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Land-use change affects the functionality of soil microbial communities: A chronosequence approach in the Argentinian Yungas

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ABSTRACT

Land-use change has drastically reduced the area of pristine forests in tropical and subtropical regions. In NW Argentina, Yungas forests were among the most affected by deforestation for the implementation of agricultural crops. Conversion of forests to croplands modified the structure and function of soil microbial communities, but its effects on soil functionality across time after land-use change are understudied. Therefore, the objective of this study was to analyze the impact of land-use change and time under cultivation on the functionality of microbial communities in these soils. We established a 30-year old chronosequence comprising 4 stages (forest and short-, mid- and long-term agriculture) in 3 independent farms. Together with soil physicochemical properties, we measured microbial biomass carbon, basal respiration, ammonification, acid and alkaline phosphomonoesterase activities, and community-level physiological profiling (CLPP). During the first years of cultivation, the functionality and biomass of soil microbial communities were strongly affected. Compared to forest soils, short-term agricultural soils exhibited a reduction on microbial biomass (~45%), ammonification (~67%) and acid phosphomonoesterase activity (~41%). Moreover, increased basal respiration (up to 94%) and metabolic quotient in those soils suggested radical changes in functionality at the beginning of the chronosequence. However, CLPP evidenced that older agricultural sites had an increased global catabolic response per unit biomass, while it detected no differences in physiological diversity of soil microbial communities along the chronosequence. The lack of differences detected between mid- and long-term agricultural sites, in addition to a reduced inter-site variability, evidences an apparent stabilization and homogenization of soil microbial communities towards the end of the chronosequence. In contrast with physicochemical variables, microbiological variables had a greater performance for characterizing the different stages of the agricultural chronosequence and the impact of land-use change on soil functionality.

1. Introduction

In the last decades, agricultural and farming surface of tropical and subtropical regions has increased at the expense of deforestation of pristine forests, a phenomenon that is particularly relevant in South America, Africa and Asia (Achard et al., 2014; Food and Agriculture Organization of the United Nations, 2012). The most representative cases in Argentina take place in the ecoregions of Yungas and Chaco, where large areas of native forest have been removed and deforestation is still in progress (Gasparri and Grau, 2009; Viglizzo et al., 2011; Volante et al., 2012). Yungas forests, the southern limit of the Andean subtropical rainforests of South America, constitute one of the major hotspots of biodiversity (Myers et al., 2000). Within the Yungas, the lowest altitudinal vegetation level, also known as pedemontane forests, was the most affected by land-use change, mainly for sugarcane, citrus and, during the last decades, soybean production (Brown and Malizia, 2004; Gasparri et al., 2008). When forest lands are deforested and substituted by agricultural crops, and especially when monoculture-based systems predominate, vegetation is drastically modified and soils are exposed to radical changes in their below-ground environment, mainly in temperature, water balance, and carbon and nutrient dynamics (Don et al., 2011; Gasparri et al., 2008; Murty et al., 2002; Volante et al., 2012). What is more, land clearing

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and agriculture involve many mechanical and chemical disturbances which modify several aspects of soil physics, chemistry and, of course, its living communities.

Since microorganisms have a key role in soil functioning, disturbance will lead to concatenated effects on soil ecosystem processes and, thus, the ecosystem services provided (Brussaard, 2012). In the last years, concern in soil ecology has grown significantly and many authors have reported clear effects of land-use change (i.e., cultivated vs. adjacent pristine soils) on soil microbial communities, regarding their abundance, diversity and activity (e.g., Bossio et al., 2005; Nogueira et al., 2006; Upchurch et al., 2008; Montecchia et al., 2011; Navarrete et al., 2013; Rodrigues et al., 2013; Brackin et al., 2013). In particular microbial biomass, respiration and enzymatic activity were usually reduced in cultivated soils when comparing them with adjacent non-cultivated sites (e.g., Nogueira et al., 2006; Trasar-Cepeda et al., 2008a,b; Chaer et al., 2009; Bissett et al., 2011). However, the dynamics of soil microbial communities after land-use change and with increasing time under cultivation are understudied.

Yungas pedemontane forests are highly suitable to build a chronosequence scheme due to the progressive "colonization" of pristine lands by agricultural activity (Gasparri and Grau, 2009; Viglizzo et al., 2011). As space-for-time substitutions, chronosequences contribute to mimic temporal dynamics where long-term studies are not accessible (Walker et al., 2010) and, thus, would help to search for temporal patterns or identify critical stages in the response of soil microbial communities to land-use change. This approach has been widely applied in studies concerning soil formation or succession gradients, mainly in the field of aboveground ecology but also in soil biota (e.g.: Freedman and Zak, 2015; Kuramae et al., 2010; Orwin et al., 2006). Despite this, soil agricultural chronosequences, studying the effect of increasing time under cultivation, are scarce in the literature, probably due to the lack of suitable scenarios. This approach has been usually applied in studies of soil quality based on chemical and physical properties (Bahr et al., 2014; Lemenih et al., 2005; Moebius-Clune et al., 2011), while those based on the response of soil microbial communities have barely been carried out (An et al., 2008; Eleftheriadis and Turrión, 2014; Melo et al., 2012; Montecchia et al., 2015; Tischer et al., 2014).

In previous studies, we observed marked differences in structural and functional aspects of microbial communities when comparing agricultural and pristine soils from Yungas, which evidenced how they respond to deforestation for extensive cropping (Montecchia et al., 2015, 2011). Microbial communities from agricultural soils exhibited a modified genetic structure, lower viable biomass and diversity of phospholipid fatty acids, and increased respiratory response to several carbon substrates (Montecchia et al., 2011). Furthermore, when we analyzed the structure of bacterial communities from soils differing in time under cultivation, we found changes in community composition and also a decrease in phylogenetic beta diversity in long-term agricultural soils compared to forest soils (Montecchia et al., 2015). Now, in order to better understand the relationship between microbial structure and function, which remains a major gap in soil microbial ecology (Bissett et al., 2013), we focused on the functionality of microbial communities from those soils. Moreover, since our studies are observational and, thus, randomization is restricted to sampling and many factors cannot be manipulated, we decided to improve the reliability of our results with a more complete sampling design.

For all the above mentioned, we aimed to analyze the functionality of soil microbial communities inhabiting soils from a 30-year old agricultural chronosequence, where the predominant activity is soybean monoculture under no-tillage practices. The sampling design comprised four stages of the chronosequence (forest sites and short-, mid- and long-term agricultural sites) obtained in three different farms constituting true replicates of each situation. We focused on microbial functionality using tools that have been widely accepted to evaluate soil biological quality (Alef and Nannipieri, 1995; Bloem et al., 2005; Schinner et al., 2012). We assessed basal respiration, nutrient-releasing activities (ammonification and phosphomonoesterase activities) and community-level physiological profiling (CLPP), together with measurements of microbial biomass carbon. A multivariate approach was applied to analyze the relationship of these traits among themselves and with soil physicochemical data, in order to attain a more complete interpretation of the phenomena taking part in these soils. We hypothesized that the impact of deforestation and agricultural practices will reduce biomass and activity of soil microbial communities early in the agricultural chronosequence, when the soil environment is more dramatically modified. We expected that after many successive years of cropping under the same management microbial communities would adapt to the agricultural environment and microbial traits would stabilize after many years under the same cropping system, with the possibility of a recovery in some functional aspects, at least to some extent.

2. Materials and methods

2.1. Site description

The study area is located in the district of Anta (latitude 24°48′–24°52′S, longitude 64°11′–64°19′W, elevation 370–580 m a.s.l.), Salta province, northwestern Argentina. We selected three farms (F1, F2 and F3) located in the Yungas pedemontane forest, a transition area between the ecoregions of Yungas and Chaco that determines a W-E gradient in vegetation composition (Cabrera, 1976). The climate is subtropical with a dry season (in winter). Mid-temperature in summer is 26.5 °C and 14 °C in winter and annual precipitation reaches 600–750 mm.

According to USDA soil taxonomy, the dominant soils in the sampled area are Udic Argiustolls, Fluventic Hapludolls and Haplusterts (http://geointa.inta.gov.ar/visor). Spatial heterogeneity in soil properties is expectable, due to the fluvic origin of these soils. Slopes and strong summer rain can lead to erosion that can be accelerated after native vegetation is replaced by annual crops.

2.2. Soil sampling and physicochemical analyses

Samples were collected in early May 2011, after the wet season and at the end of summer crops. Soils from three farms were selected, in order to recreate three replicates of a chronosequence comprising forest, short-, mid- and long-term agriculture stages (0, 3–5, 11–14 and 28–30 years under cultivation, respectively) (Table 1). Agricultural sites were plots of 45 to 75 ha, cultivated with soybean (*Glycine max* (L.) Merr.) at the moment of sampling, while pristine, non-disturbed, sites were non-deforested areas adjacent to agricultural plots. Sampling was carried out following previous schemes (Montecchia et al., 2011). Briefly, in each agricultural or pristine site, 5 sub-sites (each of them a composite sample of a delimited ~0.25 m^2 area) were selected along a diagonal transect, saving *ca.* 70 m between sub-sites. Composite samples from each sub-site consisted of 10 soil cores (15 cm depth, 5 cm diameter) collected from inter-row zone, previous removal of organic litter. In pristine sites, samples were taken avoiding the presence of plants or plant roots. Due to the intrinsic heterogeneity of soils, within each site, samples were withdrawn from a subplot with homogeneous characteristics at glance (slope, position in the landscape, etc.) in order to limit the variability of soil properties.

Soil samples were sieved through a 2 mm mesh and then stored at 4 °C until biological analysis. Composite samples of each site

Table 1
Description of sampling sites with different land uses from three farms located in Salta province, NW Argentina.

Farm	Land use	Description	Coordinates and elevation (m a.s.l.)
F1	Forest	Pristine pedemontane forest	24°52'26.4"S 64°12'10.6"W, 441 m
	Short-term agriculture	Soybean monoculture for 3 years	24°51'49.0"S 64°14'51.7"W, 487 m
	Mid-term agriculture	Soybean monoculture for 13 years	24°52′38.6"S 64°12′00.3"W, 441 m
	Long-term agriculture	30 years agricultural use, soybean monoculture during the last 26 years	24°52'30.5"S 64°11'38.0"W, 514 m
F2	Forest	Pristine pedemontane forest	24°51'59.4"S 64°19'09.9"W, 540 m
	Short-term agriculture	Soybean monoculture for 5 years	24°51'39.8"S 64°19'02.6"W, 503 m
	Mid-term agriculture	Soybean monoculture for 11 years	24°51′41.0"S 64°18′44.5"W, 546 m
	Long-term agriculture	30 years of agricultural use, soybean monoculture during the last 23 years, rotation with maize 2 years ago	24°52'34.5"S 64°19'00.2"W, 538 m
F3	Forest	Pristine pedemontane forest	24°48'43"S 64°18'11"W, 577 m
	Short-term agriculture	Soybean monoculture for 5 years	24°48'32"S 64°18'05"W, 574 m
	Mid-term agriculture	Soybean monoculture for 14 years	24°48'02"S 64°16'06"W, 503 m
	Long-term agriculture	30 years agricultural use, soybean monoculture during the last 26 years	24°48'41"S 64°11'49"W, 464 m

were air-dried to carry out a routine physicochemical analysis. Soil organic C (SOC), total N, extractable P, exchangeable cations, texture, pH, electrical conductivity (EC) and water holding capacity (WHC) were measured by a commercial laboratory (Laboratory of Soil and Water, INTA-Salta) using standard methods (Sparks et al., 1996). To estimate labile carbon content, we used K_2SO_4-extractable carbon data obtained from non-fumigated samples from the microbial biomass carbon assay. The main physicochemical properties of soils are summarized in Table 2.

2.3. Microbial biomass carbon and respiration

Microbial biomass carbon (MBC) was measured according to the fumigation-extraction method (International Organization for Standardization, 1997; Vance et al., 1987). Fumigated and non-fumigated samples were measured in triplicate and MBC ($\mu g\,C\,g^{-1}$ dry weight soil) was calculated as the difference between fumigated and non-fumigated samples, using a conversion factor (kEC) of 0.38 (Joergensen, 1996).

Microbial basal respiration (BR) was measured according to the method of Isermeyer (Alef, 1995a; International Organization for Standardization, 2002) with slight modifications. Briefly, 25 g of soil at 66% WHC were incubated for 72 h at 25 °C in darkness in a sealed jar containing a vial with NaOH. After incubation and titration with HCl, BR ($\mu g\,CO_2\,g^{-1}$ dry weight soil d^{-1}) was calculated as the difference between blanks without soil and the average value of the triplicates for each sample.

2.4. Ammonification and phosphomonoesterase activities

Ammonification (Ammo) was measured under anoxic conditions according to Kandeler (1996a,b). In the absence of oxygen, nitrification is inhibited and accumulated ammonia can be used as an estimator of potential nitrogen mineralization. Briefly, soil samples (5 g) were incubated in waterlogged conditions for 1 week at 37 °C. At the end of the incubation period, ammonia was extracted with 1 M KCl, subjected to a modified Berthelot reaction and quantified in spectrophotometer at A_{660} (Kandeler, 1996b).

Table 2
Physicochemical properties of soils from forest sites and cultivated sites with short-, mid- and long-term agricultural history belonging to three farms from Salta province, NW Argentina. For site descriptions see Table 1.

Soil parameter[a]	Farm F1				Farm F2				Farm F3				Mean values			
Textural class	forest Silt loam	short Silt loam	mid Loam	long Loam	forest Loam	short Loam	mid Loam	long Sandy loam	forest Loam	short Loam	mid Sandy loam	long Sandy loam	forest	short	mid	long
Sand (%)	22	38	33	47	48	40	38	56	34	40	65	64	34.8 b	39.3 ab	45.3 ab	55.7 a
Silt (%)	55	50	46	37	39	44	49	33	49	46	25	27	47.7 a	46.7 a	40.0 a	32.3 a
Clay (%)	23	12	21	16	13	16	13	11	17	14	10	9	17.5 a	14.0 a	14.7 a	12.0 a
WHC (%)	43	32	31	28	33	32	33	29	29	30	25	26	35 a	31 ab	30 ab	28 b
pH	5.7	8.0	6.5	6.8	7.5	6.2	7.5	6.6	7.0	7.3	6.5	6.7	6.7 a	7.2 a	6.8 a	6.7 a
EC ($mmhos\,cm^{-1}$)	0.44	0.80	0.24	0.36	0.92	0.28	0.80	0.16	1.08	1.04	0.16	0.32	0.8 a	0.7 a	0.4 a	0.3 a
SOC (%)	2.53	2.11	1.30	1.34	2.03	1.73	1.57	1.27	3.66	2.73	1.17	0.97	2.7 a	2.2 ab	1.3 b	1.2 b
Labile C (ppm)	43	118	42	60	42	101	79	50	123	146	63	70	69 b	121 a	61 b	60 b
Total N (%)	0.20	0.18	0.14	0.10	0.18	0.17	0.14	0.10	0.37	0.27	0.10	0.07	0.25 a	0.21 a	0.13 b	0.09 b
C:N	12	12	9	13	11	10	11	12	10	10	12	13	11 a	11 a	11 a	13 a
P (ppm)	62	55	31	29	45	16	26	8	119	114	5	12	75 a	62 a	21 a	16 a
K ($meq\,kg^{-1}$)	15.0	18.5	13.6	12.2	10.7	9.1	2.4	8.2	5.9	3.2	6.8	5.8	1.1 a	1.0 a	0.8 a	0.9 a

For each variable, different letters denote significant differences among mean values ($P < 0.05$).
[a] WHC: water holding capacity; EC: electrical conductivity; SOC: soil organic C.

Ammonification was expressed as μg $N\text{-}NH_4^+$ g^{-1} dry weight soil d^{-1}, according to calibration curves. Blanks were performed with samples stored at −20 °C during the whole incubation period.

Acid and alkaline phoshomonoesterase activites (AcP and AkP, respectively) were determined according to Dick et al. (1996) using *p*-nitrophenyl phosphate as a substrate. Yielded *p*-nitrophenol (μg g^{-1} dry weight soil h^{-1}) was determined by A_{410} using calibration curves. Assays were carried out in triplicate, and two blanks, without soil and without substrate, were run.

2.5. Physiological profiling of soil microbial communities

Community-level physiological profiling (CLPP) (Garland and Mills, 1991) was carried out using BIOLOG EcoPlates following the manufacturer's instructions. Conditioning of soil samples, pre-incubation, loading plates and all procedures were performed according to Correa et al. (2009). EcoPlates were incubated at 28 °C and the A_{590} was measured with a Multiskcan1 EX (Thermo) at 0, 24, 48, 72, 96, 144 and 168 h. Absorbance values were blanked against the control well without any C source, values equal or less than 0.25 were considered as zero activity. After preliminary analysis, data taken at 48 h were retained for further analyses since they were representative of all the observations in the 48–168 h period. For profiling analysis, we carried out preliminary analysis with data from individual C-sources but then, to improve visualization, we grouped them in carbohydrates, carboxylic acids, amino acids, amines, polymers, phenolic acids and miscellaneous (Campbell et al., 1997). AWCD (average well color development) was calculated as mean substrate utilization for each sample. As AWCD represents the utilization of different C-sources by soil microbial communities, we used it to estimate potential metabolic activity.

2.6. Data analysis

To improve data interpretation we calculated the metabolic quotient or qCO_2 (BR:MBC) and the MBC per unit SOC ratio (MBC:SOC) (Anderson, 2003), along with other ratios relating microbial activity with MBC, SOC and soil N content. We also calculated diversity indices from CLPP data with the software PAST (Hammer et al., 2001): Richness (number of utilized C-sources), Shannon-Weaver (H), Simpson (1-Dominance), Evenness ($e^{H/S}$) and Equitability (H richness^{-1}).

All data was analyzed using R 3.0.1 (R Core Team, 2014). Mixed-effects models (package 'nlme' - Pinheiro et al., 2015) were used to evaluate biochemical variables individually; the 'site' (years under agricultural use) factor was loaded as a fixed effect, and both 'farm' and 'sub-site' were treated as random effects. Nesting was considered following the hierarchy 'farm > site > sub-site'. Then one way ANOVA and *post hoc* Tukey test (package 'multcomp'-Hothorn et al., 2008) were run.

Separate multivariate analyses were carried out for biochemical, CLPP and physicochemical datasets using principal component analysis (PCA). For soil physicochemical variables, after preliminary analyses that included the whole dataset, the data matrix was modified in the following way: SOC, total N and extractable P contents were summed up in a single variable (CNP), texture was represented only by sand content, and K was excluded from the analysis due to high co-linearity with clay content. To evidence the relationship between biotic and abiotic variables we calculated Pearson correlation coefficients with the software PAST (Hammer et al., 2001).

3. Results

3.1. Soil physicochemical properties

Five soil physicochemical properties out of twelve exhibited changes along the chronosequence (Table 2). In terms of soil texture, long-term agricultural soils showed ~60% higher sand content and ~20% lower WHC than pristine soils ($P = 0.037$ and 0.041). No differences were detected between the other land-use categories. Mean SOC content was 23–74% smaller in mid- and long-term agricultural soils in comparison to the pristine soils ($P = 0.007$ and 0.002, respectively). Similarly, total N was 14–73% lower in mid- and long-term agricultural soils than in pristine ($P = 0.001$) and short-term agricultural soils ($P = 0.01$). Finally, labile C, despite being highly variable within sites, was significantly higher in short-term agricultural soils. The latter differed mainly from mid- and long-term cultivated soils, being around 50% higher

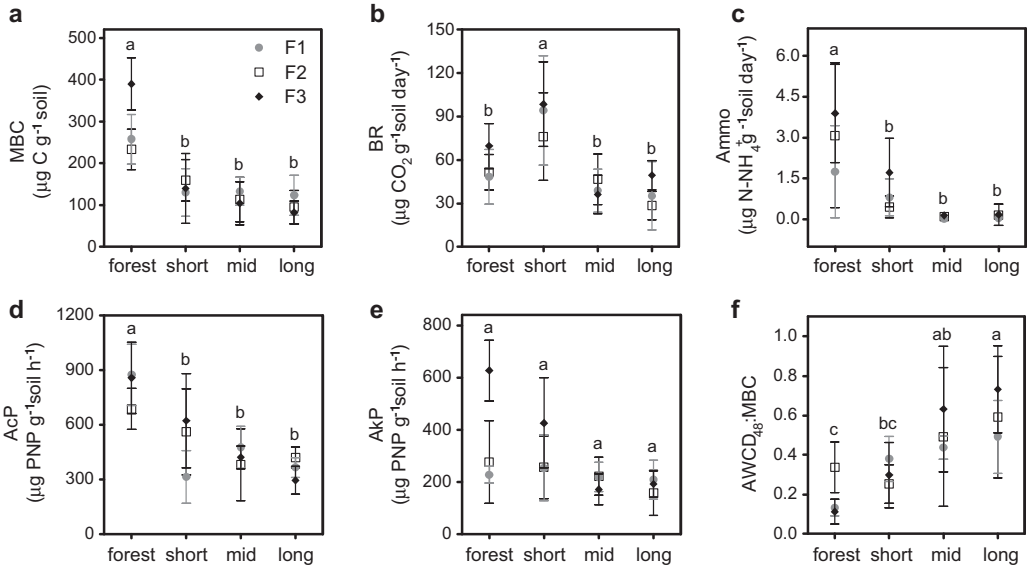

Fig. 1. Biological properties of soils from forest sites and cultivated sites with short-, mid- and long-term agricultural history belonging to three farms (F1, F2 and F3) from Salta province, NW Argentina. (a) microbial biomass carbon (MBC), (b) basal respiration (BR), (c) ammonification (Ammo), (d) acid phosphomonoesterase activity (AcP), (e) alkaline phosphomonoesterase activity (AKP) and (f) potential metabolic activity from CLPP data ($AWCD_{48}$:MBC) Mean values with different letters are significantly different between land uses. Error bars represent the standard deviation of the mean.

($P = 0.017$ and 0.008, respectively), but also from the pristine soil ($P = 0.037$).

3.2. Microbial biomass carbon and respiration

Microbial biomass carbon (MBC) in all agricultural soils was 20–50% of the values found in their respective pristine soils ($P < 0.001$, Fig. 1a). Although pristine soils from different farms had different MBC levels (F3 > F1 $P = 0.014$, F3 > F2 $P = 0.003$), once agriculture was implemented there were no detectable differences among soils with different years under cultivation or among farms, thus remaining stable at low values. MBC:SOC tended to decline during the first agricultural period, although it appeared to recover and stabilize in mid- and long- agricultural sites (Table 3).

Microbial basal respiration (BR) was significantly higher in short-term agricultural soils ($P < 0.001$), and this pattern was more pronounced in F2 farm (Fig. 1b). BR in mid- and long-term agricultural sites, on the other side, was on average just slightly smaller to the pristine soils values. In all farms, short-term agricultural plots exhibited the highest intra-site variability. No differences were found between mid- and long-term agriculture sites or between farms within these two agricultural stages.

qCO_2 (BR:MBC) data showed high variability in all cultivated soils, particularly in short-term sites (Table 3). Nonetheless, we were able to find that qCO_2 peaked in short-term agricultural soils compared to the forest and long-term sites ($P < 0.001$ and $P = 0.039$, respectively). In farms F1 and F3, qCO_2 was also higher in short- than in mid-term sites (data not shown). Finally, mid- and long-term agricultural soils showed similar qCO_2 values that tended to be higher than those from forest soils.

3.3. Ammonification and phosphomonoesterase activities

Pristine soils exhibited higher potential ammonification activity (Ammo) than all agricultural soils, while all mid- and long-term agricultural sites showed almost negligible Ammo values (Fig. 1c). Soils with high Ammo were also the ones with higher initial ammonia levels (data not shown). Ammo:SOC and Ammo:total N behaved similarly to Ammo, being higher in pristine soils than in cultivated soils ($P < 0.003$ in all cases, Table 3). Ammo:MBC was also higher in pristine soils but dropped significantly in mid- and long-term agricultural sites ($P = 0.0181$ and 0.0196, respectively) and not in short-term sites (Table 3).

Similarly to ammonification, acid phosphomonoesterase activity (AcP) fell in response to conversion to agriculture ($P < 0.01$) and remained at similar values regardless of the time extension of cultivation (Fig. 1d). In farm F1, the significant decrease was observed immediately after conversion while in farms F2 and F3 after short-term cultivation, the latter showing high intrinsic variability. Regarding alkaline phosphomonoesterase activity (AkP), we found no response to time under agriculture (Fig. 1e). However, we observed a clear decreasing pattern in F3 farm, which, analyzed separately, showed higher AkP in pristine than in mid- and long- term agricultural soils ($P < 0.001$). Both enzyme activities were not only lower but also less variable in soils with a long-term agricultural history. Regarding specific enzymatic activities (per unit SOC and per unit MBC), both AcP and AkP showed no response to land-use and time under cultivation except for AkP:MCB when forest and short-term sites were compared (Table 3).

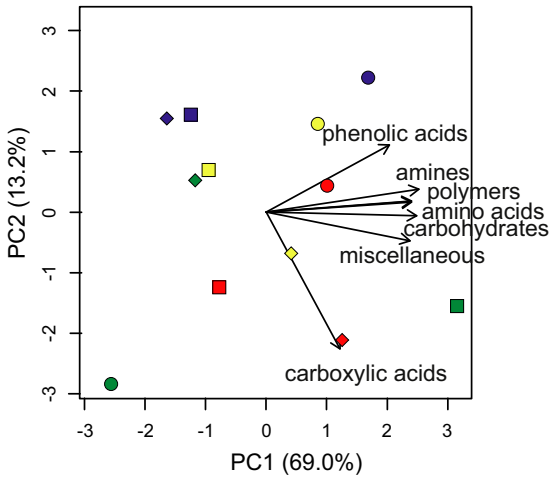

Fig. 2. Principal component analysis (PCA) of community-level physiological profiling of soils from forest sites (green symbols) and cultivated sites with short- (yellow), mid- (blue) and long-term (red) agricultural history, belonging to three farms: F1 (circles), F2 (squares) and F3 (diamonds). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. Physiological profiling of soil microbial communities

The AWCD raw data did not show a common response to land use and presented high intra- and intersite variability, particularly in older agricultural sites (data not shown). However, AWCD:MBC ratio did show a pattern and was higher in mid- and long-term agricultural soils than in forest soils (Fig. 1f). Additionally, long-term agricultural soils had higher AWCD:MBC than short-term agricultural soils.

PCA of physiological profiles using data from the 48 h measurement is shown in Fig. 2. Since all variables had positive eigenvectors for PC1 (and, thus, were relatively collinear among each other), ordination was mostly determined by global substrate utilization. Regarding site ordination, we could not detect any clustering according land use or farms. However, in F1 and F2

Table 3
Calculated ratios of biological properties from forest soils and cultivated soils with short-, mid- and long-term agricultural history.

	Ratio[a]								
	qCO_2 = BR:MBC ($\mu g\ CO_2\ mg^{-1}\ h^{-1}$)	MBC:SOC (%)	AcP:SOC ($\mu g\ PNP\ mg^{-1}\ h^{-1}$)	AcP:MBC ($\mu g\ PNP\ \mu g^{-1}\ h^{-1}$)	AkP:SOC ($\mu g\ PNP\ mg^{-1}\ h^{-1}$)	AkP:MBC ($\mu g\ PNP\ \mu g^{-1}\ h^{-1}$)	Ammo:SOC ($\mu g\ N\text{-}NH_4^+\ mg^{-1}\ d^{-1}$)	Ammo:MBC ($ng\ N\text{-}NH_4^+\ \mu g^{-1}\ d^{-1}$)	Ammo:total N ($\mu g\ N\text{-}NH_4^+\ mg^{-1}\ d^{-1}$)
forest	8.70 b	1.02 a	30.60 a	3.05 a	13.30 a	1.33 b	0.11 a	10.55 a	1.21 a
short	35.30 a	0.64 a	24.10 a	4.17 a	14.10 a	2.77 a	0.04 b	8.72 ab	0.45 b
mid	20.90 ab	0.84 ab	32.30 a	4.34 a	15.20 a	2.13 ab	0.01 b	1.05 b	0.08 b
long	19.50 b	0.80 ab	30.30 ab	4.42 a	15.90 a	2.45 ab	0.01 b	1.45 b	0.16 b

[a] qCO_2: metabolic quotient; BR: basal respiration; MBC: microbial biomass C; SOC: soil organic C; AcP: acid phosphomonoesterase activity; AkP: alkaline phosphomonoesterase activity; Ammo: ammonification. Mean values for three farms are shown. Different letters show significant differences among land-use categories for each variable ($P \leq 0.01$).

farms, the three agricultural sites were grouped and distant from the corresponding forest site along PC1 (69.0%). This was not observed for F3 farm, where the location of sites suggested gradual changes along the chronosequence.

Physiological diversity of soil microbial communities did not differ along the chronosequence, with no significant changes in the calculated diversity indices (Table S1).

3.5. Multivariate interactions among biotic and abiotic traits

PCA obtained from biotic variables displayed a clear grouping of sites according to land use (Fig. 3a). Sites were distributed along PC1 (73.4%) according to the chronosequence, with mid- and long-term agricultural sites clustered together. PC1 was mainly explained by AWCD:MBC and Ammo, the latter with higher levels in pristine soils. MBC and AcP were highly collinear and positively associated with Ammo. PC2 (15.9%) was mainly explained by BR and, thus, short-term agricultural sites were located towards positive values of this axis. This variable was not correlated with the other biological traits included in the analysis.

In contrast to biological properties, the PCA of soil physicochemical properties displayed a diffuse ordination of sites according to land use, except for the clustering of long-term agricultural sites (Fig. 3b). PC1, mainly determined by CNP (SOC, N and P contents) followed by EC, explained 46.3% of the total variability. PC2 (28.6%) appears to represent differences in pH, sand and WHC between soils.

Pearson correlation coefficients between both sets of variables (biotic and abiotic) are shown in Table S2. Excepting AWCD, all biological variables were positively correlated with SOC, P and total N. AWCD:MBC was related to sand content, WHC and C:N. BR, and also AkP, showed a positive correlation with labile C. These two biological variables, together with Ammo, were positively correlated with EC. Finally, AcP, besides being positively correlated to SOC, was negatively associated to sand content.

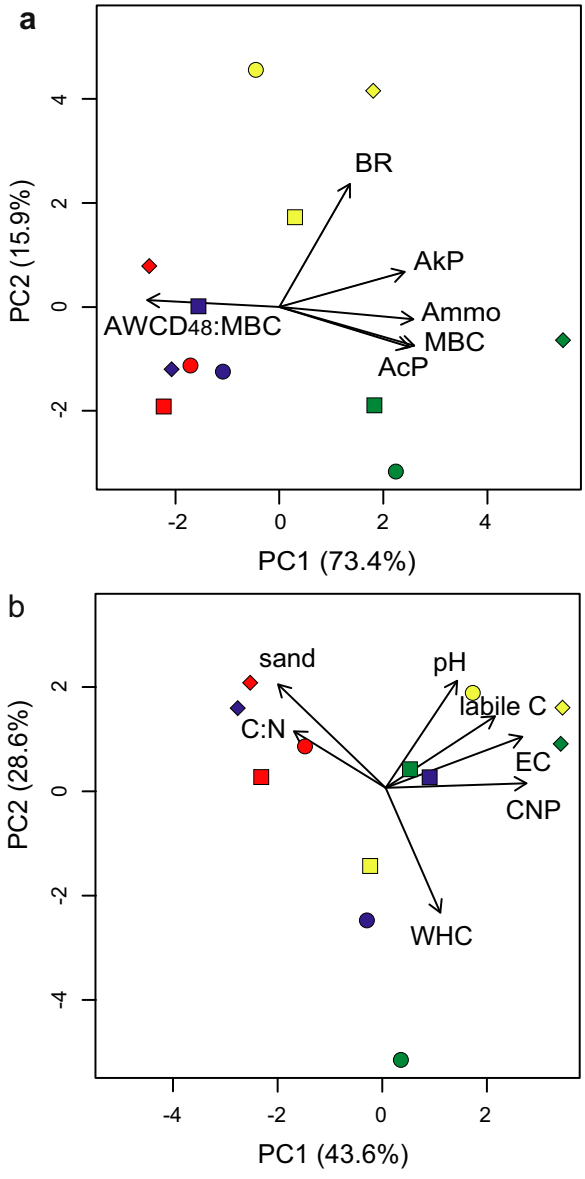

Fig. 3. Principal component analyses of biological (a) and physicochemical (b) properties of soils from forest sites (green symbols) and cultivated sites with short- (yellow), mid- (blue) and long-term (red) agricultural history, belonging to three farms: F1 (circles), F2 (squares) and F3 (diamonds). Symbols represent unique values for physicochemical data and centroids for biological data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Our agricultural chronosequence approach evidenced that soil microbial biomass was more affected by deforestation and land-use change than time under cultivation. Despite the different MBC content found in the three pristine soils, once agriculture was implemented values among cultivated sites were fairly similar. In the soils we analyzed, there seems to be no further decrease of MBC below ~77–126 $\mu g\,C\,g^{-1}$ soil, even under the continuous disturbance imposed by agriculture. This range could be a threshold MBC value for agricultural soils in this area, which could result from an intrinsic mechanism of soils, like physical protection of labile and microbial carbon (Six et al., 2002), or from a microbial strategy to overcome stressful conditions. This threshold could also be caused by management practices, since land managers implement no-tillage after two years of conventional tillage that follow deforestation. A *meta*-analysis by Kaschuk et al. (2010) indicated that no-tillage could reduce disturbance to a level that does not affect the remaining MBC or even promote its accumulation. Remarkably, in our study MBC levels remained stable in the agricultural chronosequence between 3 and 30 years of agriculture. In studies carried out in South Africa and Greece, MBC was also stabilized in agricultural soils, but the threshold value was reached after 20–25 years of agriculture (Dominy et al., 2002; Eleftheriadis and Turrión, 2014). This could be explained by intrinsic differences between soils and land management, but also by climate: compared to Mediterranean climate, in subtropical regions, disturbance caused by agricultural practices would be enhanced by higher temperature and water content in soils by leading to higher mineralization rates and, thus, earlier losses in MBC and other SOC fractions.

Even though MBC could increase during the first months after deforestation, due to an increase in available carbon and nutrients (Feigl et al., 2008), in the majority of studies microbial biomass eventually decreases to similar or lower values than in the forest soils (Brackin et al., 2013; Frazão et al., 2010; Islam and Weil, 2000; Kaschuk et al., 2010; Pérez-Brandán et al., 2014; Trasar-Cepeda et al., 2008b). That reduction in microbial populations could be a consequence of the changing soil environment and the disturbances caused by agricultural practices. In fact, in our study, disturbance is higher during the first two years, when conventional tillage is applied. Nevertheless, land-use change does not always lead to losses in MBC; for example, forest conversion to pastures appeared to have no detrimental effect (Frazão et al., 2010;

Sparling et al., 1994) or even to increase (Islam and Weil, 2000) MBC in soils.

SOC also decreased with land-use change, as reported by other authors (Eleftheriadis and Turrión, 2014; Islam and Weil, 2000; Murty et al., 2002), although it fell later in the chronosequence than MBC. It is widely recognized that MBC is more sensitive than SOC to predict changes in soil biogeochemical cycles and carbon flows (Gil-Sotres et al., 2005; Henrot and Robertson, 1994; Kaschuk et al., 2010; Sharma et al., 2004). In our case, MBC seems to predict the losses in SOC we observed in mid-term agricultural plots, as proposed by other authors (Insam and Domsch, 1988). Land-use pressure might determine the organic matter losses in soil we observed after many years of extensive cropping. In fact, it was widely observed that deforestation followed by grasslands, as opposed to croplands, generally have a lower impact in SOC stocks (Don et al., 2011; Murty et al., 2002). Coincidentally, in another study carried out in Yungas which analyzed forests soils converted to pastures, the losses of SOC were smaller than those we observed (Ripley et al., 2010).

To inquire on the functionality of soil microbial communities, we first measured BR as an estimator of global heterotrophic activity (Alef, 1995a). While we found an increased BR in short-term agricultural soils, many studies found that BR is lower in agricultural soils compared to forest soils (Anderson and Domsch, 1990; Eleftheriadis and Turrión, 2014; Islam and Weil, 2000; Trasar-Cepeda et al., 2008b). In our chronosequence, the observed increase in BR cannot be explained by microbial biomass since MBC was lower in short-term than in forest sites. Moreover, the relative changes in BR and MBC determine an increased qCO_2 in short-term agricultural soils. A similar result was found in tropical forests from Bangladesh, where soils with around 12 years under agriculture had higher qCO_2 than pristine soils (Islam and Weil, 2000). In another study, 1–2 year old *Brachiaria* pastures had higher qCO_2 than native forest and older pasture soils, which the authors attributed to a disequilibrium and re-stabilization of microbial populations (Melo et al., 2012).

Higher qCO_2 values could be explained by microbial communities with dominance of *r*-strategists (copiotrophs) over *K*-strategists (oligotrophs) (Montecchia et al., 2011), an expected disequilibrium in disturbed environments like the one resulting from deforestation and agricultural activity (Anderson, 2003). In fact, in a previous work studying the structure of bacterial communities in the same soils, Betaproteobacteria, a phylum that comprises many copiotrophic taxa, appeared as an indicator of short-term agricultural soils (Montecchia et al., 2015). This is a clear link between structure and function of soil microbial communities, where functionality is at least partially explained by changes in structure. Other studies also attributed high qCO_2 to a predominantly copiotrophic microbial community but they explained this as a consequence of a great supply of readily degradable carbon (Dinesh et al., 2003; Leirós et al., 2000). This hypothesis is in agreement with the fact that deforestation liberates nutrients and labile C in soil and, actually, labile C was increased in short-term agricultural soils from our study. Microbial communities with low C-utilization efficiency can eventually lead to SOC losses, and this is consistent with the fact that SOC decreased in agricultural soils after the observed increase in microbial basal respiration.

Anaerobic ammonification is considered a good estimator of the whole N mineralization process (Kandeler, 1996a; Nannipieri and Paul, 2009), as it is carried out by a large number of heterotrophic microorganisms under aerobic or anaerobic conditions (Alef, 1995b). Forest soils exhibited the highest Ammo rates, although with high intra-site variability, while mid- and long-term agricultural soils had almost undetectable levels. In agreement, in southern Brazil soils, higher ammonification rates were measured in native forest and reforested soils than in cultivated soils (Nogueira et al., 2006). Thus, agricultural soils appear to have lost traits associated with N mineralization.

Acid and alkaline phosphomonoesterases, commonly known as phosphatases, are intra- and extracellular enzymes that catalyze the hydrolysis of organic forms of phosphorus into phosphates. In our soils, only AcP responded to deforestation and time under cultivation. Higher AcP in forest soils was also found in other soils from the same region (Tosi et al., 2010) and in tropical soils from Kenya and the Caribbean (Acosta-Martínez et al., 2007; Bossio et al., 2005), suggesting that land-use change affects the capability of soils to regulate P bioavailability. Conversely, AkP was insensitive to land use, as found in a study by Monreal and Bergstrom (2000) and the chronosequence study of An et al. (2008). The differences between both phosphatase activities cannot be explained by soil pH or, at least not exclusively, by the fact that AcP are synthesized by microorganisms and plants while AkP are exclusively of microbial origin (Deng and Tabatabai, 1997). In fact, it has been proved that the contribution of roots to acid phosphatases in soils is smaller than predicted (Nannipieri et al., 2011) and there are studies were both enzymes reduced their activity in response to the implementation of agriculture (Bossio et al., 2005; Gil-Sotres et al., 2005; Salam et al., 1998).

Our results suggest there are other factors, different from land use, regulating the amount and/or activity of AkP. For example, organomineral complexes adsorb and stabilize extracellular enzymes and could buffer the effect of any disturbance in soil hydrolytic capacity (Deng and Tabatabai, 1997; Nannipieri et al., 2011). Therefore, extracellular enzymes might not respond in the same way than living microbial biomass to soil management or use (Paz-Ferreiro et al., 2010). Moreover, microbial biomass and activity are intimately linked with soil organic matter and, thus, together with the mentioned protective mechanism, it is a key property to be considered when interpreting enzymatic data. In this sense, we found that both phosphatase activities were positively correlated with SOC and, in fact, AcP and AkP per unit SOC showed no differences between land-use categories. We did not observe an enrichment in hydrolytic enzyme activities (i.e., higher activity per unit SOC) in agricultural soils as observed by other authors (Bossio et al., 2005; Trasar-Cepeda et al., 2008a).

Although global utilization of C-sources (AWCD) by soil microbial communities did not respond to land use or time under cultivation, we did find a consistent pattern after removing the bias of microbial biomass, with higher AWCD:MBC in older agricultural soils than in forest soils. Nevertheless, we would have predicted higher AWCD:MBC in short-term agricultural soils, the ones whose behavior we associated with copiotrophic microorganisms. This unexpected result could be a consequence of the limited fraction of the microbial community represented in AWCD data, which are rapid-growth heterotrophic bacteria able to grow with a single C source (Garland et al., 2010; Nannipieri et al., 2003). Therefore, even though both qCO_2 and AWCD:MBC refer to microbial activity per unit biomass, data from BR and BIOLOG Ecoplates appear not to be comparable. Also, the higher availability of labile C in short-term sites might explain the increased BR and qCO_2 in those soils, while having less influence in the results obtained with BIOLOG Ecoplates.

Multivariate analysis of CLPP data did not result in any consistent ordination according to land use, in agreement with other studies (Bissett et al., 2013; Waldrop et al., 2000). Moreover, we did not find any response in diversity indices or the relative consumption of different C-sources, as opposed to other authors who found higher C-source utilization diversity and higher response to more complex substrates in forest than in cultivated soils (Bossio et al., 2005; Brackin et al., 2013; Pérez-Brandán et al., 2014). The lack of differences found in our study could be explained

by the fact that high intrinsic heterogeneity of soil microbial communities at the fine scale leads to a wider metabolic capacity at the site scale, with no differences between sites under different land use (Bissett et al., 2013).

Regarding abiotic properties, coarser textures and lower WHC and CNP (SOC, N and P), were the most influential in characterizing long-term agricultural sites, which clustered in the multivariate analysis, in agreement with other studies (Bahr et al., 2014; Lemenih et al., 2005; Moebius-Clune et al., 2011). Within CNP, extractable P data must be interpreted carefully because standardized protocols require soil air-drying and this process can lead to biases resulting from the liberation of P from the microbial biomass (Sparling et al., 1985). Despite the relationship found between physicochemical traits and land use, the comparison of both PCAs showed that physicochemical traits were less associated to land-use change than microbial traits. This fact highlights the high sensitivity of microbial communities to alterations in the soil environment.

The chronosequence approach allowed us to inquire on the dynamics of soil microbial communities over time, with a farm-based sampling design that provided more reliability to our results. Microbial communities from short-, mid- and long-term agricultural sites behaved similarly, suggesting radical changes in functionality at the beginning of the chronosequence. This result reinforces a previous finding in the region, which had been obtained using CLPP by BD™ Oxygen Biosensor System but with data from a single site (Montecchia et al., 2011). Additionally, we were able to detect a higher variability of most microbial traits in short-term agricultural sites, probably due to the disturbance applied during deforestation. Despite this initial heterogeneity, there seems to be a homogenization of the functionality of soil microbial communities in mid- and long-term agricultural plots from all farms, no matter how different the pristine situation was. A similar result was found in terms of structure and diversity of soil bacterial communities, reported in a previous study (Montecchia et al., 2015). Future studies focusing on even earlier stages of agriculturization will provide useful information to better understand the response of microbial communities and to manage them properly in order to avoid detrimental effects on soil functioning.

5. Conclusions

Our chronosequence approach in a farm-based sampling design allowed us to infer on the temporal dynamics of soil microbial communities over the 30 years since deforestation. The impact of land-use change was evident early in the chronosequence, with a reduction in microbial biomass C and some nutrient-releasing activities. These soils were characterized by relatively small-sized microbial communities with a potentially high heterotrophic activity, which appear to be inefficient in terms of C utilization and, thus, imply a high potential for SOC losses. Thereafter, towards the end of the chronosequence, soil microbial communities evidenced an apparent stabilization and homogenization in the measured attributes. Together, these results suggest that soil functionality is more affected by land-use change than time under cultivation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2016.08.012.

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