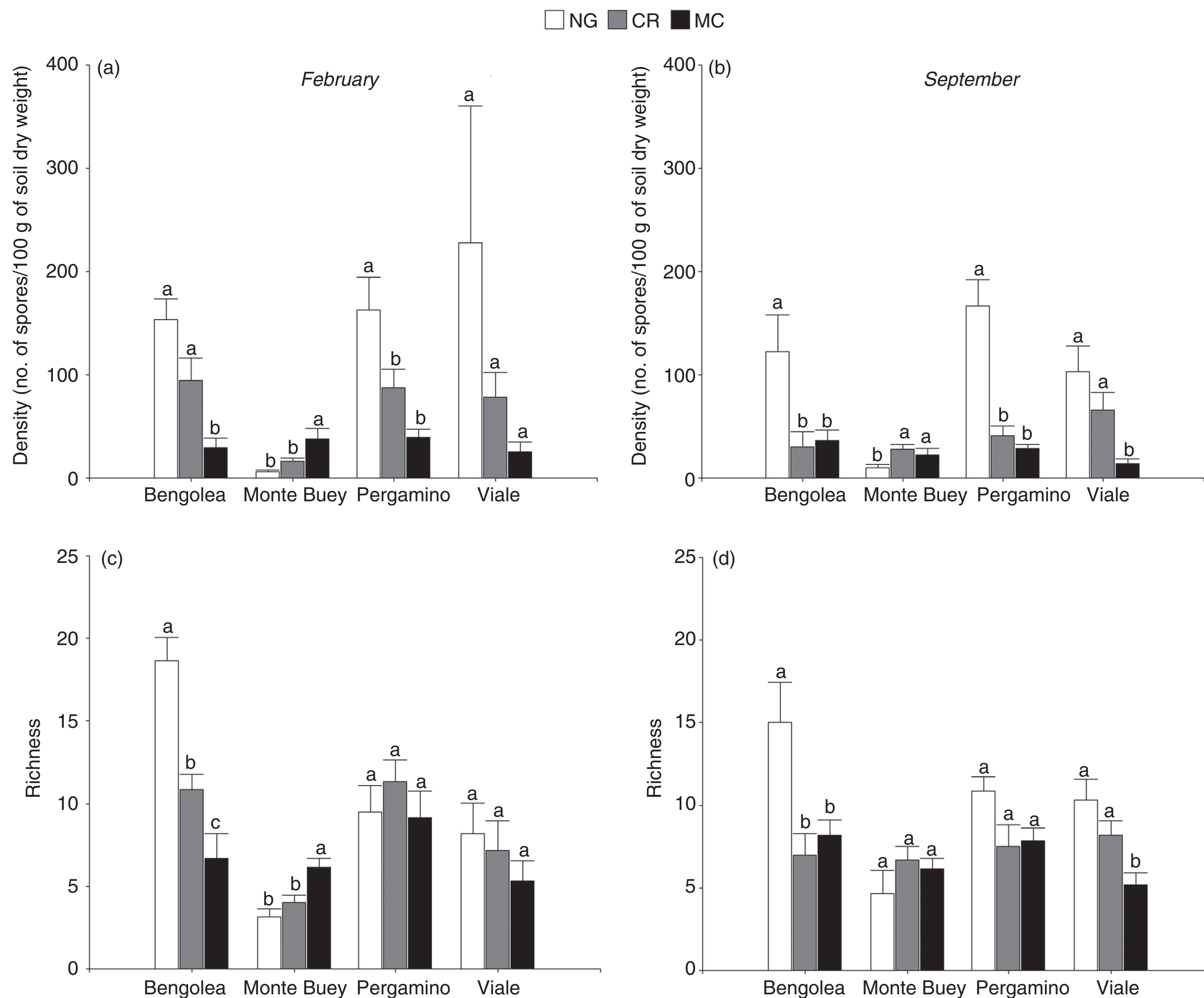

Figure 3 Variation patterns of mean values and standard error (±SE) (n = 6) of AMF spore density and richness in different cropping systems: natural grassland (white bar, NG), crop rotation (grey bar, CR) and soya bean monoculture (black bar, MC) for each geographical location studied, during February (a, c) and September (b, d). Different letters indicate significant differences between crop managements in each geographical location (DGC test, $P < 0.05$).

this study, the significant effects of cropping systems on spore density were consistent in the four geographical locations, but each location showed different patterns of variation. In comparison with no-till practices, NG showed greater values for spore density in Bengolea (only in September) and in Pergamino in both seasons. In Bengolea (in February) and Viale, both NG and CR resulted in greater spore density compared with MC. This pattern was reversed at Monte Buey, suggesting some degree of context dependency of the AMF responses (Hedlund *et al.*, 2003).

Spore density, richness and PLFA *16:1ω5c* showed particularly small values in NG at Monte Buey, where extractable phosphorus was greater than in other combinations of treatments and geographical locations. This is consistent with the negative relationship observed between P availability and AMF (Treseder, 2004).

While AMF spore density was clearly affected by cropping systems at the four locations, changes in AMF species richness were not consistent among seasons. Moreover, similar diversity was found under agricultural and natural soils. These results agree with those found by Säle *et al.* (2015) in other agricultural system, but differed with those observed in natural ecosystems from central Argentina (Soteras *et al.*, 2012).

In comparison with NG, CR and MC contained less WCFA and NLFA *16:1ω5c* fatty acids, but only at Bengolea and Pergamino, whereas PLFA showed a similar pattern at Bengolea, Pergamino and Viale. The latter fatty

© 2017 British Society of Soil Science, *Soil Use and Management*

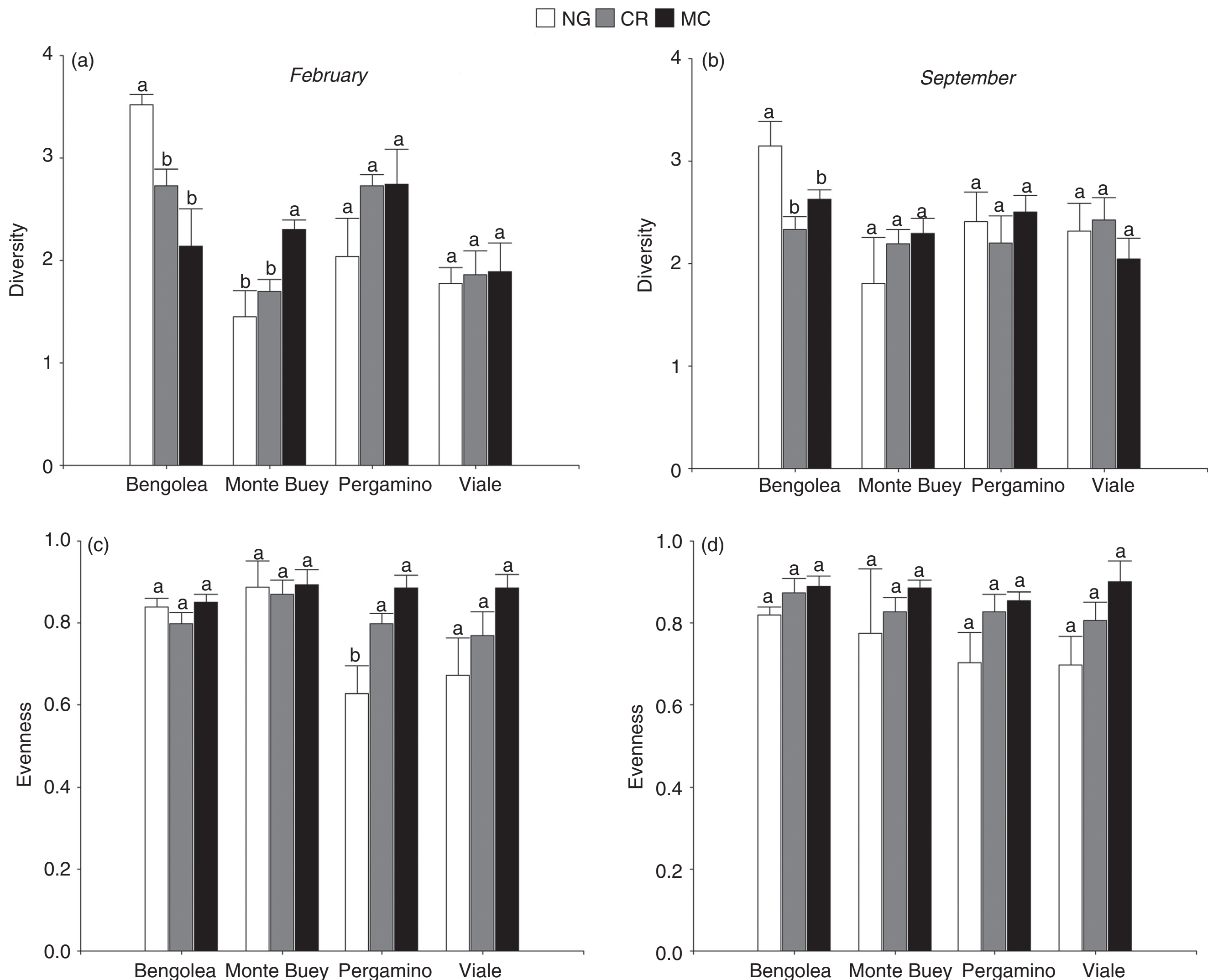

Figure 4 Variation patterns of mean values and standard error (±SE) (n = 6) of AMF diversity (Shannon–Weaver diversity index) and evenness (Pielou's evenness) in different cropping systems: natural grassland (white bar, NG), crop rotation (grey bar, CR) and soya bean monoculture (black bar, MC) for each geographical location studied, during February (a, c) and September (b, d). Different letters indicate significant differences between crop managements in each geographical location (DGC test, $P < 0.05$).

acid showed larger values under CR than NG or MC at Monte Buey. It is known that some background concentrations of this fatty acid can also come from Gram-negative bacteria (Olsson, 1999). However, in this study, the ratio of *16:1ω5c* NLFA/PLFA was greater than 1 in all treatments, which supports its mycorrhizal origin (Joergensen & Wichern, 2008). Moreover, fatty acids were positively correlated with spore density. In contrast, Gryndler *et al.* (2006) reported negative or null correlations between WCFA *16:1ω5c* and spore density. However, their study involved long term of ploughing and fertilizer application. These differences suggest that the development of AMF in no-tillage systems may be more traceable by WCFA and NLFA than in tilled soils. Accordingly, in our study, WCFA, NLFA, PLFA and spores showed the same trends, regularly decreasing in the direction NG>CR>MC, and in most cases, the NG showed the highest values for those variables.

The PCA showed positive associations between spore density, species richness, diversity, TOC, TN, P and pH, with NG having the largest values observed for these variables. Importantly, these analyses showed that arable plots tended to be grouped separately from NG for all locations. Natural grassland showed a greater spatial distribution along the axis, suggesting a strong selection pressure caused by the anthropic intervention which tends to homogenize the cultivated soils.

It is worth mentioning that all NT plots (both CR and MC) at different locations differed in agrochemical applications, such as thiram, carbendazim, glyphosate. This

© 2017 British Society of Soil Science, *Soil Use and Management*

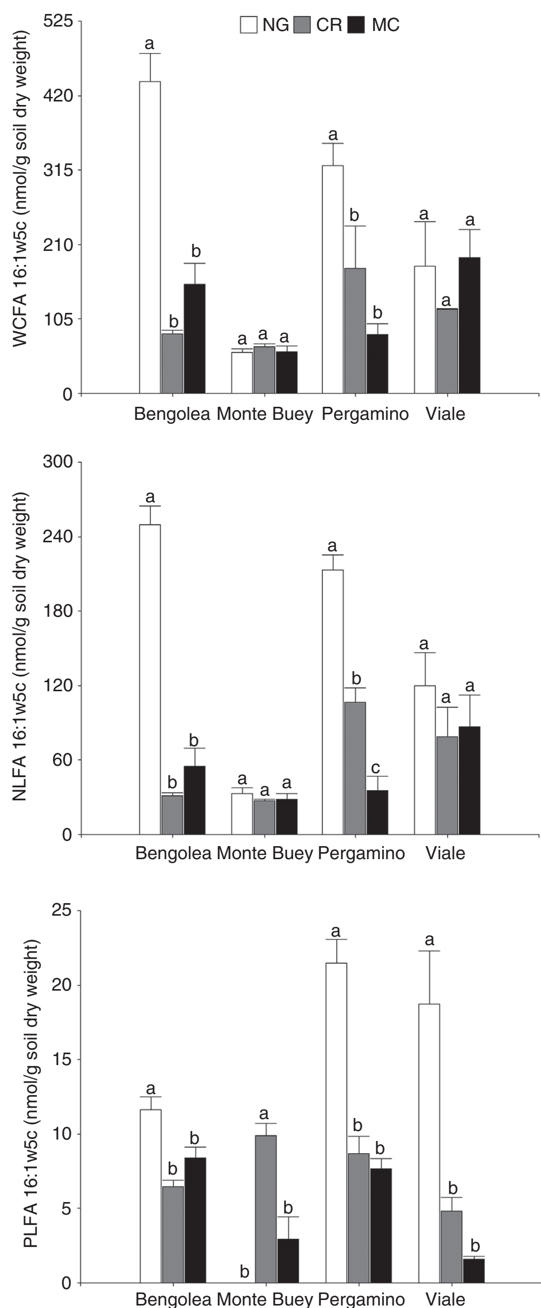

Figure 5 Variation patterns of mean values and standard error (±SE) (n = 3) of fatty acid *16:1ω5c* of AMF for the different cropping systems: natural grassland (NG), crop rotation (CR) and soya bean monoculture (MC) and each geographical location, in the Argentinean Pampas. WCFA, whole-cell fatty acid; NLFA, neutral lipid fatty acid; PLFA, phospholipid fatty acid. Different letters indicate significant differences between crop managements within each geographical location (DGC test, $P < 0.05$). WCFA and NLFA showed differences between cropping systems in Bengolea ($F = 43.63$, $P < 0.001$ and $F = 99.51$, $P < 0.001$, respectively), and Pergamino ($F = 9.07$, $P < 0.05$ and $F = 58.89$, $P < 0.001$, respectively). PLFA showed differences between cropping systems in Bengolea ($F = 13.9$, $P < 0.01$), Monte Buey ($F = 25.42$, $P < 0.001$), Pergamino ($F = 39.94$, $P < 0.001$) and Viale ($F = 18.44$, $P < 0.01$).

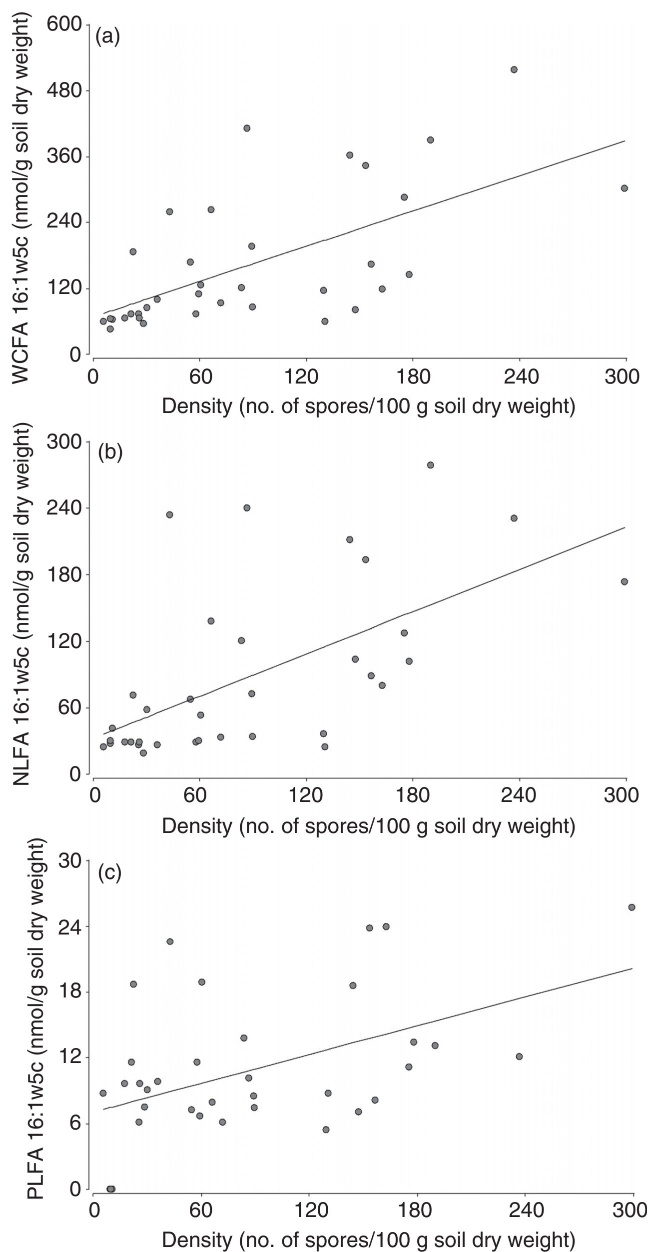

Figure 6 Relationship between AMF spore density (no. of spores/100 g of soil dry weight) and (a) WCFA *16:1ω5c* (nmol/g of soil dry weight, $r = 0.64$, $P < 0.001$); (b) NLFA *16:1ω5c* (nmol/g of soil dry weight, $r = 0.66$, $P < 0.001$); and (c) PLFA *16:1ω5c* (nmol/g of soil dry weight, $r = 0.45$, $P < 0.01$) from arbuscular mycorrhizal fungi (AMF) present in three cropping systems.

might explain the variations between locations, together with climate and abiotic properties of soils.

It must be noted that, although spore abundance varies with soil depth (Oehl *et al.*, 2005), in this study, soil samples were taken at only 10 cm depth. This may be considered as a limitation, but most studies select this depth because most mycorrhizal activity is expected to develop there.

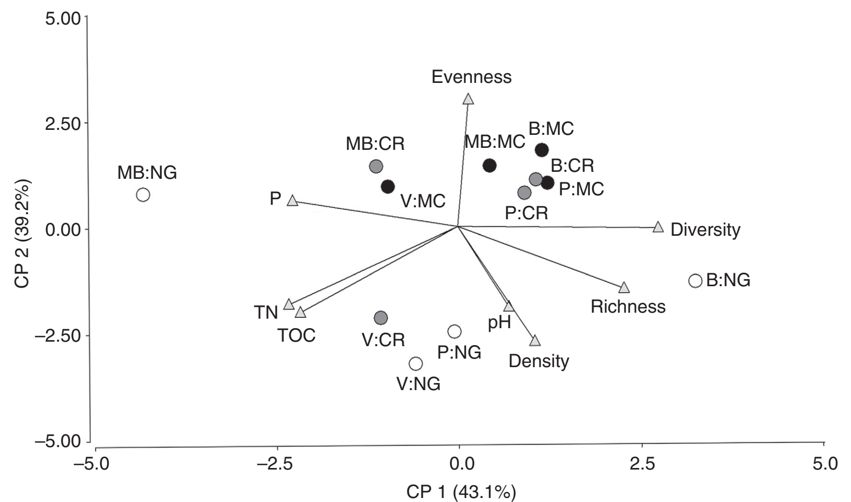

Figure 7 Principal component analysis (PCA) of AMF spore variables, and chemical soil properties, for cropping systems (natural grassland, NG (○); crop rotation management, CR (●); and soya bean monoculture, MC (●); and geographical locations (B Bengolea, B; Monte Buey, MB; Pergamino, P; and Viale, V) as classification criteria on the Argentinean Pampas.

Finally, it is worth mentioning that monocropping was restricted to soya bean. Therefore, results should be framed in this context, and further studies, considering other crops such as maize, would allow testing the generality of patterns found here.

Conclusions

In this study, we show that AM spore communities and fatty acids in soils are suitable indicators of soil management involving different cropping systems. In particular, spore richness measured at a regional scale proved to be sensitive to different NT agricultural managements. Moreover, certain morphospecies proved to be good candidates as bioindicators for no-till practices based on cropping systems in the Argentinean Pampas. Importantly, soil management systems that include practices based on crop rotation keep AMF richness at levels comparable to those observed in soils from natural environments. Given that the loss of soil biodiversity is ultimately linked to a loss of soil functions that support ecosystem services (Wagg *et al.*, 2014), we suggest that agricultural practices that halt or reverse this loss of soil biodiversity should be encouraged.

Acknowledgements

This work was supported by grants PAE-36976-PID53 and PID89 (Agencia Nacional de Promoción Científica y Tecnológica, Argentina) and Secyt-UNC. We thank CONICET and the UNC (Argentina) which supported the facilities used in this investigation. A.B., L.W., N.C. and C.U. are members of CONICET. We are particularly grateful to Dr. Larry Peterson (Canada) for critical comments and language revision of previous versions of manuscript. We are grateful to Dr. Galantini and Lic. Duval (CERZOS-CONICET-UNS-Argentina) for helping with TOC, P and TN determinations. We thank Dr. Harguinteguy C. for helping with map design.

References

Albertengo, J., Belloso, C., Giraudo, M.B., Peiretti, R., Permingeat, H. & Wall, L. 2013. Conservation agriculture in Argentina. In: *Conservation agriculture: Global prospects and challenges* (eds J.R.A.K.L. Sahrawat & A. H Kassam) pp. 352–374. CABI, Boston, USA.

Boddington, C.L. & Dodd, J.C. 2000. The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. I. Field studies in an Indonesian ultisol. *Plant and Soil*, **218**, 137–144.

Bray, R.H. & Kurtz, L.T. 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, **59**, 39–45.

Colombo, R.P., Fernández Bidondo, L., Silvani, V.A., Carbonetto, M.B., Rascovan, N., Bompadre, M.J., Pérgola, M., Cuenca, G. & Godeas, A.M. 2014. Diversity of arbuscular mycorrhizal fungi in soil from the Pampa Ondulada, Argentina, assessed by pyrosequencing and morphological techniques. *Canadian Journal of Microbiology*, **60**, 819–827.

Colwell, R. 2013. EstimateS: statistical estimation of species richness and shared species from samples. Version 9 and earlier. http://purl.oclc.org/estimates.

Copley, J. 2000. Ecology goes underground. *Nature*, **406**, 452–454.

Duval, M.E., Galantini, J.A., Iglesias, J.O., Canelo, S., Martinez, J.M. & Wall, L. 2013. Analysis of organic fractions as indicators of soil quality under natural and cultivated systems. *Soil & Tillage Research*, **131**, 11–19.

Ferrari, A.E., Ravnskov, S., Larsen, J., Tønnersen, T., Maronna, R.A. & Wall, L.G. 2015. Crop rotation and seasonal effects on fatty acid profiles of neutral and phospholipids extracted from no-till agricultural soils. *Soil Use and Management*, **31**, 165–175.

Figuerola, E.L.M., Guerrero, L.D., Rosa, S.M., Simonetti, L., Duval, M.E., Galantini, J.A., Bedano, J.C., Wall, L.G. & Erijman, L. 2012. Bacterial indicator of agricultural management for soil under no-till crop production. *PLoS ONE*, **7**, e51075.

Finckh, M.R. & Wolfe, M.S. 2006. Diversification strategies. In: *The epidemiology of plant diseases* (eds B.M. Cooke *et al.*), pp. 269–307. Springer, Dordrecht, the Netherlands.

Frostegård, Å., Tunlid, A. & Bååth, E. 1993. Phospholipid fatty acids composition, biomass and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Applied Environmental Microbiology*, **59**, 3605–3617.

González-Cortés, J.C., Vega-Fraga, M., Varela-Fregoso, L., Martínez-Trujillo, M., Carreón-Abud, Y. & Gavito, M.E. 2012. Arbuscular mycorrhizal fungal (AMF) communities and land use change: the conversion of temperate forests to avocado plantations and maize fields in central Mexico. *Fungal Ecology*, **5**, 16–23.

Grigera, M.S., Drijber, R.A., Shores-Morrow, R.H. & Wienhold, B.J. 2007. Distribution of the arbuscular mycorrhizal biomarker C16:1cis11 among neutral, glyco and phospholipids extracted from soil during the reproductive growth of corn. *Soil Biology and Biochemistry*, **39**, 1589–1596.

Gryndler, M., Larsen, J., Hršelová, H., Řezáčová, V., Gryndlerová, H. & Kubát, J. 2006. Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment. *Mycorrhiza*, **16**, 159–166.

Hartmann, M. & Widmer, F. 2006. Community structure analyses are more sensitive to differences in soil bacterial communities than anonymous diversity indices. *Applied and Environmental Microbiology*, **72**, 7804–7812.

Hedlund, K., Santa Regina, I., Van der Putten, W.H., Lepš, J., Díaz, T., Korthals, G.W., Lavorel, S., Brown, V.K., Gormsen, D., Mortimer, S.R., Rodríguez Barrueco, C., Roy, J., Smilauer, P., Smilauerová, M. & Van Dijk, C. 2003. Plant species diversity, plant biomass and responses of the soil community on abandoned land across Europe: idiosyncracy or above-belowground time lags. *Oikos*, **103**, 45–58.

van der Heijden, M.G.A., Bardgett, R.D. & van Straalen, N.M. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, **11**, 296–310.

Jansa, J., Erb, A., Oberholzer, H.R., Smilauer, P. & Egli, S. 2014. Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. *Molecular Ecology*, **23**, 2118–2135.

Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K. & Barea, J.-M. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils*, **37**, 1–16.

Joergensen, R.G. & Wichern, F. 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. *Soil Biology & Biochemistry*, **40**, 2977–2991.

Longo, S., Cofré, N., Soteras, F., Grilli, G., Lugo, M. & Urcelay, C. 2016. Taxonomic and Functional Response of Arbuscular Mycorrhizal Fungi to Land Use Change in Central Argentina. In: *Recent advances on mycorrhizal fungi* (ed. M.C. Pagano), pp. 81–90. Fungal Biology, Springer International Publishing, Switzerland.

Magurran, A.E. & McGill, B.J. 2011. *Ecological diversity: Frontiers in measurement and assessment*. Oxford University Press, New York.

McGonigle, T.P. & Miller, M.H. 2000. The inconsistent effect of soil disturbance on colonization of roots by arbuscular mycorrhizal fungi: a test of the inoculum density hypothesis. *Applied Soil Ecology*, **14**, 147–155.

Mummey, D.L., Stahl, P.D. & Buyer, J.S. 2002. Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation. *Applied Soil Ecology*, **21**, 251–259.

Oehl, F., Sieverding, E., Ineichen, K., Ris, E.A., Boller, T. & Wiemken, A. 2005. Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. *New Phytologist*, **165**, 273–283.

Oehl, F., Laczko, E., Bogenrieder, A., Stahr, K., Bösch, R., van der Heijden, M. & Sieverding, E. 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology & Biochemistry*, **42**, 724–738.

Olsson, P.A. 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology*, **29**, 303–310.

Omar, M.B., Bolland, L. & Heather, W.A. 1979. P.V.A. (polyvinyl alcohol). A permanent mounting medium for fungi. *Bulletin of the British Mycological Society*, **13**, 31–32.

Öpik, M., Metsis, M., Daniell, T.J., Zobel, M. & Moora, M. 2009. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. *New Phytologist*, **184**, 424–437.

Redecker, D., Schüßler, A., Stockinger, H., Stürmer, S., Morton, J. & Walker, C. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza*, 1–17.

Säle, V., Aguilera, P., Laczko, E., Mäder, P., Berner, A., Zihlmann, U., van der Heijden, M.G.A. & Oehl, F. 2015. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. *Soil Biology & Biochemistry*, **84**, 38–52.

Sasser, M. 1990. Identification of bacteria through fatty acid analysis. In: *Methods in phytobacteriology* (eds Z. Clement, K. Rudolph & D. C Sands), pp. 199–204. Akadémiai Kiadó, Budapest.

Soteras, F., Becerra, A., Cofré, N., Bartoloni, J. & Cabello, M. 2012. Arbuscular mycorrhizal fungal species in saline environments of Central Argentina: seasonal variation and distribution of spores at different soil depths. *Sydowia*, **64**, 301–311.

Soteras, F., Grilli, G., Cofré, N., Marro, N. & Becerra, A. 2015. Arbuscular mycorrhizal fungal composition in high montane forests with different disturbance histories in Central Argentina. *Applied Soil Ecology*, **85**, 30–37.

Treseder, K.K. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. *New Phytologist*, **164**, 347–355.

Wagg, C., Bender, S.F., Widmer, F. & van der Heijden, M.G.A. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences*, **111**, 5266–5270.

Walker, C., Mize, C.W. & McNabb, H.S. Jr 1982. Populations of endogonaceous fungi at two locations in central Iowa. *Canadian Journal of Botany*, **60**, 2518–2529.

Wall, L.G. 2011. The BIOSPAS consortium: soil biology and agricultural production. In: *Handbook of molecular microbial ecology I: Metagenomics and complementary approaches* (eds F. de Bruijn), pp. 299–306. Wiley & Sons Inc, John.

© 2017 British Society of Soil Science, *Soil Use and Management*

Wang, P., Zhang, J.J., Xia, R.X., Shu, B., Wang, M.Y., Wu, Q.S. & Dong, T. 2011. Arbuscular mycorrhiza, rhizospheric microbe populations and soil enzyme activities in citrus orchards under two types of no-tillage soil management. *Spanish Journal of Agricultural Research*, **9**, 1307–1318.

Wetzel, K., Silva, G., Matczinski, U., Oehl, F. & Fester, T. 2014. Superior differentiation of arbuscular mycorrhizal fungal communities from till and no-till plots by morphological spore identification when compared to T-RFLP. *Soil Biology & Biochemistry*, **72**, 88–96.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Standing crops and phenological stages at the sampling time and standing crops during three previous years, for the different cropping systems at geographical locations in the Argentinean pampas.

Table S2. Spore density (per 100 g soil) (and relative spore density) of each arbuscular mycorrhizal fungi (AMF) species identified from the spore morphotypes isolated from soil of different cropping systems: natural grassland (NG), crop rotation (CR) and soybean monoculture (MC) in the Argentinean pampas. The values are mean density ($n = 6$) ± SD of each morphospecies in each season.

Table S3. Mean values (±SD) of AMF spore density (n° of spores/100 g of soil dry weight), richness, diversity and evenness in soils of different cropping systems: natural grassland (NG), crop rotation (CR) and soybean monoculture (MC) ($n = 3$) at each geographical location, each season and year in the Argentinean pampas.

Table S4. Eigenvectors obtained from principal component analysis of the AMF spore variables and chemical soil properties.

© 2017 British Society of Soil Science, *Soil Use and Management*