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Relationship between microbial functions and community structure following agricultural intensification in South American Chaco

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ABSTRACT

Intensification of agricultural systems through the use of intensive agriculture and the advance of deforestation have led to a decrease of soil biological quality. Soil functional and structural microbiota are sensitive parameters to monitor changes caused by agricultural use. Different sites under soybean monoculture (continuous soybean) and soybean/maize rotation practices were selected. Samples were collected from agricultural soils under different periods of implantation: 4-year rotation; 15-year rotation; 5-year monoculture; and 24-year monoculture (M24). A site of native vegetation recently under agricultural production (RUA) was also sampled. Native vegetation soils (NV) adjacent to agricultural sites were sampled as a control. In general, the results showed that RUA and M24 had lower enzyme activities, less microbial abundance and low physical and chemical soil quality than those subjected to crop rotation. In contrast, both the bacterial and total microbial biomasses were significantly higher in NV and crop rotation than in soils under monoculture systems. Although it was expected that differences in microbial activities would be due to changes in microbial community abundance, the results indicated that changes in soil management produced faster alterations to soil enzyme activities than any modifications induced in the microbial community structure. Consequently, both aspects of microbial diversity, namely function and structure, were affected independently by agricultural intensification.

Keywords: soil microbiology; phospholipid fatty acids; soil enzymes; microbial activity; sustainable management

In the last years, transgenic soybeans advanced on grazing areas that were finally transformed into permanent agriculture sites. In many world regions, especially in South American Chaco, this process of agriculturisation has generated a simplification of local production systems, characterized by a monoculture predominantly featuring soybean (Pérez-Brandán et al. 2014). For this reason, land use and land cover changes have become major issues for policymakers and are currently the subject of political disagreements (Volante et al. 2012).

Soil microorganisms mineralize, oxidize, reduce, and immobilize the mineral and organic materials in the soil, and these microbial activities are fundamental for plant growth (Wienhold et al. 2005). It is known that the implementation of crop rotation ameliorates soil structure, nutrient balance, pest regulation and crop productivity, as well as it improves soil (Bending et al. 2002, Costantini et al. 2006). Thus, quantifying soil microbiota by assessing the functional and structural diversity is an ideal tool for soil management evaluations, especially when physical and chemical parameters are involved in these studies (Pankhurst et al. 2003).

Although it is important to analyse the effects of crop management on soil microbial structure, this aspect is vast and difficult to measure, with resultant changes in the community structure being

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hard to interpret. For this reason, these effects should be related to microbial functions, by examining the key processes mediated by microbes. However, only a few studies have attempted to link the functionality of microorganisms with microbial structure.

The objectives of this investigation were to (i) assess the associations between microbial activities and community structure in the soil as a result of the intensification of agricultural systems under soybean monoculture and crop rotation; (ii) determine the relationship between soil microbial community function and structure, and chemical and physical parameters.

MATERIAL AND METHODS

Site description and soil sampling. This study was conducted at six soybean commercial sites located in Anta Department (Las Lajitas, Salta) of the north-west region of Argentina, which corresponds to the semiarid Chaco area, Argentina. The climate of the region is continental-subtropical, with little or no water deficit in January and February. The mean annual precipitation is 600–800 mm, concentrated in spring-summer, with a prolonged dry season in winter. The average annual temperature varies from 23°C in the north to 18°C in the south. Local native vegetation is a xerophytic subtropical forests dominated by *Aspidosperma quebracho-blanco*, *Schinopsis quebracho-colorado*, *Chorisia speciosa*, *Caesalpinea paraguariensis* and *Prosopis* spp. The dominant soil type of the region is loam (1.68% of organic carbon, 32% sand, 44% silt, 24% clay) Entic Haplusterts Ceibalito series with A, AC and C horizons (USDA Soil Taxonomy).

Different sites under soybean monoculture (continuous soybean) and soybean/maize rotation practices were selected. Samples were collected from agricultural soils under different periods of implantation: a 4-year rotation (R4); a 15-year rotation (R15); a 5-year monoculture (M5); and a 24-year monoculture (M24). A site of native vegetation recently under agricultural production (RUA) was also sampled. Native vegetation soils (NV) adjacent to agricultural sites were sampled as a control (Table 1). No-tillage operation was not performed in any plots during the growing seasons. The six treatments were carried out, each with three replicates, and six rhizosphere composite samples were taken per site, 30 m apart from each other in an area of 900 m^2 inside each agricultural site. Soil sampling was performed during the 2009–2010 and 2010–2011 agricultural cycles. Rhizosphere samples were collected and pooled, with roots being gently shaken to remove loosely adhering soil, before being placed in plastic bags and processed immediately.

Table 1. Chemical and physical properties of soils under different agricultural management systems (2009/10–2010/11)

	Agricultural soil			Pristine soil		
	M24	M5	R15	R4	RUA	NV
Soil chemical properties						
Organic carbon (%)	1.09^{d}	1.20^{c}	1.47^{b}	1.36^{c}	1.54^{b}	2.07^{a}
Total nitrogen (%)	0.10^{d}	0.12^{c}	0.14^{b}	0.12^{c}	0.16^{a}	0.17^{a}
Extractable phosphorus (mg/kg)	15.63^{d}	20.3^{c}	31.33^{b}	23.85^{c}	29.82^{b}	39.33^{a}
Water holding capacity (%)	25.16^{c}	25.44^{c}	28.67^{b}	26.84^{bc}	27.67^{b}	31.83^{a}
pH	6.80^{b}	7.12^{a}	7.11^{a}	6.72^{b}	7.03^{a}	5.9^{c}
Soil physical properties						
Soil aggregate stability (%)	15.73^{d}	22.67^{c}	22.11^{c}	22.7^{c}	29.00^{b}	36.20^{a}
Bulk density (g/cm^3)	1.68^{a}	1.55^{b}	1.32^{d}	1.42^{c}	1.36^{cd}	1.35^{cd}
Productivity						
Soybean yield (kg/ha)	2548^{c}	2634^{c}	4048^{a}	3584^{b}	3212^{b}	–

Data are means of 15 replicates/plot analysed. Means between treatments in the same row followed by the same letter are not significantly different according to the *LSD* test ($P \leq 0.05$). M – soybean monoculture; R – soybean/maize crop rotation; RUA – recently under agriculture; NV – native vegetation. The numbers 24, 5, 15, and 4 indicate years under the corresponding land-use intensification

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Microbial activities. To quantify soil microbial respiration (MR), potentially mineralizable C (CO_2-C respiration) was determined according to Alef (1995). Soil samples (10 g) were air-dried, sieved, and incubated with NaOH 0.2 mol/L at 28°C for 7 days. The release of CO_2 was analysed using HCl 0.2 mol/L, and control flasks without soil were also incubated with NaOH 0.2 mol/L at 28°C for 7 days.

General microbial activity was measured by hydrolysis of fluorescein diacetate (FDA), using a procedure of Adam and Duncan (2001). Briefly, 2 g of soil and 15 mL of 60 mmol potassium phosphate buffer pH 7.6 were placed in a 50-mL conical flask. Substrate (FDA, 1000 μg/mL) was added to start the reaction. The flasks were placed in an orbital incubator at 30°C and 100 rpm for 20 min. Once removed from the incubator, 15 mL of chloroform/methanol (2:1 v/v) was added immediately to terminate the reaction. The contents of the conical flasks were then centrifuged at 2000 rpm for 5 min. The supernatant was then filtered and measured at 490 nm on a spectrophotometer (Massachusetts, USA).

Dehydrogenase activity (DHA) was determined according to García et al. (1997). To carry this out, 1 g of soil at 60% of its field capacity was exposed to 0.2 mL of 0.4% INT (2-p iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22°C in darkness. The INTF (iodonitrotetrazolium formazan) formed was extracted with 10 mL of methanol by shaking vigorously for 1 min, before filtering through a Whatman N°5 filter paper and measuring spectrophotometrically at 490 nm.

Acid phosphatase (PHA) was assayed using 1 g soil, 4 mL 0.1 mol/L universal buffer (pH 6.5), and 1 mL 25 mmol p-nitrophenyl phosphate (Tabatabai and Dick 2002). After incubation at 37°C for 1 h, the enzyme reaction was stopped by adding 4 mL 0.5 mol/L of NaOH and 1 mL 0.5 mol/L of $CaCl_2$ to prevent the dispersion of humic substances. Absorbance was measured in the supernatant at 400 nm, and enzyme activity was expressed as micrograms of p-nitrophenol released per h/g/soil.

Microbial community structure. Phospholipid fatty acid (PLFA) analysis was carried out as described by Bossio et al. (2005). A standard nomenclature was used. Branched fatty acids i15:0, a15:0, i16:0, i17:0 and a17:0 were chosen to represent gram-positive bacteria, and the monoenoic and cyclopropane fatty acids 16:1ω9, 16:1ω11, cy17:0, 18:1ω9c, 18:1ω9t, and cy19:0 were selected to represent gram-negative bacteria. The fatty acids 10 methyl 18:0, 16:1ω9c and the polyenoic 18:2ω6, 9 were used as indicators of actinomycetes, arbuscular mycorrhizal fungi (VAM) and fungal biomass, respectively. Total microbial biomass (total PLFAs) was estimated as the sum of all the extracted PLFAs.

Soil chemical and physical properties. To analyse soil chemical properties, soil samples were air-dried and sieved (2 mm) to determine the organic carbon (OC) by wet oxidation, following the Walkley and Black procedure (Black 1965), with total nitrogen (TN) being quantified by the micro-Kjeldhal method (Bremner 1996). Other parameters also determined were: available phosphorus (Pe) using the Bray-Kurtz method (Bray and Kurtz 1945); pH, using a potentiometer in a 1:2.5 soil:water suspension; and electrical conductivity (EC) with a conductivity meter in a 1:2.5 soil:water suspension. The water holding capacity (WHC) was measured by the gravimetric method.

Aggregate stability (AS) was quantified following the method of micro-sieves (1–2 mm) according to Corvalán et al. (2002), and bulk density (BD) was measured by the core method (Blake and Hartge 1986), using cores of 3 cm in diameter, 10 cm in length, and 70.65 cm^3 in volume.

Statistical analysis. The statistical significance of the differences between treatments was assessed by the analysis of variance (ANOVA) and *LSD* (least significant difference). In addition, a principal component analysis (PCA) was performed to determine separation among treatments, and to identify the PLFAs, chemical, and physical variables that best contributed to the separations of treatments.

RESULTS AND DISCUSSION

Agricultural management: soil microbial activity, physical responses and crop yield. Soil MR is the most widely used parameter to evaluate microbial activity as a whole, as it shows the beneficial shifts produced by agricultural practices that allow microorganisms to increase their activities. In our study, MR had the lowest values in M24, R4 and RUA (Figure 1), suggesting that nutrient mobilization occurs in the soil matrix and is caused by the constant supply of plant material from the residue

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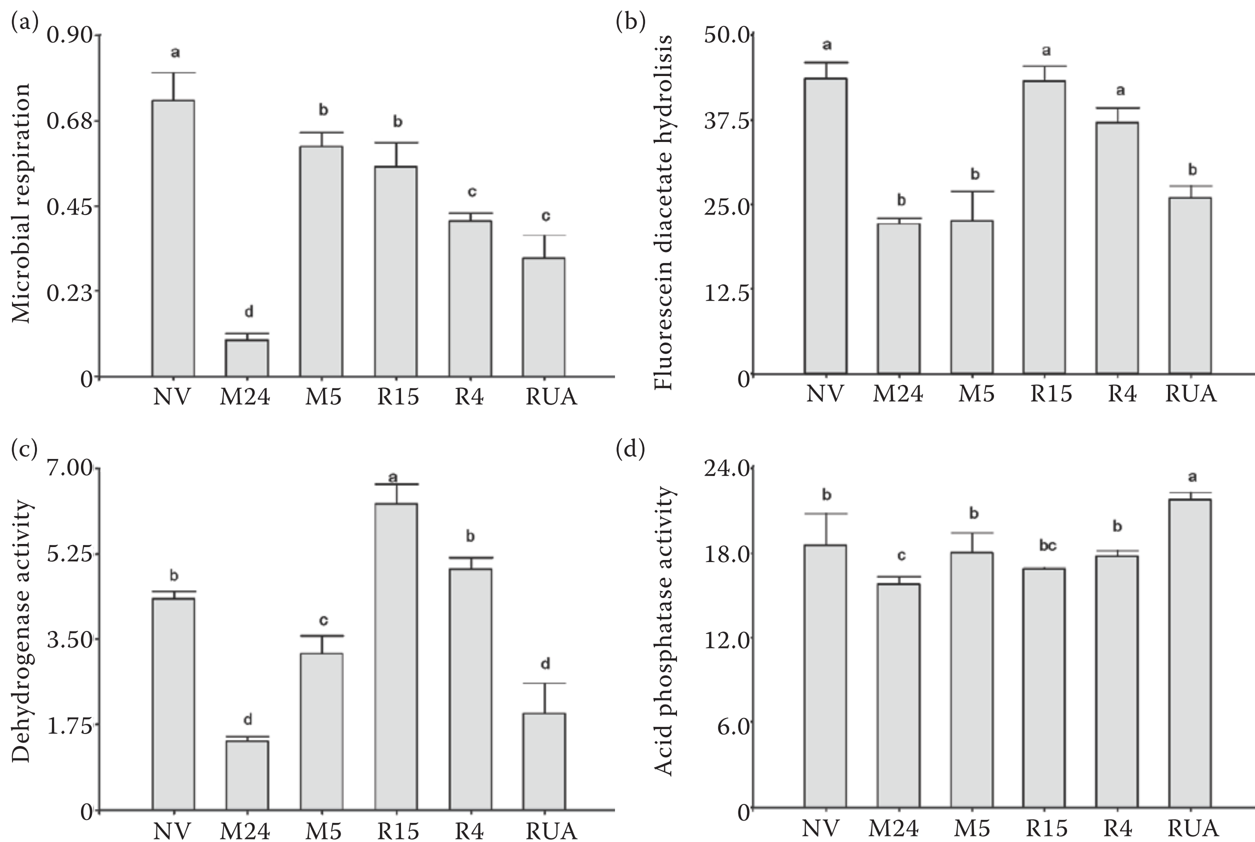

Figure 1. Soil microbial activities in a sandy loam soil under different agricultural management systems (2009/10–2010/11). M – soybean monoculture; R – soybean-maize crop rotation; RUA – recently under agriculture; NV – native vegetation. The numbers 24, 5, 15, and 4 indicate years under the corresponding land-use intensification. (a) Microbial respiration (mg CO_2/g soil/week); (b) fluorescein diacetate hydrolysis (µg fluorescein/g soil/h); (c) dehydrogenase activity (mg INTF (iodonitrotetrazolium formazan)/g soil/h), and (d) phosphatase activity (µmol p-nitrophenol/g soil). Bars with the same colour followed by the same letter are not significantly different according to the *LSD* test ($P \leq 0.05$)

left by the previous crop (particularly important in R15) or residue from the native vegetation in NV, with a shift in soil microbial activities usually corresponding to changes in soil nutrient sources (Spedding et al. 2004).

Microbial enzyme activities are also sensitive indicators of resource demands because their production increases in response to resource limitation (Sinsabaugh et al. 2009) (Figure 1). For example, FDA activity plays an important role as an indicator to measure the overall soil microbial activity, because lipases, proteases and esterases exhibit a high ubiquity in soils and are involved in the hydrolysis of FDA. In tropical forests, these enzymes decompose the complex biopolymers present in the soil matrix, with an increase in the activity of these enzymes in this type of system having been observed. This may explain why the FDA activity was higher in NV than in M24 or M5 (Figure 1), which further suggests that increased production of extracellular enzymes is not a limiting factor in this type of soil.

Although less pronounced, a similar trend was observed for DHA activity. It is widely known that DHA is susceptible to low pH (Velmourougane et al. 2013), then in NV this enzymatic activity was probably performed by different microbial groups than those found in the agricultural treatments, which may explain the lower values obtained in NV compared to crop rotation treatments (R15 and R4).

It is probable that the acidic characteristics of NV soils may favour the establishment of P solubilizing microorganisms due to the greater contribution of vegetal residues (Tiecher et al. 2012). Although we noted a different response in the PHA activity in RUA, this response may be due to changes in

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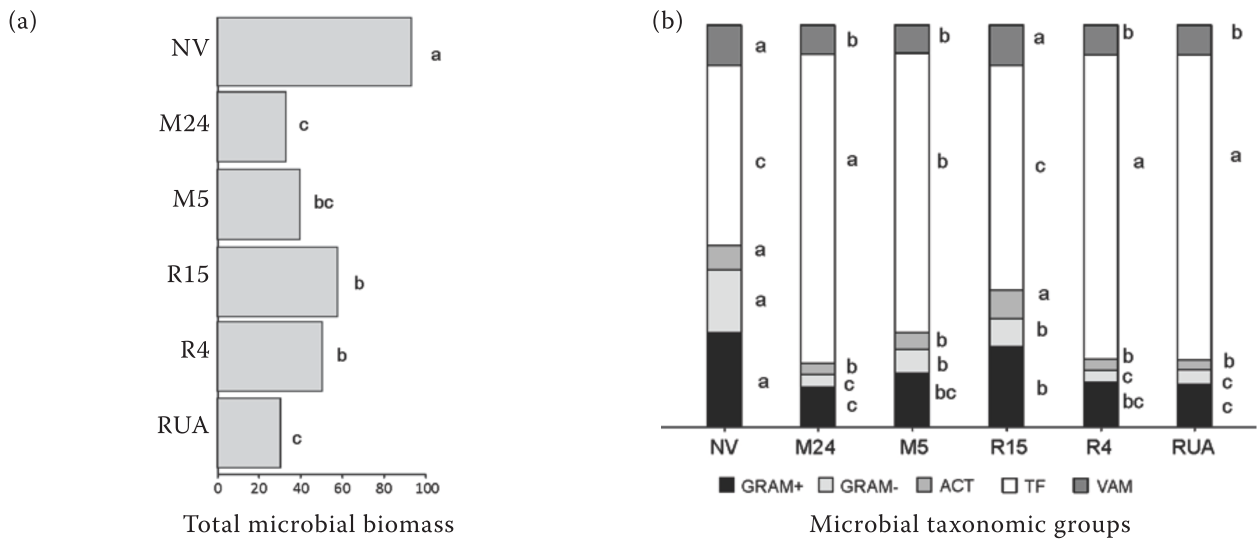

Figure 2. Phospholipid fatty acid (PLFA) profiles (a), total microbial biomass (nmol PLFA/g soil) and (b) molar percentages of specific microbial groups (nmol PLFA%/g soil) in a sandy loam soil under different agricultural management systems (2009/10–2010/11). M – soybean monoculture; R – soybean-maize crop rotation; RUA – recently under agriculture; NV – native vegetation. The numbers 24, 5, 15, and 4 indicate years under the corresponding land-use intensification. Error bars are standard errors. Bars with the same colour followed by the same letter are not significantly different according to the *LSD* test ($P \leq 0.05$). Gram+ – gram-positive bacteria; gram– – gram-negative bacteria; ACT – actinobacteria; TF – total fungi; VAM – vesicular-arbuscular mycorrhiza

the soil during the burning and deforestation of trees and shrubs of the native vegetation, which facilitated a relative and temporary increase of this enzymatic activity.

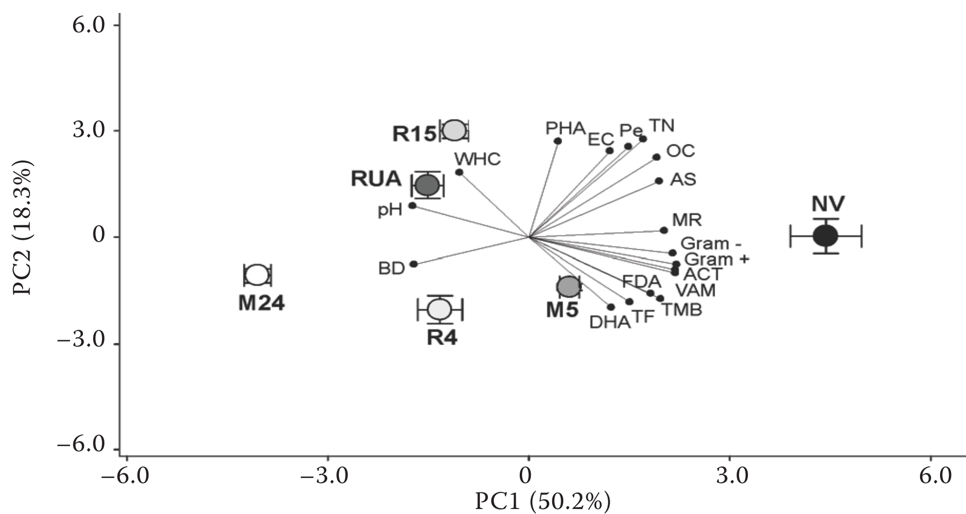

Figure 3. Principal component analysis (PCA) of the microbial activities, soil chemical-physical properties and phospholipid fatty acid data (PLFA) in a sandy loam soil under different agricultural management systems (2009/10–2010/11). Error bars are standard errors. M – soybean monoculture; R – soybean/maize crop rotation; RUA – recently under agriculture; NV – native vegetation. The numbers 24, 5, 15, and 4 indicate years under the corresponding land-use intensification. OC – organic carbon; TN – total nitrogen; Pe – extractable phosphorus; WHC – water holding capacity; EC – electrical conductivity; pH – soil pH; AS – soil aggregate stability; BD – bulk density; MR – microbial respiration; FDA – hydrolysis diacetate fluorescein; DHA – dehydrogenase activity; PHA – acid phosphatase activity; gram+ – gram-positive bacteria; gram– – gram-negative bacteria; ACT – actinobacteria; TF – total fungi; VAM – vesicular-arbuscular mycorrhiza; TMB – total microbial biomass

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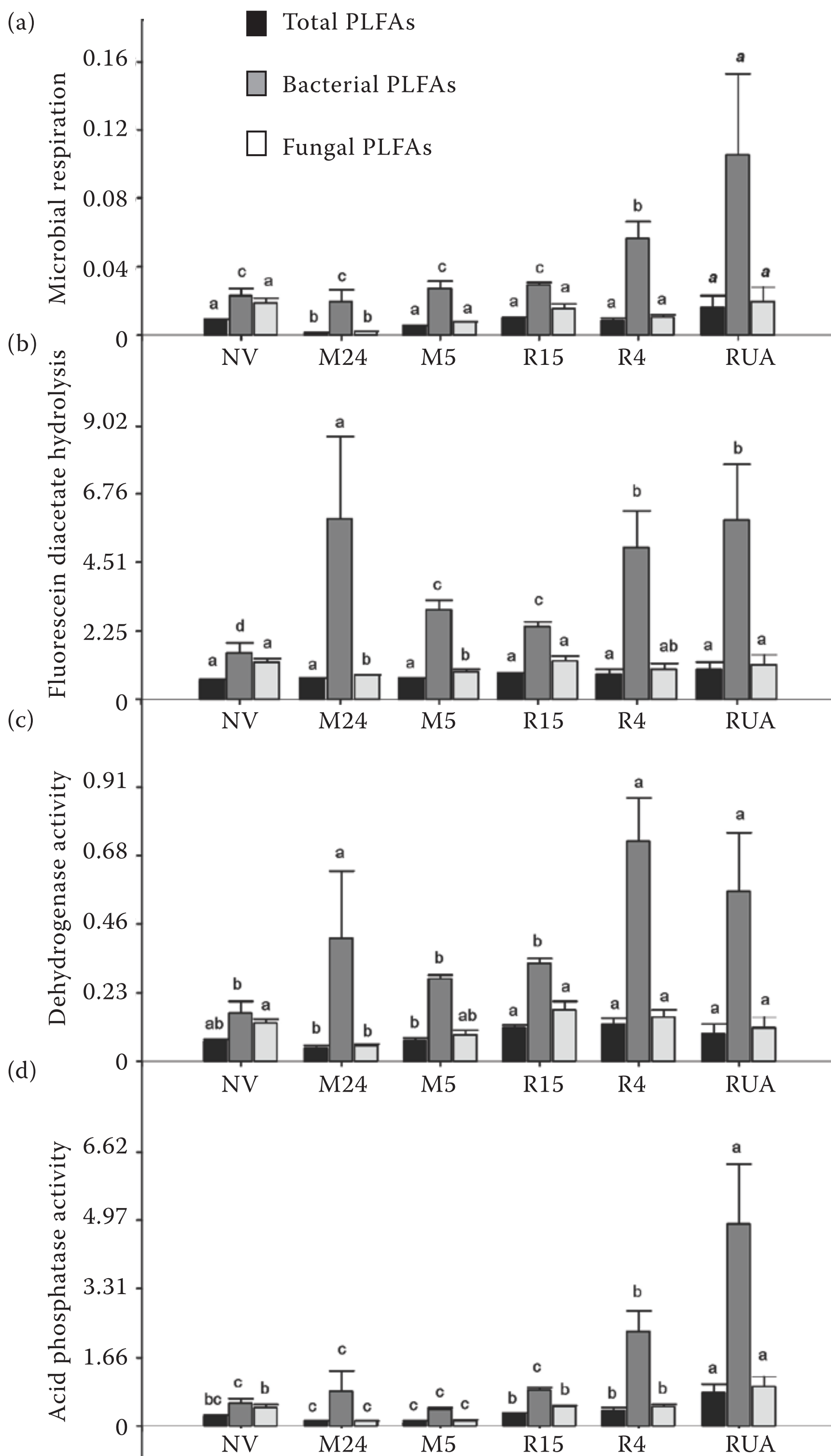

Figure 4. Ratios of enzyme activity to microbial biomass as indicated by phospholipid fatty acids (PLFAs) in sandy loam soil under different agricultural management systems (2009/10–2010/11). (a) Microbial respiration (mg CO_2/g soil/week); (b) dehydrogenase activity (mg INTF (iodonitrotetrazolium formazan)/g soil/h); (c) fluorescein diacetate hydrolysis (µg fluorescein/g soil/h), and (d) phosphatase activity (µmol p-nitrophenol/g soil). M – soybean monoculture; R – soybean/maize crop rotation; RUA – recently under agriculture; NV – native vegetation. The numbers 24, 5, 15, and 4 indicate years under the corresponding land-use intensification. Error bars are standard errors. Bars with the same colour followed by the same letter are not significantly different according to the *LSD* test ($P \leq 0.05$)

Considering soil physical aspects (Table 1), the results obtained showed that AS tended to be higher in the NV, with the opposite occurring with BD, which was significantly higher in M24. Chen et al. (2009) stated that soybean monoculture produces a great extraction of soil nutrients, a low intake of crop debris and soil compaction, thereby affecting the content of chemical and physical soil parameters. The retention of carbonaceous residues in soils can directly affect AS and BD, improving soil structure and increasing the diversity of soil microorganism populations and enzyme activities

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(Havlicek 2012). Finally, as expected, crop yield tended to be higher under soybean/maize rotation in comparison with continuous soybean.

Agricultural management: soil microbial community structure and chemical and physical responses. Our study showed differences in the structure of soil microbial communities in response to agricultural intensification, with total microbial biomass (total PLFAs) being more abundant in NV than in agricultural soils. Furthermore, crop rotation sites (R15 and R4) showed higher microbial biomass compared to continuous crop (M24 and M5), suggesting that the accumulation of maize residues in the soil surface have increased the microbial abundance. Both gram-positive and gram-negative bacteria showed the highest abundance in NV soils and R15 treatments, with the lowest values found in the M24 and RUA treatments (Figure 2). Our results are in agreement with those found by Bossio et al. (2005), who reported a greater abundance of microbial lipid groups in native vegetation soils and crop rotation soils compared with those under soybean monoculture. The R15 treatment also revealed the highest values of actinobacteria and arbuscular mycorrhizal-fungi, while the lowest abundance of total fungi was observed in NV, suggesting a change in the microbial population abundance with respect to the other treatments. As evidenced by the PC analysis, the soil chemical and physical parameters were also affected by intensive agriculture, with low values of OC, TN, AS, but high BD values, as reported elsewhere (Aziz et al. 2013) (Figure 3). Regarding these physical parameters, it was observed that the abundance of gram-positive bacteria, gram-negative bacteria, actinobacteria, vesicular-arbuscular mycorrhizae and total biomass rose with increasing AS values, with the opposite response being observed between BD and all microbial taxa. These results are consistent with those reported by Pankhurst et al. (2003), who indicated that the differences in the contributions of labile OC, TN and other nutrients provided by the debris and specific residues of each crop produced an increase in some soil microbial groups, thereby modifying the abundance of their communities (Montecchia et al. 2011).

Relationship between microbial activities and soil microbial community structure. The ratios between enzyme activities and microbial biomass were calculated to show the relative contribution of the stabilized enzyme activity associated with microorganisms (Nannipieri et al. 2012) (Figure 4). In general, the ratios of enzyme activities to total PLFAs were not statistically different between treatments, but in most cases were lower in the monoculture treatments, indicating a synchronous response of enzyme activities to agricultural intensification (Zhang et al. 2014). Furthermore, although the ratios of the enzyme activity to bacterial PLFAs did not show a clear trend, for most enzymes, the highest values were observed in RUA treatments. This suggests that bacterial communities are more resistant than fungi to changes in their environment, which permitted their growth under conditions of extreme disturbance. Finally, the ratios of microbial activity to fungal PLFAs were lower in monoculture systems than in NV and R treatments, indicating that the increase in enzyme activities was mainly derived from a rise in the enzyme activities associated with fungal microorganisms.

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