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Long-term legacy of land-use change in soils from a subtropical rainforest: Relating microbiological and physicochemical parameters

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Abstract

Tropical and subtropical ecosystems are widely affected by the expansion of agriculture over pristine lands. Despite research efforts, knowledge of the impact of land-use change on soil is still limited by intrinsic variability, inconsistent results and inadequate replication. This study aimed to better understand the consequences of land-use change by focusing on long-term effects on both soil biotic and abiotic parameters. For this purpose, we selected three productive farms under similar management, each of them with pristine forest sites and agricultural sites that had been deforested for ~15 and ~30 years. In each site, we analysed soil microbiological (phospholipid fatty acids [PLFAs], biomass and activity) and physicochemical parameters. Long-term land-use change caused a detriment in soil microbial biomass, activity and fungal abundance, but only small changes in PLFA composition. In fact, PLFA composition was more affected by soil physicochemical properties such as carbon-to-nutrient ratios and labile carbon than by land use. Some physicochemical parameters (e.g., organic carbon and nutrients) were also negatively affected by land-use change and were more sensitive to time under agricultural use than microbiological parameters. The lower sensitivity of microbiological parameters could be the result of severe drought conditions at sampling, which may have affected soil microbial communities in both land uses. We were also able to detect associations between specific microbiological and physicochemical parameters. Among these, we identified some that seemed to result from their co-variation in response to land-use change and others that seemed to be independent of land use. Overall, our results show that soils can suffer further deterioration several years after deforestation. In order to restore soil health in these degraded lands, we need to keep on investigating the physical, chemical and biological mechanisms responsible for this deterioration.

Highlights

- Land-use change affected soil microbiological and physicochemical parameters.
- Microbiological parameters seemed to stabilize after continuous agriculture.

- Soil organic C, total N and fine particles were still reduced after long-term cultivation.
- Microbiological parameters were mostly associated with C-to-nutrient ratios and labile C.
- Drought conditions may have affected microbial response to land-use change.

KEYWORDS

deforestation, microbial activity, microbial biomass, PLFA, soil organic carbon, Yungas

1 | INTRODUCTION

Deforestation of pristine lands for agricultural purposes has been a major concern in the last decades, affecting a vast area of different ecoregions. According to the FAO (2016), between 1990 and 2015 there was a net loss of some 129 million ha of forest, mostly focused in the tropics, in particular in South America and South Africa. The Andean tropical and subtropical rainforests of South America are known as Yungas, and, within NW Argentina, this ecoregion is particularly relevant as a major biodiversity hotspot (Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000). Even though the global rate of annual net loss has slowed down (from 0.18% in the 1990s to 0.08% between 2010 and 2015) (FAO, 2016), the loss of native forest is still a major concern, especially when it is carried out with little or no regulation on remaining forest, ecological corridors or suitable land-use alternatives. Within the Yungas, for example, the lowest stratum or pedemontane forest was widely affected by the expansion of croplands over pristine lands (Gasparri & Grau, 2009; Seghezzo et al., 2011), compromising the ecosystem services provided by the land (Nanni & Grau, 2017; Volante, Alcaraz-Segura, Mosciaro, Viglizzo, & Paruelo, 2012).

It is known that deforestation alters not only aboveground but also belowground ecosystems, hence impacting soil microbial communities. Because soil microorganisms are key drivers of ecosystem processes, their response has a major impact on soil health and environmental sustainability (Singh & Gupta, 2018). Land-use change was shown to reduce soil nutrient and carbon stocks (Assefa et al., 2017; Bahr, Chamba Zaragocin, & Makeschin, 2014; Moebius-Clune et al., 2011), especially in forest-to-agriculture transitions (Don, Schumacher, & Freibauer, 2011; Houghton, 2018). Similarly, clear alterations in soil microbial communities were found between cultivated or pasture soils and their pristine counterparts (Bossio et al., 2005; Brackin, Robinson, Lakshmanan, & Schmidt, 2013; Navarrete et al., 2013; Rodrigues et al., 2013; Upchurch et al., 2008). This was not an exception for soils in the NW Argentinian Yungas, where microbial biomass and functionality, as well as bacterial structure, were affected by the transition from forest to agricultural use (Montecchia et al., 2011, 2015).

In spite of the relatively large number of publications on the response of soils to deforestation (Trivedi, Delgado-Baquerizo, Anderson, & Singh, 2016), current knowledge on the processes occurring during land-use change is still limited, especially for tropical and subtropical environments (Pajares, Bohannan, & Souza, 2016). Likewise, it is not clear how changes in biotic and abiotic parameters are related to each other, especially after a long period of cultivation. This is mostly due to the intrinsic variability of soils, inconsistent results and limited realistic replicates. Moreover, there is limited information regarding the fate of soil physicochemical and microbiological properties after several years under cultivation (Bahr et al., 2014; Recha et al., 2013; Tosi et al., 2016). According to a previous study in the Argentinian Yungas, some microbiological and physicochemical parameters could stabilize after successive years under the same agricultural practices (Tosi et al., 2016). Yet, this was not the case for soil organic carbon (SOC) in a neighbouring ecoregion (Villarino et al., 2017), and further evidence is needed to describe long-term effects of deforestation and continuous agriculture on soils. Understanding biotic–abiotic feedbacks in these degraded soils is key to elaborating suitable soil health restoration practices.

For all the above-mentioned reasons, this study aimed to analyse the consequences of prolonged agricultural use (~15 and ~30 years) for soils from deforested lands from Yungas pedemontane forest, Salta province, using neighbouring pristine sites as a reference of initial conditions. To make our results more representative, we collected samples from each land-use stage in three neighbouring farms under similar agricultural management. Both microbiological and physicochemical parameters were analysed using standardized and accessible tools, with the goal of detecting biotic-abiotic associations at an intra-site and inter-site scale. According to previous findings, we expected drastic effects of land-use change (forest vs. agriculture) on soil microbial communities and soil physicochemical parameters. Yet, we hypothesized

TABLE 1 Description of sampled sites according to land use: pristine forest and agriculture for approximately 15 or 30 years

Land use	Site i.d.	Years since deforestation	Sampled area[a] (ha)	Coordinates and elevation (m a.s.l.)	Farm i.d. [b]
Forest	1	—	6	24°52′25″S, 64°12′10″W, 441	F1
	2	—	5	24°51′52″S, 64°19′05″W, 540	F2
	3	—	3	24°48′43″S, 64°18′11″W, 577	F3
Agriculture	4	13	46	24°51′41″S, 64°18′41″W, 546	F2
~15 years	5	15	57	24°52′28″S, 64°11′59″W, 441	F1
	6	16	37	24°48′02″S, 64°16′06″W, 503	F3
Agriculture	7	30	50 [c]	24°48′41″S, 64°11′49″W, 464	F3
~30 years	8	32	45	24°52′31″S, 64°11′38″W, 514	F1
	9	32	74	24°52′41″S, 64°18′58″W, 538	F2

[a]In agricultural soils, sampling area is equivalent to plot area unless indicated.
[b]Sites are spatially grouped in farms.
[c]Section of a 244-ha plot.

that, because agricultural management practices were relatively stable, abiotic and biotic parameters would stabilize over time. However, because microbial communities are highly sensitive to fluctuations in their surrounding environment, microbiological parameters could still show some changes between ~15 and ~30 years of agriculture. Those additional 15 years of agricultural use could not only further reduce microbial biomass and potential activity, but also favour the proliferation of some microbial groups over others, hence shifting the microbial community structure.

2 | MATERIALS AND METHODS

2.1 | Study area

Soil samples were taken from an agricultural area located in the district of Anta, Salta province, Argentina (Table 1). The landscape is known as Yungas pedemontane forest, a transition between the ecoregions of Yungas and Chaco from W to E (Cabrera, 1976). This transition is determined by a gradient in precipitation and altitude, which is reflected in the flora and fauna. The climate is subtropical with a dry period in winter (between May to September), with average temperatures of 26.5°C in summer and 14°C in winter, and an annual precipitation of 600–750 mm concentrated in summer (96% of the annual total). The most widespread soil series is "Las Lajitas" (Lj), in which the dominant soils are Udic Argiustolls. Subordinated soils are Typic Ustipsament ("Apolinario Saravia" series) and Typic Hapludalf (Mollinedo series), named according to the USDA soil taxonomy (http://visor.geointa.inta.gob.ar). Soils in the area have a fluvic origin and thus are expected to show spatial heterogeneity.

Our study comprised three productive farms with similar management practice history. Within each of the farms, we selected three sites representing a pristine forest and two agricultural sites of approximately 15 and 30 years cultivation (Table 1). All agricultural sites presented harvested soybean (*Glycine max* (L.) Merr.) at the time of sampling, and all pristine sites were part of the remnant native forest adjacent to the agricultural plots.

2.2 | Soil sampling and storage

In concordance with previous studies, sampling was carried out in early May. Samples were collected from three sites per farm (one site per land-use category) and two subsites per site. In each subsite we collected a composite sample made of ~10 cores (0–10 cm, Ø = 2 cm). These cores were taken from the inter-row zone, after removing all organic litter from the surface. Similarly, in forest soils, plant roots and litter were avoided. All soil samples were immediately kept in refrigerated containers, in which they were transported to the laboratory. Once in the laboratory, soils were sieved through a 2-mm mesh and stored at 4°C, except for a subsample, which was freeze-dried and stored at −50°C for phospholipid fatty acid (PLFA) analyses.

2.3 | Physicochemical parameters

Physicochemical analyses were carried out by a commercial laboratory (Laboratorio de Manejo y Conservación de Suelos, Facultad de Agronomía, Universidad de Buenos Aires) and included soil organic carbon (SOC) (Walkley-Black), total nitrogen (Kjeldahl), texture (Bouyoucos),

electrical conductivity , exchangeable cations, extractable phosphorus (P) (Bray-Kurtz) and pH (1:2.5 in water), all according to standardized protocols (Sparks et al., 1996). We also measured gravimetric water content, although weight changes were negligible due to severe drought at the sampling time.

2.4 | Phospholipid fatty acid (PLFA) analysis

Soil PLFAs were extracted using the protocol from Palojärvi (2006). From 4 g of freeze-dried soil, ester-linked fatty acids were obtained using the lipid monophasic extraction method. The obtained lipids were fractioned into glycolipids, neutral lipids and phospholipids using silica-based solid phase extraction (Agilent Bond Elut, Agilent Technologies, Santa Clara, CA, USA), and the phospholipid fraction was recovered and subjected to mild alkaline methanolysis (Chowdhury & Dick, 2012). The detection and quantification of methyl-PLFAs was carried out by gas chromatography–mass spectrometry (GC–MS) (Agilent 7890A and 5997A, respectively). We used an HP5 autoID-1 mod. 19,091J 413 (30 m × 250 μm × 0,25 μm) column and He as a carrier gas. The injector was set at 260°C and the temperature programme was: 60°C for 1 min, 10°C min^{-1} until 130°C, 15°C min^{-1} until 260°C and finally 260°C for 15 min. The amount of sample used was 1 μL with a 1:50 split ratio. Finally, the identification and quantification of peaks were carried out using the software Enhanced ChemStation, MSD ChemStation F.01.00.1903 (Agilent Technologies, Santa Clara, CA, USA), together with the NIST library and two commercial standards (BAME-47080 U and FAME37-CRM47885, Supelco Inc., Bellefonte, PA, USA). The identified fatty acids were named following the structure A:BωC, where A is the carbon atoms in the chain, B is the number of double bonds, and C is the position of double bonds from the aliphatic end. Following that nomenclature, we used the following prefixes: cy-, cyclopropyl group; *i*- and *a*-, ramifications in the penultimate (iso) and antepenultimate (anteiso) carbon; n° + Me, central ramification, located in the carbon atom indicated by the number. Finally, suffixes were used to indicate the geometry of the molecule (c, cis; t, trans). Because most of the PLFA taxonomic and physiological markers have been shown to be ambiguous, we included only the most robust taxonomic markers together with the different chemical structures, as suggested by Quideau et al. (2016). We used the fungal marker 18:2ω6,9, which has proved to be more consistent with fungal abundance than 18:1ω9c, known to be present in some bacteria (Frostegård, Tunlid, & Bååth, 2011; Quideau et al., 2016). The abundance of bacteria was westimated as the sum of the fatty acids commonly associated with Gram-negative bacteria (cyclopropyl), Gram-positive bacteria (iso and anteiso), and actinobacteria/sulphate-reducing bacteria (mid-chain branched). The abundance of each PLFA was first expressed as pmol g^{-1} soil and also as a proportion of total microbial PLFAs to focus on compositional differences. All variables obtained from the PLFA analysis are enumerated in Table S1.

2.5 | Microbial biomass carbon and microbial activity

Total microbial biomass was estimated as microbial biomass carbon (MBC) using the fumigation-extraction method (ISO standard 14240-2:1997), which consists of the fumigation of a batch of soil samples with chloroform and the estimation of the organic carbon derived from cellular lysis. Organic carbon was extracted from all subsamples (25 g, in triplicate) using K_2SO_4 0.5 M, and extracts were stored at −20°C until determination. The amount of organic carbon in each sample was obtained from a digestion with H_2SO_4 in the presence of $K_2Cr_2O_7$, and titration with $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$ using ferroin as an indicator. MBC was calculated as: $(OC_F - OC_{NF}) * k_{EC}^{-1}$, where OC_F and OC_{NF} = organic carbon extracted from fumigated and non-fumigated samples, respectively, and $k_{EC} = 0.38$, indicating the extractable part of MBC (Joergensen, 1996). OC_{NF} was used as an estimator of K_2SO_4-extractable carbon, which we will refer to as labile C.

Microbial activity was studied through basal respiration (BR) and hydrolysis of fluorescein diacetate (HFDA). BR was measured as previously described (Tosi et al., 2016) using a standardized procedure (ISO-standard 16702:2002). Soil samples (25 g, in triplicate) were previously brought to 60% WHC with distilled water and then incubated for 72 h at 25°C in darkness. BR (μg CO_2 g^{-1} dry soil day^{-1}) was calculated from the difference from blanks without soil. HFDA, on the other hand, was used to estimate the potential global hydrolytic activity (intracellular and extracellular hydrolytic enzymes). Following the protocol by Alef (1995), 1 g soil was incubated with a 4.9 mM fluorescein diacetate solution in acetone and 60 mM phosphate buffer pH 7.6, for 3 hr at 37°C. Fluorescein, the product of the reaction, was extracted with acetone and quantified at A_{490}. HFDA was expressed as μg of fluorescein g^{-1} dry soil hr^{-1}. Three technical replicates were carried out per sample.

Microbial variables are enumerated and briefly described in Table S1.

2.6 | Data analysis

Statistical analyses were carried out in R 3.6.3 (R Core Team, 2020). Univariate physicochemical and microbial data were analysed using linearized mixed-effects models in the package "nlme" (Pinheiro, Bates, DebRoy, Sarkar, & Core Team, 2018). For PLFA-derived variables expressed as a proportion of total PLFA, the data were logit-transformed before applying linear models (Warton & Hui, 2011). The explanatory variable "land use" was considered a fixed effect. We used a random-intercept model to account for the spatial hierarchy or nesting of our sampling design ("farm > site > subsite"). All models were tested for normality and homogeneity of variance, and different VarIdent variance structures were tested if the latter was not satisfied. Using the final model, one-way ANOVA and post hoc Tukey test were used to test the effect of land use and compare the three categories. Several ratios were calculated to improve data interpretation, such as the metabolic quotient (qCO_2) and MBC:SOC ratio (Anderson, 2003), and they were analysed in the same way as individual variables. Variance explained by random effects in each of the analysed parameters can be seen in Tables S2–S4.

Phospholipid fatty acid multivariate analyses were carried out with the package "vegan" (Oksanen et al., 2018). PLFA composition data were analysed using the Aitchison distance calculated from a centred log-ratio-transformed table (Aitchison, Barceló-Vidal, Martín-Fernández, & Pawlowsky-Glahn, 2000). Changes in PLFA composition in response to land use were visualized with non-metric multidimensional scaling (NMDS) using *metaMDS* and analysed with permutational multivariate analysis of variance (PERMANOVA) using *adonis*. In order to account for spatial nesting of the data, we used the *strata* argument to constrain permutations within farms. Additionally, in order to analyse the influence of soil physicochemical parameters on PLFA composition, we used distance-based redundancy analysis (db-RDA) with the Aitchison distance matrix calculated for the previous analysis. To exclude farm or location effects, we included this factor as a conditioning variable in the db-RDA model. Before carrying out this constrained analysis, a preselection of soil physicochemical variables was carried out to focus on ecologically relevant properties and to avoid multicollinearity. For example, because SOC and nutrients were highly collinear, we used C:N:P to summarize both in the model. The variance inflation factor (VIF) of the selected constraining variables was within the suggested threshold (1.23–2.29). We also calculated the contribution of different factors (soil properties, land use and farm) to PLFA composition using a variance partition analysis (*varpart* function).

To compare the evolution of both microbiological (biotic) and physicochemical (abiotic) parameters in response to land-use change, we first carried out a separate principal component analysis (PCA) for each dataset and then analysed the response of the first two principal components. Following that comparison, we related the same biotic and abiotic datasets using a "PCA-PCA" co-inertia analysis (COIA) with the package "ade4" (Dray & Dufour, 2007). The analysis was carried out with the function *coinertia* and the significance of the test was corroborated with *randtest*. COIA is a symmetric method that allows us to relate two datasets by maximizing their covariance and the concordance between them in a new common ordination space. It is a robust method that allows us to relate two datasets with no constraints regarding the number of variables in each dataset. We complemented this analysis with a graphical display of Pearson correlation coefficients between variables from both datasets. Correlations were calculated with and without Holm's *p*-value correction for multiple inference.

3 | RESULTS

3.1 | Abiotic: soil physicochemical parameters

Both total SOC and total N decreased with agriculture and time under cultivation ($p < .001$ for all contrasts and variables) (Table 2). After ~15 years, SOC was reduced by 29% and total N by 40%, on average, and after ~30 years both variables were ~50–54% lower than in forest soils. Similar results were found for labile C, but differences between agricultural sites were only a trend ($p = .051$). Extractable P, on the other hand, exhibited a clear decrease with respect to forest soils only after ~30 years of agriculture ($p < .001$), with only marginal trends after ~15 years ($p = .075$). Forest soils presented the lowest C:N:P ratio ($p < .01$) and lower C:N than ~15-year-old agricultural soils ($p < .01$). Table 2 also shows that ~30 years of agriculture, but not ~15 years, resulted in coarser soil texture, with increased sand ($p < .05$) and lower silt content ($p < .01$) compared to both the other sites. No

TABLE 2 Physicochemical parameters of pristine forest soils and soils cultivated for approximately 15 and 30 years

		Agriculture	
Soil parameters	**Forest**	**~15 years**	**~30 years**
SOC (%)	3.23 (0.63)a	2.29 (0.29)b	1.60 (0.26)c
Labile C (ppm)	186.4 (81.5)a	93.4 (29.0)b	71.5 (33.0)b
Total N (%)	0.35 (0.07)a	0.21 (0.03)b	0.16 (0.03)c
Ext. P (ppm)	24.9 (4.6)a	14.1 (11.5)ab	6.8 (3.7)b
C:N	9.3 (0.8)b	11.0 (1.1)a	10.2 (0.9)ab
C:N:P	0.39 (0.10)b	3.04 (5.51)a	2.91 (3.70)a
Sand (%)	33.9 (8.7)b	35.2 (5.0)b	50.1 (12.9)a
Silt (%)	52.7 (6.4)a	51.8 (2.6)a	36.1 (11.3)b
Clay (%)	13.4 (3.0)a	13.0 (4.0)a	13.8 (2.6)a
pH (water, 1:2.5)	6.5 (0.6)a	7.1 (0.6)a	6.9 (0.3)a
EC (mmhos cm^{-1})	1.16 (0.47)a	0.76 (0.29)a	0.39 (0.15)b
Exc. K (meq kg^{-1})	1.98 (0.52)b	2.59 (0.47)a	1.83 (0.45)b
Exc. Na (meq kg^{-1})	0.40 (0.09)a	0.40 (0.07)a	0.43 (0.09)a

Note: Mean values and standard deviation (between parentheses) are shown. For each variable, different letters show significant differences between land-use categories ($p < .05$).

EC: electrical conductivity; Ext. P: extractable phosphorus; Exc. K/Na: exchangeable potassium/sodium; SOC: soil organic carbon.

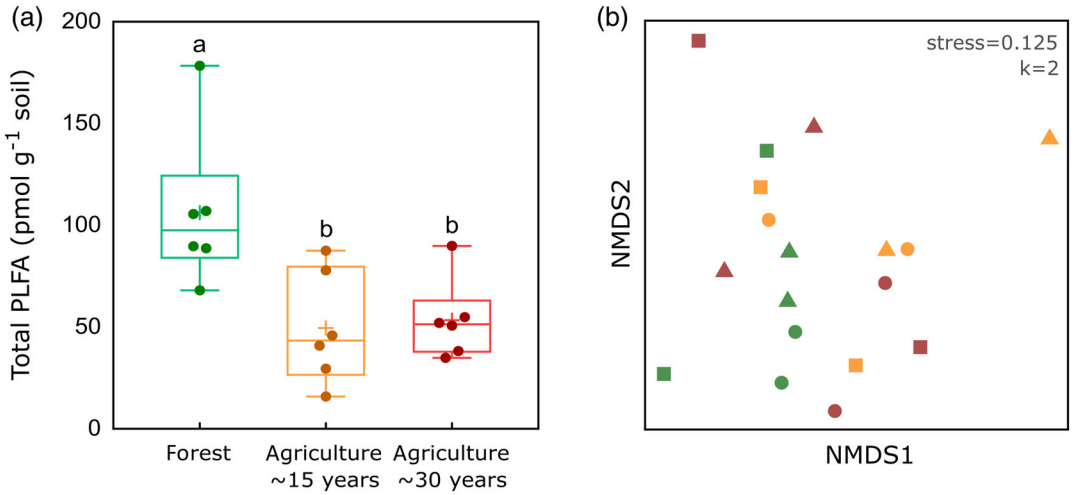

FIGURE 1 Changes in phospholipid fatty acid (PLFA) abundance (a) and PLFA composition (b) of soil microbial communities from forest sites and long-term cultivated sites. PLFA composition is presented as a non-metric multidimensional scaling (NMDS) plot of Aitchison distances. Symbol colours are coded by land use: forest soils (green), ~15-year-old agricultural soils (yellow), ~30-year-old agricultural soils (red). In (a) different letters show significant differences between land-use categories ($p < .001$). In (b) symbol shapes represent the three sampled farms. PERMANOVA found significant land-use change effects (forest vs. agriculture) on PLFA composition with $R^2 = 0.138$ ($p = .005$) (see Table S1 for more details) [Color figure can be viewed at wileyonlinelibrary.com]

differences in clay content were found. Electrical conductivity was also only affected after ~30 years of agriculture ($p < 0.001$), whereas exchangeable K was higher in ~15-year-old agricultural soils. pH showed the same trend as exchangeable K (~15-year-old agriculture > forest, $p = .07$) (Table 2).

3.2 | Biotic: microbial PLFA, biomass and activity

PLFA data were analysed with a multivariate approach, but also by extracting data on total PLFAs (i.e., viable microbial biomass), relative abundance of specific

TABLE 3 Relative abundance of taxonomic group markers and chemical structures obtained from PLFA data of pristine forest soils and soils cultivated for approximately 15 and 30 years

PLFA[a]	Forest	Agriculture	
		~15 years	~30 years
Specific group markers			
Bacteria	51.7 (5.30)a	51.5 (5.00)a	51.8 (4.40)a
Gram-positive	32.1 (4.20)a	32.3 (6.70)a	32.0 (5.70)a
Gram-negative	10.75 (2.90)a	10.41 (3.74)ab	9.69 (5.03)b
Gram-positive:Gram-negative	2.37 (0.68)b	2.56 (1.29)ab	3.05 (1.31)a
Fungi	1.90 (0.69)a	1.24 (0.44)ab	1.10 (0.37)b
Fungi:Bacteria	0.04 (0.02)a	0.02 (0.01)b	0.02 (0.01)b
Chemical structures			
Saturated	64.5 (4.3)a	64.6 (5.2)a	64.9 (3.4)a
Straight chained	23.6 (1.9)a	23.5 (1.9)a	22.8 (2.5)a
Mid-chain branched [b]	17.0 (3.4)b	18.7 (3.0)a	18.1 (3.4)ab
Iso-branched [c]	17.6 (2.8)a	16.4 (4.6)a	17.1 (3.2)a
Anteiso-branched [c]	6.3 (0.9)a	6.0 (1.3)a	6.9 (1.4)a
Cyclopropyl	10.8 (2.9)a	10.4 (3.7)ab	9.7 (5.0)b
Unsaturated [d]	24.8 (4.8)a	25.0 (4.2)a	25.4 (2.8)a
Monounsaturated	22.9 (4.6)a	23.8 (3.8)a	24.3 (2.7)a

Note: Mean values and standard deviation (between parentheses) are shown. For each variable, different letters show significant differences between land-use categories ($p < .05$).
[a]All variables expressed as percentage (%) except for fungi:bacteria and Gram-positive:Gram-negative ratios.
[b]Saturated with mid-chain branching.
[c]Saturated with terminal branching.
[d]Sum of monounsaturated and fungal marker 18:2ω6,9.

taxonomic markers, and chemical structures relevant to the structure or physiological status of microbial communities. Figure 1 shows both changes in total PLFA abundance, an equivalent of viable microbial biomass, and changes in PLFA composition in response to long-term land-use change. Total PLFA abundance was 52.8% higher in pristine soils than in cultivated soils, regardless of their age ($p < .001$), and did not vary with time under cultivation (Figure 1a). Regarding PLFA composition, PERMANOVA detected small but significant effects of land-use categories ($R^2 = 0.138$, $F = 2.39$, $p = .005$). Yet, these were mostly explained by differences between forest and agricultural soils, but not between agricultural soils of different ages (Table S5). The high percentage of unexplained variability found by PERMANOVA ($R^2 = 0.86$) is reflected in the NMDS plot, where land-use effects can only be observed as a grouping among forest sites (Figure 1b). Figure S1 also shows how microbial PLFAs, grouped by chemical structure, responded to land use more in terms of abundance than composition.

The total abundance of both bacteria and fungi (pmol g^{-1} soil) decreased with land-use change (Figure S2), but only fungal communities were sensitive in terms of relative abundance (Table 3). This response was observed as a ~42% decrease between forest and ~30-year-old agricultural soils ($p < .01$) (Table 3). The fungi:bacteria (F:B) ratio presented a similar behaviour, but also with clear differences between forest and ~15-year-old agricultural soils ($p < .01$). Gram-negative bacteria were also lower in ~30-year-old agricultural soils compared to forest soils, but no variation was observed for Gram-positive bacteria (Table 3). In terms of chemical structure, the most abundant PLFAs were 16:0 (~16% of total PLFAs), *i*15:0 (~8%) and 18:1ω9c (~7%) (Figure S3). Saturated fatty acids were twice as abundant as unsaturated fatty acids, but were not affected by land use (Figure S1, Table 3). Yet, forest soils had a lower proportion of mid-chain branched fatty acids and a higher proportion of cyclopropyl fatty acids, compared to ~15 and ~30-year-old agricultural soils, respectively (Table 3). Notably, some monoenoic or mid-chain branched PLFAs, mostly not fully identified against the used standard, were below detectable levels in several agricultural soils (Figure S4).

Total microbial biomass, estimated as MBC, was higher in pristine soils, with no difference among the two

Microbial properties [a]	Forest	Agriculture	
		~15 years	~30 years
MBC	417 (115)a	185 (46)b	206 (38)b
BR	201 (54)a	101 (34)b	77 (10)b
HFDA	263 (40)a	181 (76)ab	148 (21)b
MBC:SOC	1.33 (0.30)ab	0.88 (0.20)b	1.44 (0.31)a
qCO_2 (BR:MBC)	19.9 (6.5)a	23.9 (13.6)a	15.3 (3.8)a
BR:SOC	0.26 (0.07)a	0.19 (0.06)a	0.22 (0.06)a
HFDA:SOC	73.2 (17)a	82 (32)a	84 (35)a

TABLE 4 Microbial biomass carbon, respiration, hydrolytic activity and associated ratios of pristine forest soils and soils cultivated for approximately 15 and 30 years

Note: Mean values and standard deviation (between parentheses) are shown. For each variable, different letters show significant differences between land-use categories ($p < .05$).
BR: basal respiration (μg CO_2 g^{-1} $soil^{-1}$ day^{-1}); HFDA: hydrolysis of fluorescein diacetate (μg fluorescein g^{-1} $soil^{-1}$ hr^{-1}); MBC: microbial biomass carbon (μg C g^{-1} soil); SOC: soil organic carbon (%); MBC:SOC: (%), qCO2: metabolic quotient (μg CO_2 g^{-1} hr^{-1}); , BR:SOC (μg CO_2 g^{-1} hr^{-1}), HFDA:SOC (μg fluorescein g^{-1} hr^{-1}).

stages of agriculture (Table 4). MBC in all agricultural soil was 51–56% below the MBC in forest soils ($p < .001$). The variability of MBC, both within and between sites, was reduced with time under cultivation. As expected, there was a significant positive correlation between MBC and PLFA biomass (Pearson $r = 0.82$, $n = 22$, $p < .001$). Microbial activity behaved similarly to MBC. Basal respiration (BR) showed a 50–62% decrease from pristine to agricultural soils ($p < .001$), again with no differences between the two stages of agriculture (Table 4). Changes in global hydrolytic activity, measured as HFDA, were only detected as a ~44% decrease between forest and ~30-year-old agricultural plots ($p = .004$). The variability of MBC, BR and HFDA was reduced in cultivated soils, especially in ~30-year-old sites. When analysing these variables per unit organic carbon or per unit biomass, most land-use effects were dissipated (Table 4).

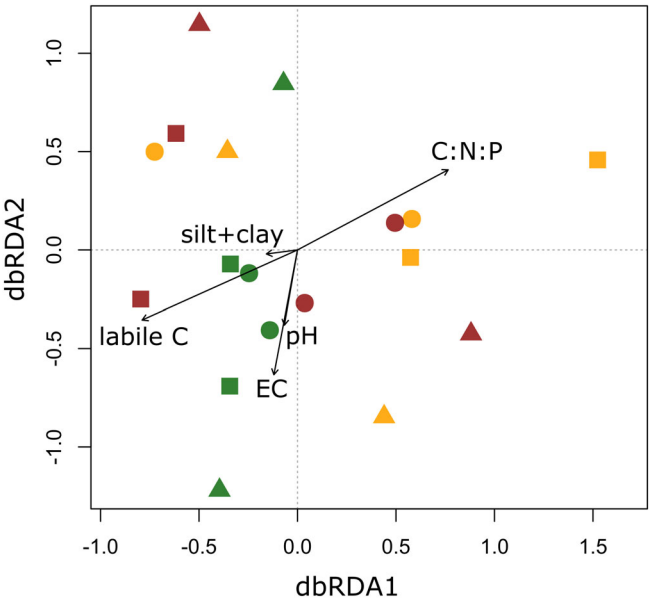

FIGURE 2 Distance-based redundancy analysis (db-RDA) biplot describing the response of soil microbial PLFA composition (Aitchison distance) to soil physicochemical parameters. Symbols are coded by land use: forest soils (green), ~15-year-old agricultural soils (yellow), ~30-year-old agricultural soils (red). The effect of farm was conditioned using partial RDA. EC, electrical conductivity; labile C presented as a fraction of SOC [Color figure can be viewed at wileyonlinelibrary.com]

3.3 | Relationship between microbiological and soil physicochemical parameters

The relationship between PLFA composition and soil physicochemical parameters was analysed using a partial db-RDA (Figure 2, Table S6). The partitioning of variance was 11.08% conditioned (i.e., farm or location effect), 37.06% constrained and 51.86% unconstrained. Overall, the model (including silt + clay, pH, labile C fraction, electrical conductivity [EC] and C:N:P) was found to be only marginally significant ($p = .012$). When looking at the importance of each soil parameter, we found that RDA1 (20.47%) was mainly explained by labile C and the C:N:P ratio, followed by pH (Figure 2, Table S6). RDA2 (9.92%) also had a large contribution of EC, followed by the C:N:P ratio and soil texture (silt + clay). In a supplementary db-RDA where land-use change effects were conditioned, the importance of C:N:P and EC decreased, whereas the importance of pH increased (Table S7).

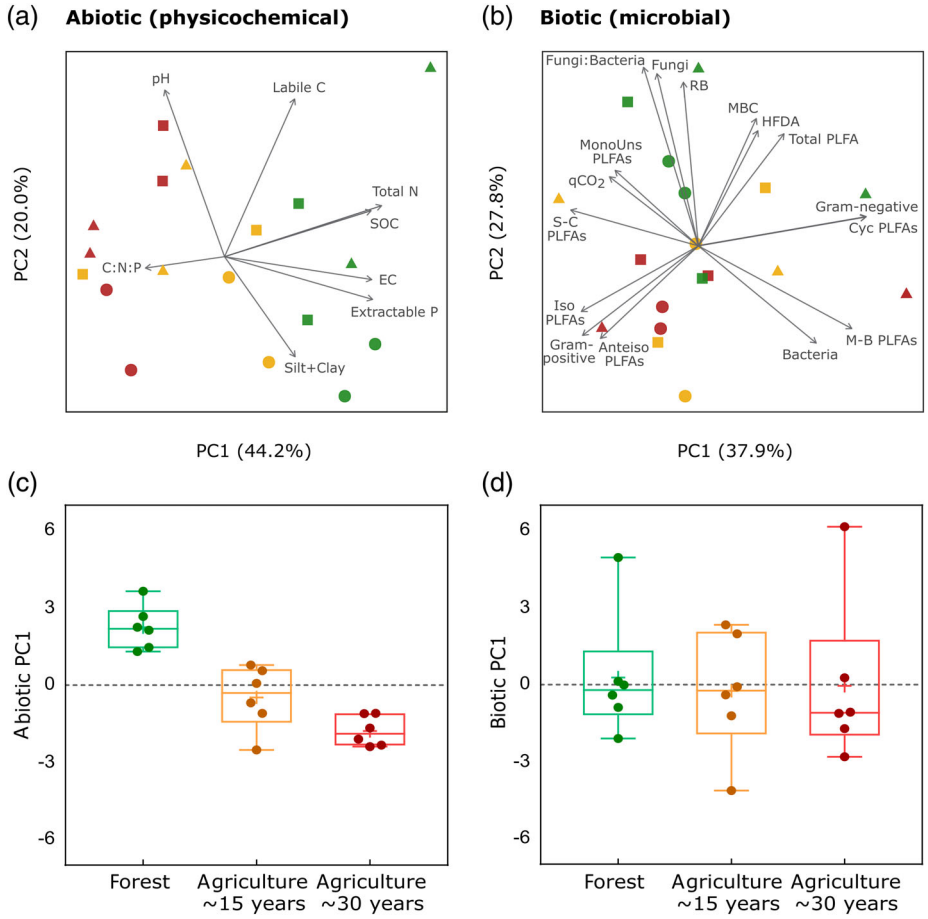

FIGURE 3 Response of soil physicochemical (abiotic) and microbiological (biotic) parameters to long-term land-use change: (a, b) biplots of principal component analyses (PCAs) summarizing the behaviour of each dataset, (c, d) boxplots showing the response of the first principal component (PC1) to land use. In all plots, symbol colours are coded by land use: forest soils (green), ~15-year-old agricultural soils (yellow), ~30-year-old agricultural soils (red). In (a) and (b), symbol shapes represent the three sampled farms. S-C/M-C PLFAs: straight-chained and mid-chain branched PLFAs, respectively. See Figure S6 for response of PC2. EC: electrical conductivity; SOC: soil organic carbon; labile C presented as a fraction of SOC; BR: basal respiration ; qCO_2: metabolic quotient; HFDA: hydrolysis of fluorescein diacetate; MBC: microbial biomass carbon; PLFA: phospholipid fatty acid; Cyc: cyclopropyl; S-C: straight-chained; M-B: mid-chain branched; MonoUns: monounsaturated [Color figure can be viewed at wileyonlinelibrary.com]

Notably, including land use in the soil db-RDA did not improve the model; however, including soil properties in a land-use db-RDA did improve the model significantly (Table S8). A variance partitioning analysis also evidenced how soil properties were more influential to PLFA composition than land use (Figure S5). Yet, both analyses found a relatively high proportion of unexplained variability, which suggests other factors were conditioning PLFA composition. (Table S6, Figure S5).

Next, we wanted to compare and relate abiotic (physicochemical) and biotic (microbial) datasets. Figure 3 summarizes the response of both datasets to land-use change and long-term cultivation. Abiotic parameters were overall more different among the three land-use categories, with SOC, total N, EC, extractable P and silt + clay being higher on forest soils, followed by ~15-year-old agricultural soils (Figure 3a). Their higher sensitivity can be better visualized mainly in PC1, which explained 44.2% of the variability (Figure 3c). The sensitivity of biotic parameters seemed less clear along PC1 (37.9%), especially between agricultural sites (Figure 3b,d). However, biotic parameters did show a clear response to land-use change along PC2 (27.8%), although with relatively high variability in forest and ~15-year-old agricultural soils (Figure S6). Microbiological parameters defining PC2 were mostly fungal abundance, microbial activity and microbial biomass (Figure 3b).

To relate abiotic and biotic datasets we used co-inertia analysis because, unlike RDA, it treats both datasets symmetrically and finds a common structure between them (Figure 4, Figure S7). The biplot in Figure 4 shows a clear association of SOC, total N, extractable P and EC, with microbial biomass, activity and fungi. These also explain the highest amount of variability. These same variables presented the highest Pearson correlation coefficients, as expected (Figure 5). However, when excluding data from forest soils, these correlations were not as strong, evidencing that their correlation is mostly explained by their strong covariation in response to land-use change. The co-inertia plot also shows another set of microbiological parameters responding mostly to a gradient of pH, C:N:P and soil texture (Figure 4). Sites with lower pH (in these soils ~6.5), lower C:N:P and finer textures were associated with higher bacterial abundance, in particular Gram-positive bacteria with iso and anteiso membrane lipids. Mid-chain branched and straight chained PLFAs were also more associated with these soil

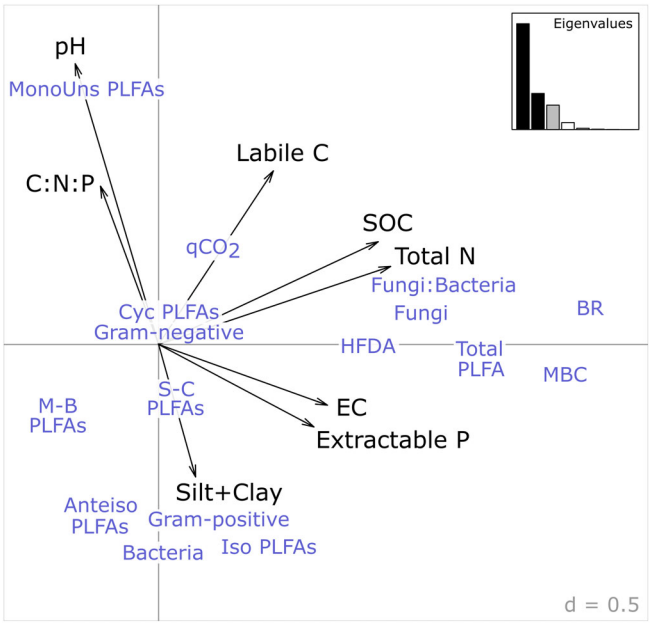

FIGURE 4 Biplot of co-inertia analysis (COIA) showing the relationship between soil physicochemical (abiotic) and microbiological (biotic) parameters. Abiotic parameters are shown in black and biotic parameters in blue. On the upper right, the scree plot of the eigenvalues shows the importance of each axis. BR: basal respiration ; EC: electrical conductivity; HFDA: hydrolysis of fluorescein diacetate ; MBC: microbial biomass carbon; PLFA: phospholipid fatty acid; Cyc: cyclopropyl; qCO_2: metabolic quotient; SOC: soil organic carbon; labile C presented as a fraction of SOC [Color figure can be viewed at wileyonlinelibrary.com]

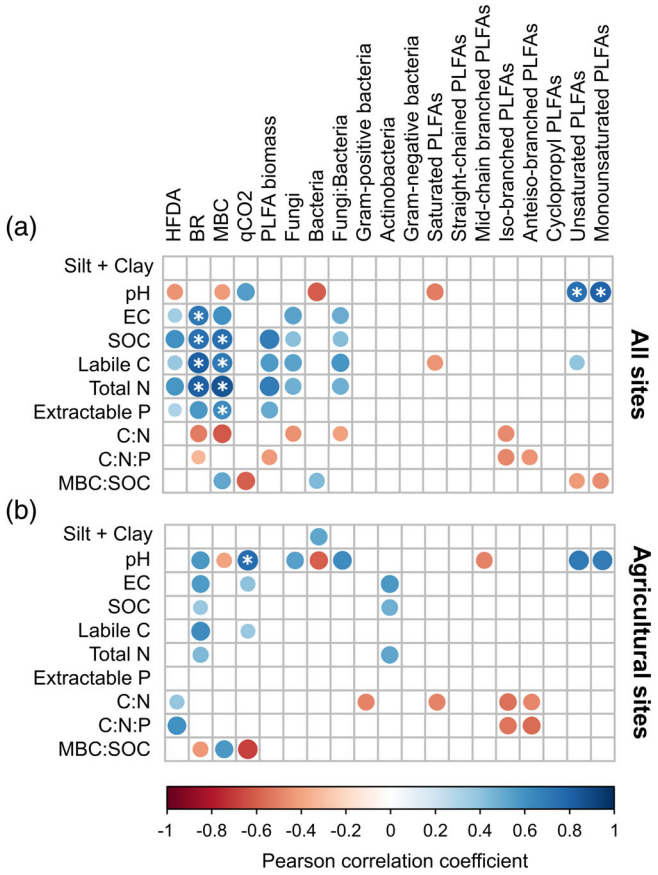

FIGURE 5 Pearson correlations between the main measured soil biotic and abiotic parameters for both the whole dataset (a) and agricultural soils only (b). The magnitude of the correlations is represented by colour intensity and circle size. For a broader assessment, all correlations with uncorrected-*p* values <.10 are shown (Holm's corrected-*p* values <.10 are indicated with an asterisk). BR: basal respiration ; HFDA: hydrolysis of fluorescein diacetate ; MBC: microbial biomass carbon ; PLFA: phospholipid fatty acid; qCO_2: metabolic quotient; SOC: soil organic carbon; EC: electrical conductivity [Color figure can be viewed at wileyonlinelibrary.com]

physicochemical conditions. In contrast, sites with higher pH, higher C:N:P and coarser textures presented a higher proportion of monounsaturated PLFAs. Correlations between pH and microbiological parameters seemed to remain when excluding forest sites from the analysis (Figure 5b). Unlike BR, the metabolic quotient or qCO_2 was less associated with physicochemical parameters, although in agricultural soils it seemed to be positively correlated with soil pH and to some extent labile C and EC (Figure 4, Figure 5b). Another interesting association that appeared exclusively within agricultural soils was a positive correlation between HFDA and carbon-to-nutrient ratios (Figure 5b).

4 | DISCUSSION

4.1 | Abiotic: soil physicochemical parameters

Several soil physicochemical parameters were sensitive to both land-use change and time under agricultural use, which suggests that further soil degradation could still occur several years after deforestation. In concordance with our results, a chronosequence study found SOC losses for up to 30 years in perennial and pasture crops in the southern Ecuadorian Andes (Bahr et al., 2014). Yet other studies reported that the highest SOC losses occurred somewhere between the first 5 and 10 years of cropping (Recha et al., 2013; Villarino et al., 2017). Major SOC losses following deforestation are usually attributed to an increased mineralization rate in response to disturbance and a reduction in C inputs from aboveground biomass, modulated by other factors such as precipitation, temperature or clay content (Fujisaki, Perrin, Garric, Balesdent, & Brossard, 2017). In the longer term,

however, disturbance became quite limited because no-tillage management was implemented 2 years after deforestation. Hence, SOC losses could have been prolonged by limited C inputs from crops (Fujisaki et al., 2017; Villarino et al., 2017) and soil erosion. The latter hypothesis is supported by coarser textures in the oldest agricultural soils. Additionally, the fact that labile C remained relatively stable between ~15 and ~30-year-old sites could suggest that more stable SOC fractions are being lost in later stages of agricultural use. However, this result is not consistent with previous findings (Sheng et al., 2015; Tosi et al., 2016; Villarino et al., 2017), and labile C might only be reflecting seasonal fluctuations in vegetation cover and environmental conditions.

Limited inputs and physical degradation may also explain reductions in soil nutrient content following land-use change and long-term agricultural use (Assefa et al., 2017; Bahr et al., 2014; Moebius-Clune et al., 2011). In our study, total N, and to a lesser extent extractable P, seemed to still occur after 15 to 30 years of cultivation. Similarly, the reduction in EC could reflect a decrease in the concentration of soluble nutrients, as well as changes in water dynamics. Overall, nutrient losses were more enhanced than SOC losses, considering the higher C-to-nutrient ratios of agricultural soils compared to forest soils. It is possible that other factors associated with agricultural activity, such as nutrient losses via stream discharge and crop uptake, are responsible for these exacerbated losses in soil nutrients.

4.2 | Biotic: microbial PLFA, biomass and activity

Most microbiological parameters were also sensitive to land-use change, although they were generally more stable between agricultural sites. An explanation for this could be the no-till management (Sekaran et al., 2020), which was implemented shortly after deforestation. Two other aspects could have made microbiological parameters less sensitive to agricultural age: (a) drought conditions at the moment of sampling, which were imposing a severe stress on soil microbial communities irrespective of their land use, and (b) limited resolution of the microbial techniques used in this study. PLFA analyses are able to detect meaningful ecological changes in soil microbial communities, targeting exclusively viable microorganisms (Orwin, Dickie, Holdaway, & Wood, 2018; Zhang et al., 2019). However, they present some limitations, such as a low taxonomic resolution and the fact that they can indistinctly reflect taxonomic and physiological changes (Frostegård et al., 2011; Willers, Jansen van Rensburg, & Claassens, 2015). For that reason, we interpreted our results cautiously, looking at overall changes in PLFA composition, chemical structures and only the most robust taxonomic markers.

Soil PLFA composition evidenced only small differences between soils under different land uses, in contrast to previous findings (Bossio et al., 2005; Drenovsky, Steenwerth, Jackson, & Scow, 2010; Zhang et al., 2016). Land-use change can affect microbial structure via several factors, such as changes in vegetation cover and composition, changes in carbon and nutrient inputs, and repeated inoculation of foreign bacteria (e.g., rhizobia) (Mawarda, Le Roux, Dirk van Elsas, & Salles, 2020). Yet in our study, soils were undergoing drought conditions at the moment of sampling, with negligible water content and ~61% lower precipitation than in the previous crop season (Tosi et al., 2016). We believe drought stress could have masked land-use effects on PLFA composition due to the high sensitivity of membrane lipids to physiological stress (Frostegård et al., 2011; Willers et al., 2015). Indeed, all samples exhibited a dominance of ramified and cyclopropyl fatty acids, both associated with stressful conditions (Hedrick, Peacock, & White, 2007). Moreover, prolonged drought conditions could favour some taxa over others (Schimel, Balser, & Wallenstein, 2007). Both taxonomic and physiological changes induced by drought stress could lead to shifts in microbial PLFA composition independently in soils under both land uses, as we corroborated in a microcosms experiment (Tosi, 2017). We cannot discard that higher resolution methods, such as high-throughput sequencing, could have detected clearer land-use patterns independently of the environmental conditions at sampling (Li et al., 2017; Montecchia et al., 2015). Nevertheless, findings by Orwin et al. (2018) suggest that PLFA profiling is comparable to 16S rRNA pyrosequencing in terms of detecting strong shifts in community structure, in spite of their different resolutions and targets.

Long-term land-use change reduced fungal abundance and the F:B ratio, as found by other authors (Bossio et al., 2005; Drenovsky et al., 2010; Zhang et al., 2016). Because we used the marker 18:2ω6,9, we can attribute those changes to a reduction in both saprophytic and ectomycorrhizal fungi. While the latter can be explained by the loss of woody plant species, negative effects on saprophytic fungi could respond to lower C inputs and physical disturbance by agricultural practices (Strickland & Rousk, 2010). Bacteria seemed less sensitive to land-use change, except for a lower Gram-negative bacteria abundance (and higher Gram-positive:Gram-negative ratio) in ~30-year-old agricultural sites. Other authors also found higher Gram-positive:Gram-negative ratios in forest-to-crop transitions (Bossio et al., 2005;

Drenovsky et al., 2010; Zhang et al., 2016). Gram-positive bacteria, due to their thicker cell walls and sporulating taxa, are considered more tolerant to drought and environmental stress (Schimel et al., 2007). These traits might also make them better adapted to agricultural soils, which are more frequently exposed to environmental fluctuations. A higher Gram-positive:Gram-negative ratio could also indicate a higher proportion of copiotrophs over oligotrophs (Montecchia et al., 2015; Orwin et al., 2018), in agreement with previous findings (Montecchia et al., 2015), and should reflect the lower resource availability in agricultural soils.

Microbial biomass and activity were also negatively affected by land-use change, as previously reported (Bossio et al., 2005; Brackin et al., 2013; Kaschuk, Alberton, & Hungria, 2010; Tosi et al., 2016). Yet, these parameters were relatively stable after a long period of agricultural use, with no changes between ~15- and ~30-year-old agricultural soils. This result supports previous results in the area, where microbial biomass seemed to stabilize after several years under cultivation (Tosi et al., 2016). Here, microbial biomass (MBC or total PLFA) losses represent a reduction in active, potentially active and dormant cells (Blagodatskaya & Kuzyakov, 2013). Moreover, considering PLFA results, these losses seem to be explained by a reduction in fungi but not bacteria.

Regarding microbial activity, previous studies have reported negative effects of land-use change on HFDA (Chaer, Fernandes, Myrold, & Bottomley, 2009; da Silva et al., 2012). This result evidences an overall decrease in the hydrolytic capacity of soils, including both intra- and extracellular enzymes (Alef, 1995). Extracellular enzymes stabilized in the soil matrix could explain why HFDA was less sensitive to agricultural activity than BR, which reflects the activity of living microorganisms. In fact, BR was lower in both agricultural soils, irrespective of their age, possibly due to the lower availability of C sources and nutrients (Chaer et al., 2009; Zhang et al., 2016). In particular, the availability of potentially mineralizable carbon is a key driver of basal respiration because no C substrates are added for its measurement (Ren et al., 2018). Unlike BR, the metabolic quotient or qCO_2 remained unaltered, which suggests it could have been affected by the above-mentioned drought stress. In fact, this quotient is known to increase in disturbed environments or under stressful conditions (Anderson, 2003) and the values registered were much higher than in our previous study (on average, 20.7 vs. 8.7 mg CO_2 mg^{-1} hr^{-1}) (Tosi et al., 2016). Most probably, the water added for the respiration assay acted as a drastic rewetting event that induced a sharp respiration peak in all soils (Schimel et al., 2007; Tosi, 2017).

4.3 | Relationship between microbiological and soil physicochemical parameters

In this study, PLFA composition was more associated with soil physicochemical properties than long-term land-use change effects. However, as mentioned above, drought conditions may have had a strong effect on soil PLFAs, which could explain the large proportion of unexplained variability found in our analyses. Among the analysed soil physicochemical parameters, microbial PLFA composition was mostly sensitive to C:N:P, in concordance with previous findings (Li et al., 2017; Zhang et al., 2016). Soil stoichiometry was found to be tightly associated with microbial processes such as organic matter decomposition and nutrient cycling, and could in turn shape microbial community structure (Delgado-Baquerizo et al., 2017). The labile fraction of SOC, which is readily available for microbial utilization, also had an important effect on PLFA composition. Similar results were found by Zhang et al. (2016) in another context of land-use change. Unlike C:N:P, the influence of labile C was not reduced when conditioning land-use effects in the db-RDA, which suggests it also reflects changes within forest or agricultural soils. In studies using DNA-based approaches, both labile C and C-to-nutrient ratios were influential drivers of microbial community structure (Delgado-Baquerizo et al., 2017; Ramírez, Fuentes-Alburquenque, Díez, Vargas, & Bonilla, 2020). However, such studies also reported pH as a key driver of microbial structure (Lauber, Hamady, Knight, & Fierer, 2009), but here its effect on PLFA composition was not as relevant. This could be related to methodological differences, but also to the narrow and neutral pH range in these soils.

When summarizing and comparing both biotic (microbiological) and abiotic (physicochemical) datasets, we confirmed the latter were overall more sensitive to long-term agricultural use. This was an unexpected finding, considering that soil microbial communities are highly responsive to environmental fluctuations, but the factors discussed at the beginning of this section could be associated with this. Next, we described the relationship between these two datasets in order to investigate the associations between soil microbial communities and their abiotic environment. The co-inertia analysis detected associations between some microbiological parameters and physicochemical gradients. The most important association was between microbial biomass, fungal abundance and activity, and SOC and nutrient content, and it was mostly reflecting the covariation of these properties in response to land-use change. This was partially confirmed by excluding forest soils from the analyses (e.g., db-RDA and correlations), which markedly

reduced the importance of these associations and increased the importance of other soil parameters such as pH and soil texture. In fact, the co-inertia analysis detected another association between some bacterial PLFAs and C:N:P, pH and soil texture. It is not totally clear why bacteria, and in particular Gram-positive bacteria, were more abundant in sites with lower C:N:P, lower pH (~6.5) and finer textures. Although C-to-nutrient ratios could be directly influencing microbial metabolism, soil texture and pH might play a secondary role, driving or reflecting other changes in the soil environment.

Clearly, in land-use change studies the covariation of different physicochemical or microbiological parameters can confound and challenge the identification of true relationships. This is particularly challenging in observational studies, where few variables can be controlled, and in land-use change scenarios, where many parameters undergo drastic changes. However, with the proper sampling design and enough replication (e.g., analysing multiple farms or spatial units), observational studies constitute highly valuable tools to explore microbial communities *in situ*.

5 | CONCLUSIONS

Consistent with previous findings, forest-to-crop transitions caused a detriment in soil microbial biomass, activity and relative fungal abundance. Unexpectedly, microbial PLFA composition was less sensitive to land-use change than microbial abundance. In fact, PLFA composition was more affected by soil physicochemical properties such as C-to-nutrient ratios and labile C, two variables that have already been reported as drivers of microbial community structure. Contrary to our hypothesis, physicochemical parameters were overall more sensitive to time under agricultural use than microbiological parameters. A possible explanation behind these results is the severe drought conditions at sampling, which may have affected soil microbial communities in both land uses. Additionally, the measured microbial parameters may have not been sensitive enough to detect differences between microbial communities from agricultural soils of different age. Finally, when relating microbiological and physicochemical parameters, we were able to distinguish a group of variables that may have been simply co-variating in response to land-use change (soil organic carbon and nutrients and microbial biomass, fungi and activity). We also found other potentially interesting associations that seemed independent of land-use change and could be further explored in future research.

Overall, our results show soils can suffer further deterioration several years after deforestation. In order to restore soil health in these degraded lands, we need to keep on investigating the physical, chemical and biological mechanisms responsible for this deterioration. Firstly, soil microbial communities of these cultivated sites seem to be relatively similar in coarse aspects such as biomass and global activity. Hence, higher resolution techniques might be necessary to understand the structural and functional changes they undergo after a long period of agricultural use. Finally, we need a more in-depth understanding of the microbial–physicochemical feedbacks that occur in these long-term agricultural soils. In this sense, studies under controlled conditions and/or at smaller spatial scales could help detect ecologically significant relationships that help assess proper soil restoration practices.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Study concept and design: O.S. Correa, M. Tosi, M.S. Montecchia and H.D. Chludil. Sampling and field work: M. Tosi and J.A. Vogrig. Laboratory analyses and statistical analysis of data: M. Tosi and H.D. Chludil. Writing–original draft and editing: M. Tosi. Writing–review and editing: M.S. Montecchia and M. Tosi. Funding acquisition: O.S. Correa, M.S. Montecchia and H.D. Chludil.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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SUPPORTING INFORMATION

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