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Drought and its legacy modulate the post-fire recovery of soil functionality and microbial community structure in a Mediterranean shrubland

María Belén Hinojosa[1] | Vito Armando Laudicina[2] | Antonio Parra[1] | Enrique Albert-Belda[1] | José Manuel Moreno[1]

[1]Departamento de Ciencias Ambientales, Universidad de Castilla–La Mancha, Campus Fábrica de Armas, Toledo, Spain

[2]Dipartimento Scienze Agrarie, Alimentari e Forestali, Università degli Studi di Palermo, Viale delle Scienze, Palermo, Italy

Correspondence
María Belén Hinojosa, Departamento de Ciencias Ambientales, Universidad de Castilla–La Mancha, Campus Fábrica de Armas, Toledo, Spain.
Email: mariabelen.hinojosa@uclm.es

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Abstract
The effects of drought on soil dynamics after fire are poorly known, particularly its long-term (i.e., years) legacy effects once rainfall returns to normal. Understanding this is particularly important for nutrient-poor soils in semi-arid regions affected by fire, in which rainfall is projected to decrease with climate change. Here, we studied the effects of post-fire drought and its legacy on soil microbial community structure and functionality in a *Cistus-Erica* shrubland (Spain). Rainfall total and patterns were experimentally modified to produce an unburned control (natural rainfall) and four burned treatments: control (natural rainfall), historical control (long-term average rainfall), moderate drought (percentile 8 historical rainfall, 5 months of drought per year), and severe drought (percentile 2, 7 months of drought). Soil nutrients and microbial community composition (ester-linked fatty acid approach) and functionality (enzyme activities and C mineralization rate) were monitored during the first 4 years after fire under rainfall treatments, plus two additional ones without them (six post-fire years). We found that the recovery of burned soils was lower under drought. Post-fire drought increased nitrate in the short term and reduced available phosphorus, exchangeable potassium, soil organic matter, enzyme activities, and carbon mineralization rate. Moreover, drought decreased soil total microbial biomass and fungi, with bacteria becoming relatively more abundant. Two years after discontinuing the drought treatments, the drought legacy was significant for available phosphorus and enzyme activities. Although microbial biomass did not show any drought legacy effect, the proportion of fungi and bacteria (mainly gram-positive) did, being lower and higher, respectively, in former drought-treated plots. We show that drought has an important impact on soil processes, and that some of its effects persist for at least 2 years after the drought ended. Therefore, drought and its legacy effects can be important for modeling biogeochemical processes in burned soils under future climate change.

KEYWORDS
climate change, enzyme activity, fire, microbial community, rainfall manipulation, resilience, soil nutrients

1 | INTRODUCTION

Droughts can alter a number of ecosystem processes, including those occurring in the soil (Evans & Wallenstein, 2012; Knapp et al., 2002). Understanding the impact of reduced precipitation on soils is an increasingly relevant research goal in ecology (Seidl, Rammer, & Spies, 2014; Smith, 2011). Drought has both direct and indirect effects on soil microbiota and nutrient cycling (Schlesinger et al., 2016; Classen et al., 2015). Soil microbes are key players in the decomposition of organic material, nutrient mineralization, and soil structure (Classen et al., 2015; Morales, Parlange, & Steenhuis, 2010). Thus, they are pivotal to our understanding of how terrestrial biogeochemical dynamics might be altered due to changes in total precipitation and patterns (Schlesinger et al., 2016).

Decreased soil water availability alters the soil microbial community owing to the challenge of finding sufficient nutrient and energy sources which become both temporally and spatially less available (Manzoni, Schaeffer, Katul, Porporato, & Schimel, 2014). Consequently, drought can lead to reduced decomposition rates and microbial growth, as well as to changes in the microbial community structure (Barnard, Osborne, & Firestone, 2013; Iovieno & Bååth, 2008; Maestre et al., 2015). While research supporting the direct effects of drought on microbial communities is common (e.g., Toberman, Freeman, Evans, Fenner, & Artz, 2008; Rousk, Smith, & Jones, 2013; Bastida et al., 2017), there is also evidence that some microbial groups are resistant to drought (Bouskill et al., 2013; Griffiths & Philippot, 2013; Pailler, Vennetier, Torre, Ripert, & Guiral, 2014). In addition, changes in the soil biota, due to drought, are associated with changes in soil biogeochemistry (Nielsen & Ball, 2015). The implications of drought for biogeochemical cycling are so far inconclusive, but several studies suggest that increased aridity has a significant impact on nutrient availability by reducing the biological breakdown of organic matter, potentially leading to a decoupling of C, N, and P cycling (Delgado-Baquerizo et al., 2013; Evans & Burke, 2013).

Changes in rainfall patterns may be particularly important in subtropical, semi-arid areas, such as the Mediterranean Basin, and other similar climate areas of the world (Giorgi & Lionello, 2008). Rainfall during winter has been decreasing in the Mediterranean Basin (Hoerling et al., 2012), and climate change projections for this region include extended seasonal summer drought, as well as an increase in the frequency and severity of drought events (Christensen et al., 2013; Collins et al., 2013). On the other hand, changes in climate projected for the future under unabated emissions have the potential to further alter other disturbance regimes (Dale et al., 2001; Seidl et al., 2014), fires being a major one, owing to the close relationship between climate and fires (Bedia, Herrera, Camia, Moreno, & Gutiérrez, 2014; Bowman et al., 2017; Urbieta et al., 2015; Williams & Abatzoglou, 2016).

Patterns of ecosystem succession after fire are generally well known. Fire generally causes a significant loss of organic matter and alters both soil structure and porosity, while important nutrient fractions are lost due to volatilization, leaching, and soil erosion. Furthermore, fire alters both the quantity and composition of the microbial community. In addition, post-fire soil properties can be significantly affected by changes in total rainfall, rainfall intensity, and rainfall patterns during the year (Certini, 2005). Among them, changes in rainfall intensity (e.g., torrential rainfall) after fire are well known, owing to its negative impacts on soil fertility due to increased runoff and erosion (Certini, 2005; Shakesby, 2011; Shakesby & Doerr, 2006). Nevertheless, the response of the soil microbial community and the dynamics of biogeochemical processes after increased fire severity and fire frequency, as projected under drier and warmer climates, are still poorly understood (Schlesinger et al., 2016). Moreover, when ecosystems experience more than one disturbance (e.g., severe fire and drought), the compound effects are also poorly known, and may lead to ecosystem shifts (Dale et al., 2001; Paine, Tegner, & Johnson, 1998).

Regarding the role of drought and fire on soil biogeochemical processes, it has been shown that soil nutrient availability, microbial activity, and biomass decrease under post-fire severe drought conditions (Potts, Suding, Winston, Rocha, & Goulden, 2012; Hinojosa et al., 2012; Hinojosa, Parra, Laudicina, & Moreno, 2016; Hanan, Tague, & Schimel, 2017). However, under moderate drought (15% rainfall reduction), Hedo de Santiago, Lucas-Borja, Wic-Baena, Andrés-Abellán, and Heras (2016) showed that soil enzyme activities, microbial biomass, and respiration were not affected during a period of 3 years in a medium-age (17 years since the last fire) forest. These studies are far from being conclusive, and there are still major gaps in our understanding of biogeochemical processes and their drivers in post-fire environments under drought.

Additionally, the legacy effect of drought, defined as the impact of previous water scarcity conditions on the current structure and functioning of ecosystems, in burned ecosystems once normal rainfall recovers is something which remains largely unexplored. Understanding this can be of the utmost relevance when predicting the future dynamics of burned systems in subtropical semi-arid areas in which the probability of having longer drought periods during the year and fires is projected to increase (Giorgi & Lionello, 2008). In fact, it has been shown that climate variability in Mediterranean type climate areas increases with reduced precipitation (Cowling, Ojeda, Lamont, Rundel, & Lechmere-Oertel, 2005). In general, legacy effects of reduced rainfall were important in affecting a number of ecosystem processes (Johnstone et al., 2016; Monger et al., 2015; Sala, Gherardi, Reichmann, Jobbagy, & Peters, 2012), while only recently these effects been studied in soil microbial community structure and functioning (Hawkes & Keitt, 2015; Wallenstein & Hall, 2012). So far, drought legacy effects have been investigated after short-term (up to 3 years) (De Vries, Liiri, Bjørnlund, Bowker et al., 2012; De Vries, Liiri, Bjørnlund, Setälä et al., 2012; Allison et al., 2013; Göransson, Godbold, Jones, & Rousk, 2013; Fuchslueger et al., 2016; Meisner, Rousk, & Bååth, 2015; Kaisermann, Vries, Griffiths, & Bardgett, 2017; Martiny et al., 2017; Legay et al., 2018), or long-term (up to 13 years) periods of drought (Evans & Wallenstein, 2012; Rousk et al., 2013). The findings suggest that the expected magnitude of the legacy effects of droughts on terrestrial ecosystems likely depends on drought intensity, as well as on the duration and timing of the drought event (Hawkes & Keitt, 2015; Huang, Wang, Keenan, & Piao, 2018). However, until now, most studies have evaluated legacies effects only after short periods subsequent to the ending of drought (a maximum of 6 months post-drought) and mostly under laboratory or mesocosm conditions (Hawkes & Keitt, 2015). To the best of our knowledge, the legacy effects of drought on soil biogeochemical processes and the microbial community after fire have not been investigated to date. Long-term legacies were found in a number of sites across the world when comparing past and current climates (Averill, Waring, & Hawkes, 2016; Delgado-Baquerizo et al., 2018, 2017; Hawkes, Waring, Rocca, & Kivlin, 2017). The legacy effects of changes in climate, including drought, on soil remain a topic of research.

The aim of this study was to examine the effect of post-fire drought on the dynamics of soil functionality and microbial structure in the field. Moreover, we were also interested in determining the legacy effect of drought on soil nutrient availability and microbial community structure and functionality 2 years after rainfall had returned to normal. To realize these objectives, we conducted a manipulative experiment in a Mediterranean shrubland in which two drought treatments (moderate and severe) were implemented in experimental plots one season before burning them, then maintained 4 years thereafter, after which the drought treatments were discontinued over two additional years. Soil nutrient availability and microbial community composition and functionality were sampled annually during the 4 years under two levels of drought, including a most severe one, mimicking a truly extreme climatic event (Smith, 2011). Additionally, sampling was continued during the two additional years after the drought treatments were discontinued, in order to test the legacy effect of the implemented drought treatments in the burned soils. We hypothesized that the legacy effect of drought in the soil nutrients and microbial community structure and functioning might have been present, even a few years after ceasing the implemented drought, due to the relevant impacts of such extremes on plant growth and community structure (Huang et al., 2018; Kaisermann et al., 2017; Mueller et al.., 2005; Parra & Moreno, 2018; Zeiter, Schärrer, Zweifel, Newbery, & Stampfli, 2016). Understanding the effects of drought and its legacy is important for a better appreciation of the impact of climate change on soil nutrient availability and on soil microbial community structure and functionality in fire-prone semi-arid regions of the world.

2 | MATERIAL AND METHODS

2.1 | Study area

The study was carried out in a *Cistus-Erica* shrubland located at Quintos de Mora Range Station (lat. 39°25′N, long. 04°04′W) in the Montes de Toledo mountain range (Central Spain). The study site was located in a northwest-facing slope at an altitude of 900 m. The climate is continental Mediterranean, with a mean annual temperature of 14.9°C and a mean annual rainfall of 622 mm. Rainfall occurs from autumn to spring (93%), while summer is dry (Los Cortijos meteorological station, 39°19′N, 4°04′W; Agencia Estatal de Meteorología, Spain). The top 5 cm of soil is sandy loam (68%, 18%, and 14% sand, silt, and clay, respectively) forming a Dystric Cambisol (FAO and IUSS Working Group WRB, 2007), with a high proportion of pebbles (40%), the parent rock being mainly quartzite, 6.5 pH, and a 11.5 C:N ratio (see Dannenmann et al., 2018, for further details about the whole soil profile of the study area).

2.2 | Experimental design, rainfall manipulation, and experimental burning

Projections for future climate change in this region anticipate reduced total precipitation and a tendency to concentrate rainfall in the winter months, with a subsequent lengthening of the summer drought (Christensen et al., 2013). Consistent with this, a rainfall manipulative experiment was initiated at the beginning of 2009, with different treatments implemented based on long-term precipitation records (1948–2006) at the nearby Los Cortijos meteorological station (10 km away). Thus, annual precipitation was modified by changing the rainfall pattern and total precipitation from spring to autumn in 6 × 6 m plots, assigned to four experimental treatments, following a randomized complete block design (with four blocks arranged parallel to the slope). The treatments were as follows: (a) environmental control (EC), natural rainfall without any manipulation, 455 mm/year fell on average during the study period, ranging from 366 to 652 mm/year); (b) historical control (HC), simulation of the biweekly long-term rainfall patterns in the study site (i.e., 600 mm/year, percentile 50 of historical data series, drought lasting 2 months, July and August); (c) moderate drought (MD), 25% reduction of HC (i.e., 450 mm/year, percentile 8, and drought lasting 5 months, from May to September); and (d) severe drought (SD), 45% reduction of HC, (i.e., 325 mm/year, percentile 2, drought lasting 7 months, from April to October) (Figures. S1 & S2).

Manipulations were implemented by means of a set of automatic rainout shelters with an irrigation facility installed in each treated plot. Based on historical precipitation records, rainfall targets were set for each 2-week period. Rainfall was allowed to naturally fall until reaching the target for the 2-week period, after which the shelters were displayed in case more rainfall fell. If, at the end of the 2-week period, rainfall had not reached the set target, the plots were irrigated to meet it (Figure S2). This approach was chosen to maximize the input of natural rainfall, while minimizing the display of the rainout shelters, and thus their effects on the microclimate of the plots. In addition, 1-m-wide roofing-type plates were displayed on the surface around each plot to increase the width of the area deprived of rainfall.

In September 2009, the rainout shelters and irrigation system were dismantled in order to allow for the individual burning (+) of each plot, with the exception of one plot per block which was kept unburned (−) and continued receiving natural rainfall, thus serving as and unburned environmental rainfall control (EC−) (Figure S1). Thus, this set of plots permitted the comparison of soil properties between unburned and burned plots under nonmanipulated rainfall, as well as between burned and drought-treated plots.

The experimental burning was similar across all plots. The intensity of the implemented fires was very high (13.5 min of average residence time above 100°C, and 710°C of mean maximum temperatures). A wider description of the experimental setup was reported by Parra, Ramírez, Resco, Velasco, and Moreno (2012). After the fire, the shelters and irrigation system were re-installed and rainfall manipulation continued for four more years, from 2010 to 2013. At the beginning of autumn 2013, the experimental system was dismantled; thereafter all plots received the same natural rainfall.

2.3 | Soil sampling

Soil samples were collected at the end of spring during the 4 years after burning, from 2010 to 2013 (28/05/2010, 24/05/2011, 04/06/2012, and 29/05/2013). Additionally, one extra sampling was carried out in spring 2015 (01/06/2015), that is, after 2 years under natural rainfall (i.e., without rainfall manipulation treatments), in order to test the drought legacy effect of the first 4 years under drought implementation. Four composite samples, each one consisting of four soil cores, were randomly collected from the top 5 cm of soil at each plot. Soil samples were transported to the laboratory in an isothermal bag (4°C), where they were immediately sieved (<2 mm) and gravimetric water content was quantified (104°C, 24 hr) before further analysis. Field-moist samples were stored at 4°C until further analyses, which were carried out within 2 weeks after sampling.

2.4 | Soil nutrient and organic matter content

Soil aliquots were used to analyze exchangeable potassium by atomic absorption (Grant, 1992), ammonium and nitrate by spectrophotometry after 2 M KCl extraction (Keeney & Nelson, 1982), as well as phosphate in 0.5 M $NaHCO_3$ (pH: 8.5) extracts (Olsen & Sommers, 1982) by Joth's (1970) colorimetric method. Soil organic matter was estimated by Walkley and Black's wet oxidation method (Nelson & Sommers, 1996), using 1.724 as correction factor.

2.5 | Soil carbon mineralization rate and enzyme activities

The soil carbon mineralization rate was determined by the alkali-trap method (Anderson, 1982), on soil samples incubated for 15 days at 24°C in the dark and under aerobic conditions. Soils samples were incubated under field-moisture conditions. Additionally, acid phosphatase (EC 3.1.3.2, orthophosphoric-monoester phosphohydrolase, acid optimum), alkaline phosphatase (EC 3.1.3.1, orthophosphoric-monoester phosphohydrolase, alkaline optimum), arylsulfatase (EC 3.1.6.1, arylsulfate sulfohydrolase), and β-glucosidase (EC 3.2.1.21, β-d-glucoside glucohydrolase) activities were determined as described by Tabatabai (1994). Briefly, 1 g of fresh soil was incubated with the corresponding substrate at 37°C for 1 hr, with the product (p-nitrophenol, pNP) measured at 410 nm in the supernatant after stopping the reaction with $CaCl_2$ and a strong alkali.

2.6 | Soil microbial community

Soil microbial community structure was analyzed by the direct extraction of ester-linked fatty acids (ELFAs) from soil samples collected in spring 2010, 2011, 2013, and 2015, according to the method proposed by Schutter and Dick (2000). Briefly, 3 g of soil (fresh weight) was mixed with 15 ml 0.2 M KOH in methanol and 3 µg of internal standard (C19:0), then shaken at 100 rpm at 37°C for 1 hr, thus allowing the release and subsequent methylation of ELFAs. Soil pH was then neutralized by the addition of 3 ml 1.0 M acetic acid, and fatty acid methyl esters (FAMEs) were extracted with 10 ml hexane. The upper hexane layer was transferred to clean tubes and evaporated in a desiccating centrifuge for 1 hr. Dried samples were re-suspended in 100 µl hexane to be analyzed by a gas chromatograph (Thermo Scientific FOCUS™), equipped with a flame ionization detector and a fused-silica capillary column Mega-10 (50 m × 0.32 mm I.D.; film thickness 0.25 µm). The gas chromatograph temperature progression was as follows: initial isotherm at 115°C for 5 min, increase at a rate of 1.5°C per minute from 115 to 230°C, and final isotherm at 230°C for 2 min. The identification of FAME peaks was based on comparing retention times with known standards (Supelco Bacterial Acid Methyl Esters mix cat no. 47080-U and Supelco 37 Component FAME mix cat no. 47885-U). The relative abundance of FAMEs was expressed as mole percent (mol %) of total fatty acids, and quantified relative to nonadecanoic acid (C19:0) as an internal standard. The fatty acid nomenclature was as described by Hinojosa, Carreira, García-Ruíz, and Dick (2005). Fatty acid methyl esters reported as typical of the various microbial groups are: fungi (18:2ω6,9c), gram-negative bacteria (17:0cy and 19:0cy), gram-positive bacteria (i15:0, a15:0, i16:0, i17:0, and a17:0), with (10Me18:0) for the actinomyces within the latter (Bossio & Scow, 1998; Zelles, 1999).

2.7 | Statistical analyses

The different response variables were first statistically analyzed using a randomized block ANOVA. Block effects were never statistically significant. Thus, they were excluded from the final analysis and plots within blocks were considered as independent in subsequent analyses. The effects of fire alone on soil variables under natural rainfall (EC- vs. EC+) were tested using repeated measures for sampling carried out in each of the first 6 years after fire (ANOVA) ($n = 4$). The effects of the drought treatments on soil variables in the burned plots (EC+/HC+/MD+/*SD*+) were tested using repeated measures ANOVA ($n = 4$) for samplings carried out in each of the first 4 years after fire. The legacy effect of the drought treatments on soil variables in the burned plots (EC+/HC+/MD+/*SD*+) was tested using repeated measures ANOVA ($n = 4$) for samplings carried out in the fourth (last year under drought treatments) and sixth years after fire, that is, 2 years under natural rainfall once the drought treatment had been halted. Differences in the burned plots, due to drought treatments in the sixth post-fire year (one-way ANOVA, $n = 4$), determined whether plots had converged or not in their soil properties. In addition, at each sampling time, one-way ANOVA was used to test all the studied rainfall treatments effects, followed by a post-hoc HSD Tukey test. Data were tested for normality and homoscedasticity. Analyses were carried out using STATISTICA 7 (StatSoft, Inc., 2004).

The effect of treatments on soil microbial community structure was tested using PERMANOVA (permutational multivariate analysis of variance), employing the relative abundance of the whole set of fatty acids present in the soil samples (Anderson, 2001). This was done for the first (2010), second (2011), and fourth (2013) year after fire, in addition to the sixth post-fire year. This analysis was carried out with PERMANOVA (Anderson, 2005) based on 9999 permutations and the Bray-Curtis distance measure of dissimilarity for untransformed and unstandardized data. To aid the interpretation of the PERMANOVA analyses, a non-metric multidimensional scaling (NMDS) analysis was performed. NMDS analysis was based on Sørensen's distance and the "slow and thorough" autopilot mode of NMDS in PC-ORD (McCune & Mefford, 1999) using randomized data for a Monte Carlo test of significance. The final stability of each run was evaluated by examining plots of stress (a measure of the dissimilarity between ordinations in the original n-dimensional space and in the reduced dimensional space) vs. the number of iterations.

3 | RESULTS

3.1 | Soil organic matter and C mineralization

Under natural rainfall, fire caused a significant reduction of both soil organic matter and carbon mineralization, an effect which was maintained 6 years after fire. In the burned plots both soil organic matter and carbon mineralization were significantly reduced after 4 years of drought (Table 1, Figure 1). However, these differences disappeared 2 years after halting the treatments, by which time all burned plots had similar values regardless of their previous drought treatment (Table 2, Figure 1).

3.2 | Available soil nutrients

In plots under natural rainfall, soil ammonium concentration significantly decreased as a result of burning during the first two post-fire years, while soil nitrate concentration increased the first year after fire (Table 2, Figure 2). However, fire effects were not significant for either ammonium or nitrate when considering the whole study period (6 years after fire) (Table 1). Ammonium concentration did not change due to drought in the burned plots (Table 1). The first year after fire, nitrate concentration significantly increased in the burned plots under moderate and severe drought conditions in relation to the HC+ treatment. However, in the second year after fire, these plots under drought conditions drastically reduced their concentration of nitrate, showing lower values than the HC+ treatment. From the third year after fire onwards, there were no differences in the

TABLE 1 *F*- and *p*-values from repeated measures ANOVAs testing the effect on soil organic matter, nutrient availability, and enzyme and microbial activity of: (a) fire (EC−/EC+) from first to sixth post-fire year and (b) drought in burned plots (EC+/HC+/MD+/SD+) from first to fourth post-fire year

	(a) Fire under natural rainfall		(b) Drought in burned plots	
	F	*p*	*F*	*p*
SOM				
Treat. (T)	10.454	**0.017**	4.848	**0.011**
Time (t)	2.495	0.069	4.602	**0.007**
Txt	0.699	0.599	2.043	**0.043**
C mineraliz.				
Treat. (T)	6.513	**0.043**	9.262	**0.003**
Time (t)	11.3640	**<0.001**	4.578	**0.009**
Txt	1.783	0.1659	1.323	0.266
Ammonium				
Treat. (T)	3.688	0.103	0.856	0.489
Time (t)	1.307	0.295	24.309	**<0.001**
Txt	1.929	0.138	0.381	0.936
Nitrate				
Treat. (T)	0.708	0.438	7.541	**0.005**
Time (t)	9.276	**<0.001**	19.727	**<0.001**
Txt	0.633	0.644	9.377	**<0.001**
Phosphate				
Treat. (T)	1.820	0.235	0.416	0.744
Time (t)	13.113	**<0.001**	45.703	**<0.001**
Txt	6.344	**0.002**	2.411	**0.029**
Potassium				
Treat. (T)	23.772	**0.003**	1.723	0.215
Time (t)	1.537	0.223	7.149	**<0.001**
Txt	4.624	**0.006**	8.091	**<0.001**
Ac. phosphat.				
Treat. (T)	12.907	**0.011**	6.401	**0.007**
Time (t)	11.786	**<0.001**	28.141	**<0.001**
Txt	1.033	0.410	0.382	0.935
Alk. phosphat.				
Treat. (T)	0.373	0.563	6.882	**0.002**
Time (t)	56.016	**<0.001**	24.434	**<0.001**
Txt	0.345	0.844	7.895	**0.001**
β-glucosidase				
Treat. (T)	2.976	0.078	15.524	**0.007**
Time (t)	2.315	0.093	2.293	0.088
Txt	0.759	0.653	0.330	0.854
Arylsulfatase				
Treat. (T)	9.741	**0.016**	3.967	**0.028**
Time (t)	3.693	**0.015**	13.882	**<0.001**
Txt	1.507	0.226	2.821	**0.007**

Note. The acronyms EC-, EC+, HC+, MD+ and SD+, respectively correspond to: environmental control unburned, environmental control burned, historical control burned, moderate drought burned and severe drought burned treatments.
p-values ≤0.05 are shown in bold typeface.

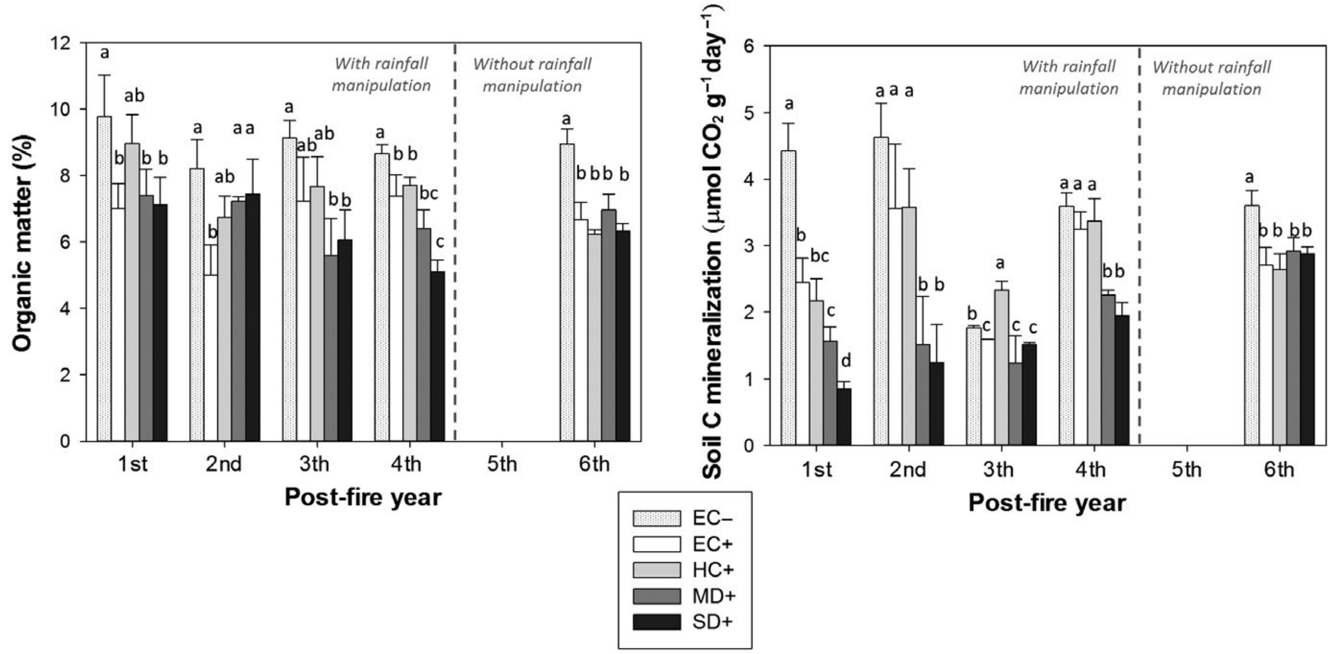

FIGURE 1 Soil organic matter and C mineralization rate from the first to the sixth post-fire year in all study plots. The acronyms EC−, EC+, HC+, MD+, and SD+, respectively correspond to: environmental control unburned, environmental control burned, historical control burned, moderate drought burned and severe drought burned treatments. From the fifth post-fire year, the entire rainfall manipulation system was dismantled and all plots received natural rainfall. For each sampling time, significant differences among the studied treatments are noted with different letters (p-values ≤ 0.05)

burned plots under manipulated rainfall conditions (Figure 2). Once the drought treatments were halted, the absence of differences in the burned plots continued in the case of ammonium and nitrate, independently of their previous rainfall treatment (Table 2, Figure 2).

There was a significant fire and time interaction effect in phosphate concentration under natural rainfall (Table 1). Thus, phosphate concentration significantly increased in burned plots in the first post-fire year, but these differences disappeared from the second year after fire onwards (Figure 2). A significant interaction between drought treatments and time was also observed (Table 1). In the first and fourth post-fire years, phosphate concentration significantly decreased in the burned plots under moderate and severe drought conditions in comparison to the HC+ treatment. These differences remained 2 years after the cessation of rainfall manipulation in the plots previously treated with severe drought (Table 2, Figure 2).

Soil exchangeable potassium concentration decreased due to fire under natural rainfall. The fire factor interacted significantly with time and the highest reduction was observed from the second to the fourth year after fire, with such differences disappearing in the sixth post-fire year. A significant interaction between drought treatments and time also affected this variable. In the first post-fire year, the exchangeable potassium concentration increased in burned soils under moderate and severe drought in relation to the HC+ treatment. Four years after fire, the direction of this effect was reversed and its concentration was lower in plots under drought conditions than in the HC+ (Table 1, Figure 2). Nevertheless, these differences due to drought disappeared after cessation of rainfall manipulation, and all burned plots reached similar values regardless of their previous rainfall pattern (Table 2, Figure 2).

3.3 | Soil enzyme activity

Under natural rainfall, soil enzyme activities, with the exception of alkaline phosphatase, initially decreased because of fire (Table 1, Figure 3). This significant effect persisted until the sixth post-fire years only in the case of acid phosphatase and arylsulfatase activities. In relation to the rainfall manipulation effects in burned plots, a significant reduction in all enzyme activities due to drought was observed (Table 1, Figure 3). However, 2 years after the cessation of rainfall manipulation, the drought effect persisted for alkaline phosphatase and β-glucosidase, which showed lower values in soils previously treated with severe drought, in comparison to those treated as historical or ECs (Table 1, Figure 3).

3.4 | Soil ester linking fatty acid profiling

The concentration of total fatty acids in soils (also used as an indicator of soil microbial biomass) under natural rainfall was significantly lower in burned than in unburned soils during the six post-fire years. It also decreased because of drought in the burned plots (Table 3, Figure 4). Nevertheless, 2 years after the cessation of rainfall manipulation, the concentration of total fatty acids in soil partially recovered in the plots previously treated with severe and moderate drought (Table 4, Figure 4).

TABLE 2 The effect on soil organic matter, nutrient availability, and enzyme and microbial activity of different rainfall patterns before and after removing rainfall manipulation systems for all burned plots (EC+/HC+/MD+/SD+), reporting the following: (a) *F*- and *p*-values from repeated measures ANOVAs testing the differences in burned plots between the fourth and sixth post-fire years in interaction with time, (b) *F*- and *p*-values from one-way ANOVAs testing differences in burned plots after removing the rainfall manipulation system on the sixth post-fire year

	(a) Differences in burned plots before and after removing rainfall manipulation (time interaction)		(b) Differences in burned plots after removing rainfall manipulation	
	F	*p*	*F*	*p*
SOM				
Treat. (T)	7.462	**0.004**	3.236	0.061
Time (t)	2.145	0.168		
Txt	9.193	**0.002**		
C mineraliz.				
Treat. (T)	0.909	0.468	0.453	0.719
Time (t)	3.763	0.078		
Txt	13.566	**<0.001**		
Ammonium				
Treat. (T)	1.824	0.196	2.084	0.155
Time (t)	5.277	0.040		
Txt	1.613	0.238		
Nitrate				
Treat. (T)	1.326	0.315	2.851	0.059
Time (t)	0.653	0.436		
Txt	4.896	**0.021**		
Phosphate				
Treat. (T)	0.448	0.722	6.091	**0.009**
Time (t)	1.981	0.184		
Txt	3.955	**0.035**		
Potassium				
Treat. (T)	0.409	0.692	2.099	0.138
Time (t)	2.318	0.153		
Txt	5.443	**0.013**		
Ac. phosphat.				
Treat. (T)	4.972	**0.018**	3.142	0.065
Time (t)	24.611	<0.001		
Txt	5.628	0.012		
Alk. phosphat.				
Treat. (T)	4.922	**0.018**	4.821	**0.019**
Time (t)	80.891	<0.001		
Txt	3.329	0.056		
β-glucosidase				
Treat. (T)	3.604	**0.045**	13.272	**<0.001**
Time (t)	14.823	**0.002**		
Txt	89.963	**<0.001**		
Arylsulfatase				
Treat. (T)	3.226	0.061	2.106	0.139
Time (t)	15.327	**0.002**		
Txt	8.374	**0.002**		

Note. The acronyms EC+, HC+, MD+, and SD+, respectively correspond to: burned plots with environmental control, historical control, moderate drought and severe drought treatments.
p-values ≤0.05 are shown in bold typeface.

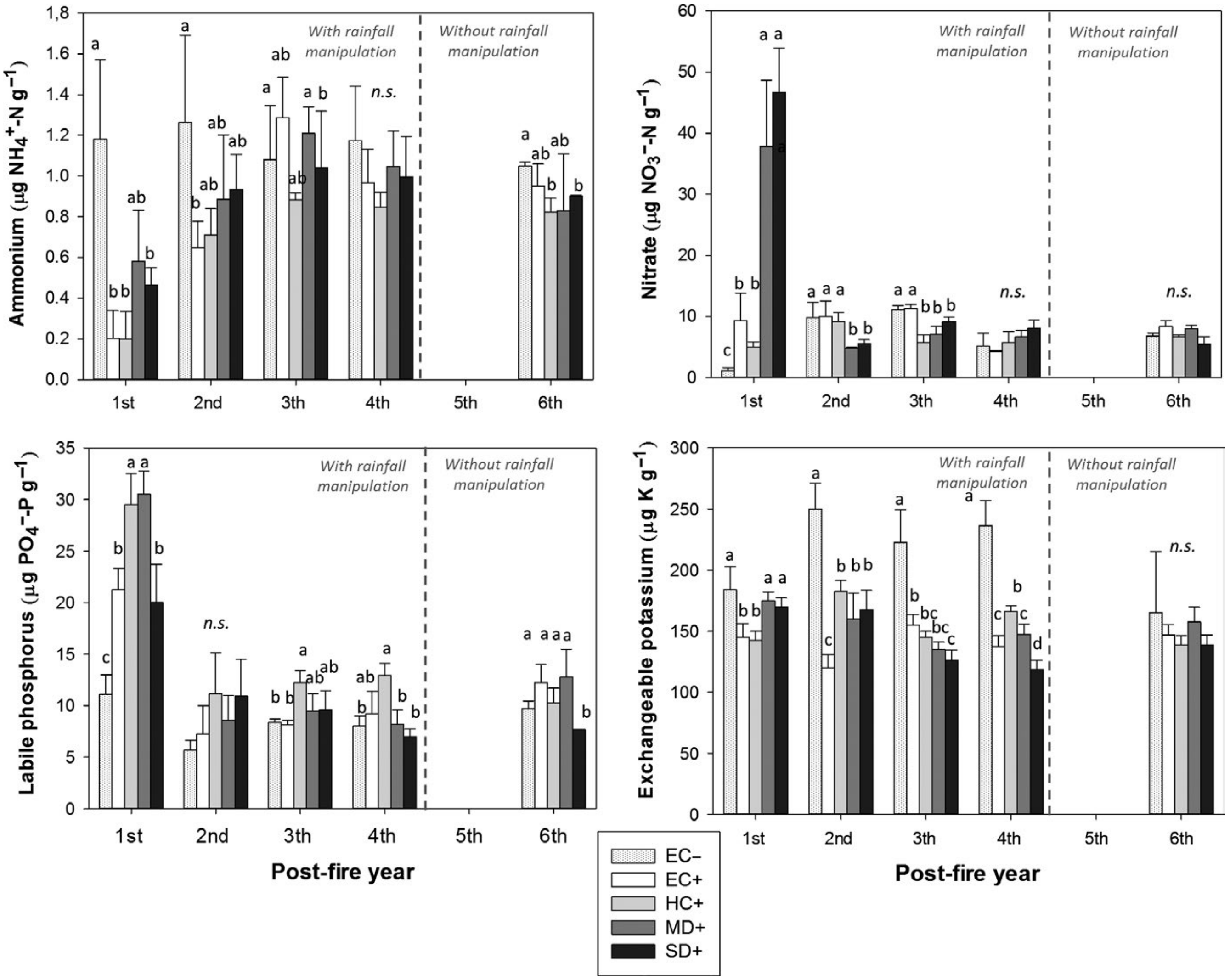

FIGURE 2 Soil concentration of ammonium, nitrate, inorganic phosphate, and exchangeable potassium from the first to the sixth post-fire year in all study plots. The acronyms EC-, EC+, HC+, MD+, and SD+, respectively correspond to: environmental control unburned, environmental control burned, historical control burned, moderate drought burned and severe drought burned treatments. From the fifth post-fire year, the entire rainfall manipulation system was dismantled and all plots received natural rainfall. For each sampling time, significant differences among the studied treatments are noted with different letters (p-values ≤ 0.05)

The relative abundance of general bacteria markers was not significantly affected by fire during the study period; likewise gram-positive or gram-negative bacteria markers. Drought caused a significant decrease in general bacteria and gram-positive markers in the burned plots the second year after fire; however, they recovered 2 years later (Table 3, Figure 5). Interestingly, after the cessation of rainfall manipulation, gram-positive bacteria markers in plots previously treated with severe and moderate drought significantly increased in comparison to natural or historical rainfall treatments (Table 4, Figure 5).

The relative abundance of fungi markers was lower in burned than in unburned plots under natural rainfall in the first two post-fire years. Nevertheless, this effect disappeared with time, and it was not observed 4 or 6 years after fire. The effect of drought in the burned plots was only significant in the second year after fire (Table 3, Figure 5). While fire initially increased the concentration of soil actinomyces under natural rainfall conditions, the effect of fire in term of its concentration was not significant when considering the whole study period (Table 3, Figure 5). In burned soils, drought caused an increase in the concentration of actinomyces markers, which was still significant for severe drought treatments 2 years after the cessation of rainfall manipulation (Tables 3 and 4, Figure 5).

The multivariate analysis of the fatty acid profile for the implemented treatments, performed using the PERMANOVA test, revealed significant differences between the burned and unburned soils under natural rainfall treatments conditions in the first and second post-fire years (Table 5). However, these differences disappeared from the fourth post-fire year onwards. The effect of drought on the burned plots was not evident in the first post-fire year. Nevertheless, significant differences were observed between HC and drought treatments in subsequent years, including after the cessation of rainfall manipulation (up to the sixth

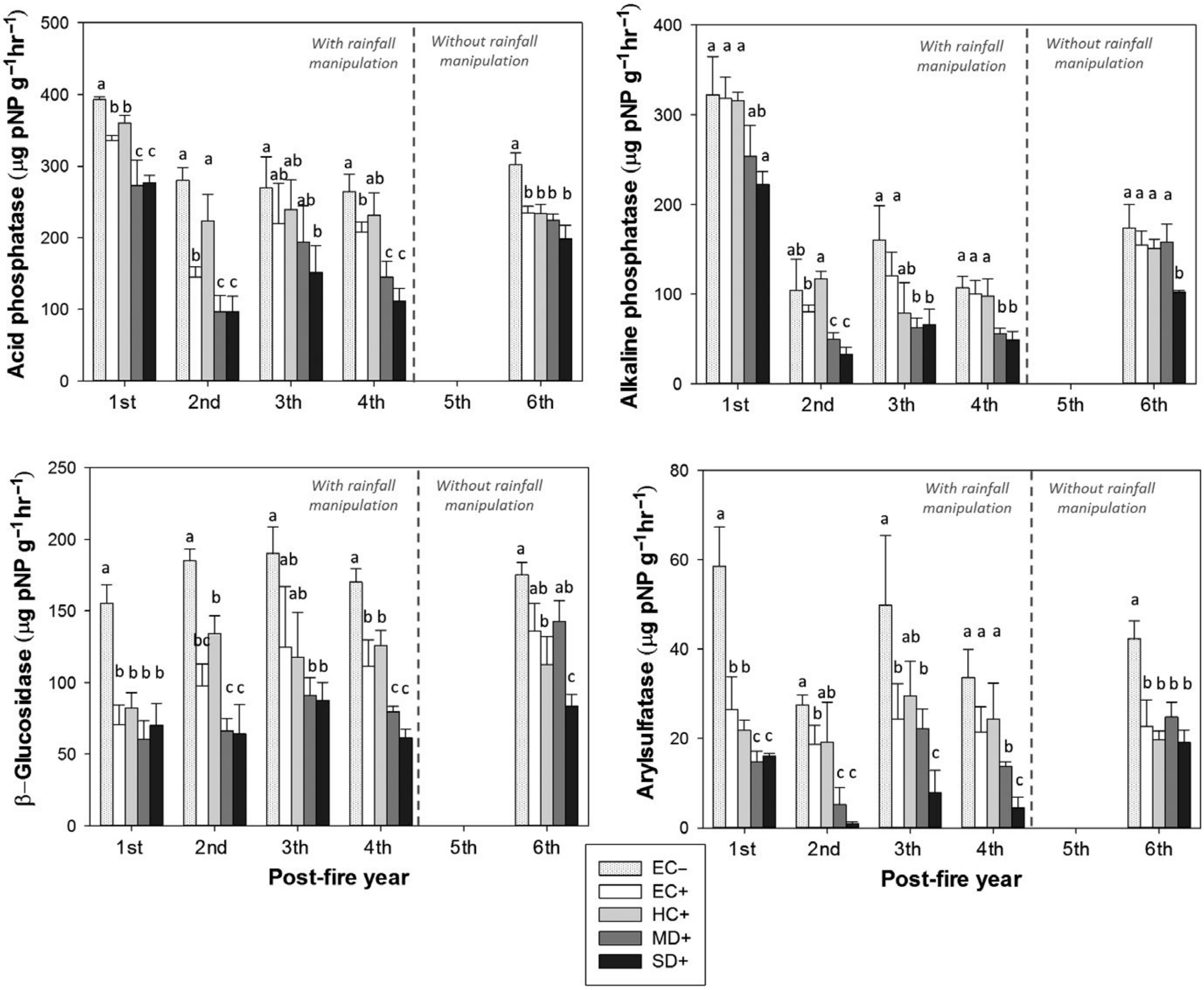

FIGURE 3 Soil enzyme activities from the first to the sixth post-fire year in all study plots. The acronyms EC−, EC+, HC+, MD+, and SD+, respectively correspond to: environmental control unburned, environmental control burned, historical control burned, moderate drought burned and severe drought burned treatments. From the fifth post-fire year, the entire rainfall manipulation system was dismantled and all plots received natural rainfall. For each sampling time, significant differences among the studied treatments are noted with different letters (*p*-values ≤ 0.05)

post-fire year). Non-metric multidimensional scaling analysis, using the whole set of ELFAs, confirmed the PERMANOVA results and differentiated between the microbial communities in burned and unburned soils in the first year after fire. The effect of drought in burned plots was evident from the second post-fire year, with no recovery being observed when rainfall manipulation was discontinued (Figure 6.).

4 | DISCUSSION

4.1 | Effect of drought

This study shows that the initial increase of nitrate and exchangeable potassium observed in the short term in burned plots due to drought (Hinojosa et al., 2016), which could be attributed to lower lixiviation and lower plant and/or microbial uptake (Johnson, Todd, & Hanson, 2008; Rouphael, Cardarelli, Schwarz, Franken, & Colla, 2012), was short lived. Although nutrient lixiviation was not evaluated in this study, this hypothesis could be supported by the fact that, during the first two post-fire years, total plant cover was 35%–40% lower in the burned plots under drought treatments (SD+ and MD+) than for the control treatment (HC+) (Figure S3). Additionally, a significant negative correlation was observed between soil nitrate concentration and microbial biomass (determined as the total amount of fatty acids) in the first post-fire year (Pearson's correlation coefficient, $r = -0.678$, $p < 0.05$, $n = 20$). Nitrate values returned to those observed in the HC+ treatment 3 years after fire. With time, exchangeable potassium decreased in the burned soil due to drought, showing lower values than in the case of the HC+. Concerning phosphorus, Hinojosa et al.

TABLE 3 *F*- and *p*- values from repeated measures ANOVAs testing the effect on soil fatty acid amount and relative abundance of microbial groups markers of: (a) fire (EC−/EC+) from first to sixth post-fire year and (b) drought in burned plots (EC+/HC+/MD+/SD+) from first to fourth post-fire year

	(a) Fire under natural rainfall		(b) Drought in burned plots	
	F	*p*	*F*	*p*
Total fatty acid				
Treat. (T)	8.941	**0.026**	4.848	**0.011**
Time (t)	1.854	0.142	3.602	**0.019**
Txt	0.452	0.794	2.543	**<0.001**
Bacteria				
Treat. (T)	6.626	0.061	20.786	**<0.001**
Time (t)	4.775	**0.021**	32.104	**<0.001**
Txt	1.777	0.205	2.411	0.061
Fungi				
Treat. (T)	7.539	**0.045**	3.267	0.062
Time (t)	1.994	0.102	34.469	**<0.001**
Txt	3.624	**0.012**	0.780	0.594
Gram+				
Treat. (T)	7.003	0.057	15.014	**<0.001**
Time (t)	1.863	0.189	17.786	**<0.001**
Txt	2.030	0.163	3.268	**0.018**
Gram−				
Treat. (T)	0.010	0.923	1.923	0.184
Time (t)	18.242	**<0.001**	27.961	**<0.001**
Txt	1.359	0.302	0.700	0.652
Actinomyces				
Treat. (T)	4.437	0.103	6.813	**0.007**
Time (t)	3.444	0.052	0.395	0.678
Txt	1.715	0.217	1.428	0.248

Note. The acronyms EC−, EC+, HC+, MD+, and SD+, respectively correspond to: environmental control unburned; environmental control burned, historical control burned, moderate drought burned and severe drought burned treatments.
p-values ≤0.05 are shown in bold typeface.

(2012), Hinojosa et al. (2016) indicated that a drier environment limits the extent of post-fire peak in soil phosphorous availability in the short term. However, the results showed that, 4 years after fire, plots subjected to drought had an even lower phosphorous availability than the burned soils under HC+ rainfall conditions. This result could be explained by a lower decomposition rate for plant detritus input, coupled with a lower soil water availability. This hypothesis is supported by the strong reduction in the soil respiration rate and enzyme activities, as observed in this experiment in burned soils under drought conditions. Our results complement the findings of Peñuelas et al. (2017), who, based on revision of data from field experiments and long-term monitoring of field gradients, concluded that drought has an important effect

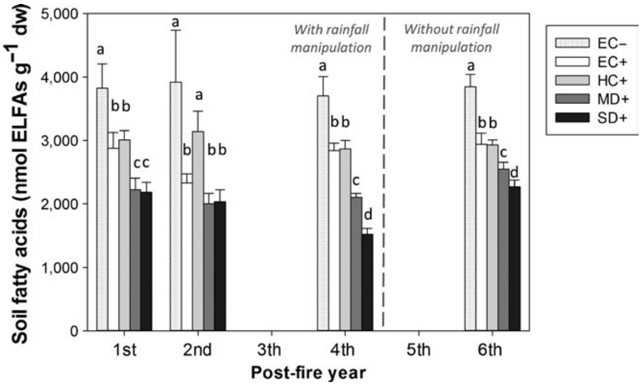

FIGURE 4 Total amount of soil fatty acids (ester-linked fatty acid [ELFAs]) from the first to the sixth post-fire year in all study plots. The acronyms EC−, EC+, HC+, MD+, and SD+, respectively correspond to: environmental control unburned, environmental control burned, historical control burned, moderate drought burned and severe drought burned treatments. From the fifth post-fire year the entire rainfall manipulation system was dismantled and all plots received a natural rainfall pattern. For each sampling time, significant differences among the studied treatments are noted with different letters (*p*-values ≤ 0.05)

on nutrient cycling in the plant-soil system of Mediterranean terrestrial ecosystems.

Consistent with the above results, the observed reduction in soil respiration rate under drought conditions suggests that the carbon cycling could be slowed down due to drought in burned soils. Such results agree with those of Wu, Dijkstra, Koch, Peñuelas, and Hungate (2011) and Vicca et al. (2014), who observed a reduction of soil microbial respiration in throughfall manipulation experiments carried out in unburned soils. Our results allow us to argue that the reduction in soil respiration as a consequence of drought could be largely driven by a reduction in microbial biomass and soil organic matter in the short term, which is supported by a strong correlation between these variables (Table S1). However, the relationships between microbial biomass and C mineralization tended to disappear with time. Therefore, with time, other factors, such as a reduction of the amount and quality of soil organic matter (Vargas et al., 2012) or an alteration to the soil microbial community structure (Canarini, Kiær, & Dijkstra, 2017; Goberna, García, Insam, Hernández, & Verdú, 2012) may cause decreased soil microbial activity. The observed reduction in enzyme activities is consistent with the positive correlation between these changes and soil moisture reported in many studies (Alster, German, Lu, & Allison, 2013; Baldrian, Merhautová, Petránková, Cajthaml, & Šnajdr, 2010; Xue et al., 2017). However, not all studies found such an explicit link between drought and enzyme activity (Bell et al., 2009; Steinweg, Dukes, & Wallenstein, 2012).

The soil microbial community plays an important role in the recovery of post-fire ecosystem functioning, but how reduced rainfall due to climate change might affect the post-fire community is poorly understood. In unburned soils, it has been reported that an increase in the intensity and frequency of droughts can lead to a decrease in microbial growth as well as to changes in microbial community

TABLE 4 The effect on soil fatty acid amount and relative abundance of microbial groups markers of different rainfall patterns before and after removing rainfall manipulation systems for all burned plots (EC+/HC+/MD+/SD+), reporting the following: (a) *F*- and *p*-values from repeated measures ANOVAs testing the differences in burned plots between the fourth and sixth post-fire years in interaction with time, (b) *F*- and *p*-values from one-way ANOVAs testing differences in burned plots after removing the rainfall manipulation system in the sixth post-fire year

	(a) Differences in burned plots before and after removing rainfall manipulation (time interaction)		(b) Differences in burned plots after removing rainfall manipulation	
	F	*p*	*F*	*p*
Total fatty acid				
Treat. (T)	11.005	**0.002**	4.852	**0.018**
Time (t)	0.541	0.480		
Txt	2.838	0.063		
Bacteria				
Treat. (T)	12.719	<0.001	5.082	**0.012**
Time (t)	1.576	0.238		
Txt	1.241	0.346		
Fungi				
Treat. (T)	3.024	0.080	2.854	0.090
Time (t)	0.910	0.362		
Txt	0.231	0.872		
Gram+				
Treat. (T)	10.637	**0.002**	3.781	**0.043**
Time (t)	0.530	0.483		
Txt	1.300	0.327		
Gram−				
Treat. (T)	2.748	0.098	1.674	0.234
Time (t)	6.610	0.028		
Txt	0.838	0.503		
Actinomyces				
Treat. (T)	3.428	0.060	3.575	**0.048**
Time (t)	0.746	0.408		
Txt	1.321	0.322		

Note. The acronyms EC+, HC+, MD+, and SD+, respectively correspond to the burned plots with environmental control, historical control, moderate drought and severe drought treatments.
p-values ≤0.05 are shown in bold typeface.

structure (e.g., Iovieno & Bååth, 2008; Barnard et al., 2013; Evans, Wallenstein, & Burke, 2014; Bastida et al., 2017). In the burned soils of this experiment, microbial biomass was significantly reduced because of drought, while its effects persisted over the 4 years under drought treatments. Drought directly affects soil microorganisms by creating osmotic stress, which can bring them to a dormant state (Blagodatskaya & Kuzyakov, 2013) or death (Xue et al., 2017). In addition, indirect changes in belowground inputs, due to the response of the plant community to drought stress, could influence its functional structure (Classen et al., 2015; Milcu, Paul, & Lukac, 2011; Sanaullah, Blagodatskaya, Chabbi, Rumpel, & Kuzyakov, 2011). In line with this, Parra and Moreno (2018) showed that drought differentially affected different groups of plants, whereby it negatively affected seeder plant species but not resprouter ones, while causing a greater abundance of herbs.

This rainfall-manipulation experiment demonstrates that, in the short term (up to 2 years), drought produces asymmetrical changes in the microbial community, with a decrease of fungi and bacteria (mainly gram-positive), and an increase of actinomyces (Hinojosa et al., 2016). However, some of the main microbial groups, such as gram-positive bacteria and fungi, tended to recover after 4 years of post-fire drought. Gram-positive bacteria are considered to be more resistant to drought due to their cell wall composition (Manzoni, Schimel, & Porporato, 2012). Many of them have strong cell walls and form spores, which are resistant to desiccation and can survive under drought conditions (Singh, Munro, Potts, & Millard, 2007; Zhang & Xu, 2008). In the same way, fungi are more tolerant to dry periods than bacteria (Manzoni et al., 2012; Schimel, Balser, & Wallenstein, 2007; Strickland & Rousk, 2010). Indeed, fungi can create large hyphal networks capable of facilitating nutrient and water transfer over long distances, in turn allowing for the exploration of water-filled soil pores not accessible to plant roots, which have lower nutrient requirements than bacteria (Strickland & Rousk, 2010). Overall, our results highlight the differentiated response of the microbial community to drought after fire.

4.2 | Drought legacy effects

Our results suggest that some of the effects of drought will persist over time after the end of such a disturbance. Indeed, we found a significant drought legacy effect in the case of microbial biomass (as indicated by the total fatty acid amount). Additionally, the legacy effect was different for the various microbial groups analyzed. While gram-positive bacteria and actinomyces maintained similar levels ~~than under~~ drought conditions, gram-negative bacteria and fungi did not change as they had done previously, even under the long-term drought conditions. In agreement with our findings, several studies have previously confirmed that gram-positive bacteria (including actinomyces) are more resistant to drought stress and further rewetting than the gram-negative bacteria. The general morphology and life history strategies of microbial groups suggest that the soil drying and rewetting shock should select for ~~gram-negative bacteria~~ against ~~gram-positive bacteria~~ and fungi (Schimel et al., 2007).

On the other hand, given the important role of soil microorganism in the release of nutrients from soil organic matter, impacts on soil nutrients could be considered another important component of the drought legacy effects (Cavagnaro, 2016). In our study, although no legacy effect in the case of soil mineral nitrogen or potassium was found, soil labile phosphorus showed a drought legacy effect, with lower values in burned plots previously exposed to drought. This effect could be explained by the legacy effect of drought on

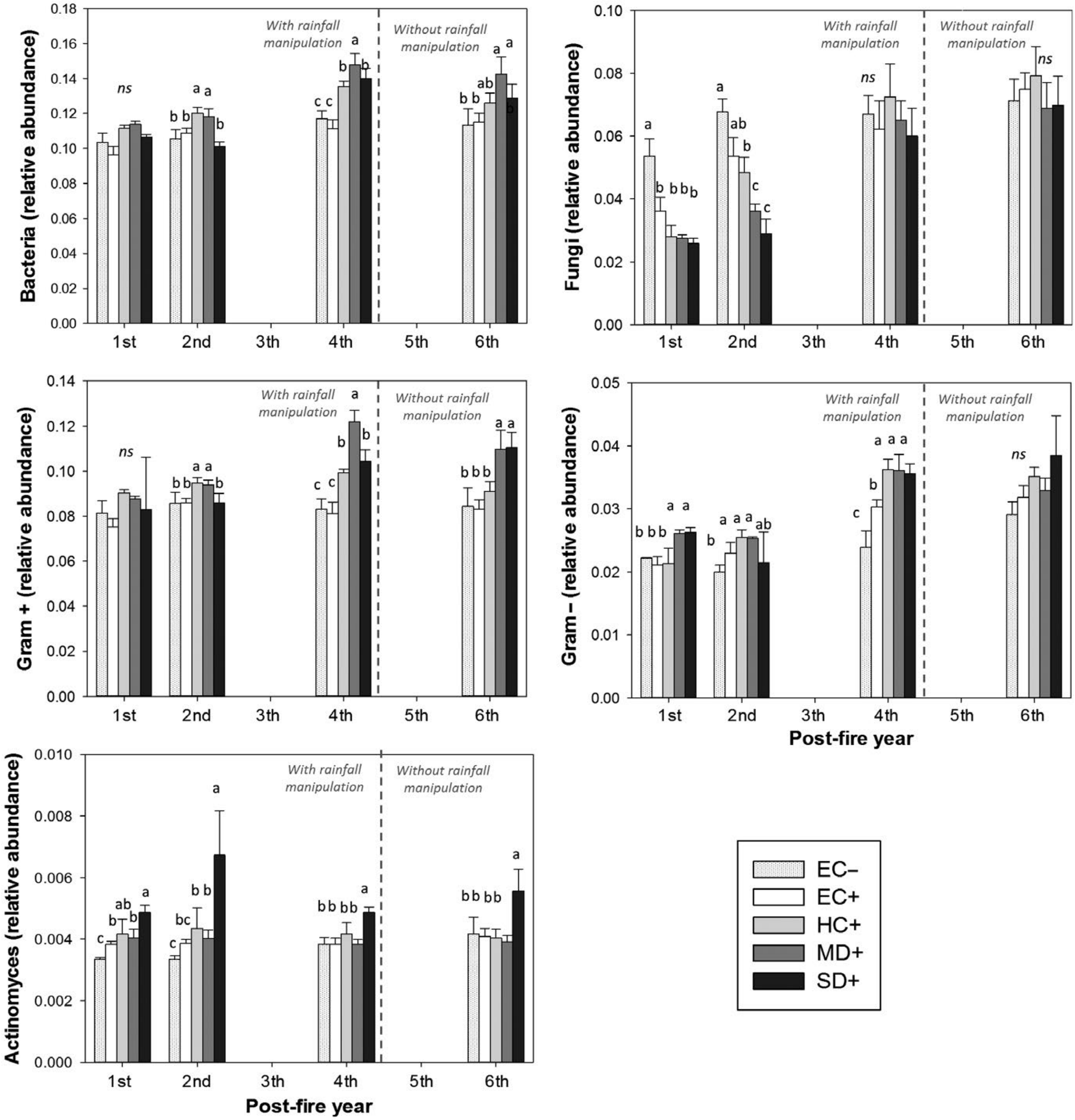

FIGURE 5 Relative abundance of the main fatty acid groups (markers) from the first to the sixth post-fire year in all study plots. The acronyms EC−, EC+, HC+, MD+, and SD+, respectively correspond to: environmental control unburned, environmental control burned, historical control burned, moderate drought burned and severe drought burned treatments. From the fifth post-fire year the entire rainfall manipulation system was dismantled and all plots received a natural rainfall pattern. For each sampling time, significant differences among the studied treatments are noted with different letters (p-values ≤ 0.05)

the capacity of soil to mineralize soil organic matter. Some of the studied soil enzyme activities (acid phosphatase, among them) maintained reduced potential activity in soils previously exposed to drought, even 2 years after recovering the natural rainfall pattern. Kaisermann et al. (2017), using a short-term mesocosm experiment, also provided evidence that legacy effects of drought on soil microbial communities alter their functional capabilities when faced with subsequent drought. In addition, the legacy effect of drought observed in this study could also be due to the direct and indirect effect of drought on plant growth and community structure during the drought (Anderegg et al., 2015; Huang et al., 2018; Walter, Jentsch, Beierkuhnlein, & Kreyling, 2013), which is consistent with the findings of Parra & Moreno (2017; 2018) for this experimental site. Such changes in the plant community, due to drought, may in turn, affect

TABLE 5 Results of the PERMANOVA test based on values of the relative abundance of fatty acids (ester-linked fatty acids) present in soil samples from the study treatments (i.e., unburned environmental control, EC−; burned environmental control, EC+; burned historical control, HC+; burned moderate drought, MD+; burned severe drought, SD+) at the four sampling times

			Pairwise comparison subsets				
	F	*p*	EC−	EC+	HC+	MD+	SD+
1st post-fire year	1.596	0.041	a	b	b	b	b
2st post-fire year	2.197	0.004	a	ab	b	c	c
4st post-fire year	1.683	0.027	a	a	a	b	c
6st post-fire year[a]	1.559	0.046	a	a	a	b	b

Note. Different letters in the pairwise comparison subset denote significant differences in treatments.
[a]Two years without rainfall manipulation.

the quantity and quality of the input of plants into the burned soils (Legay et al., 2018; Preece & Peñuelas, 2016).

Previous studies in unburned soils also found legacy effects of short- or long-lasting drought on microbial biomass, activity, and community structure (Evans & Wallenstein, 2012: De Vries, Liiri, Bjørnlund, Bowker et al., 2012; De Vries, Liiri, Bjørnlund, Setälä et al., 2012; Martiny et al., 2017; Meisner, Jacquiod, Snoek, Hooven, & Putten, 2018; Legay et al., 2018). On the other hand, Rousk et al. (2013) reported no legacy effects of long-term experimental summer drought on microbial community size, composition, growth rates, or respiration rates. Nevertheless, this study was carried out with soils sampled in winter, when drought treatments were inactive and soil temperatures were very low. This complicates any discernment between a true legacy effect and a seasonal effect. Fuchslueger, Bahn, Fritz, Hasibeder, and Richter (2014) also described a highly resilient microbial community to a severe drought after a week of rewetting. That said, in a further study, these authors reported that drought treatments altered microbial functioning, particularly in the turnover of recent plant-derived carbon, during and after further drought periods (Fuchslueger et al., 2016).

In summary, the legacy effect of drought on soil microorganisms under field conditions depends on the intensity and length of the drought event and the sensitivity of the soil system to change, which may affect the duration of the effects (Banerjee et al., 2016; Evans & Wallenstein, 2012; Meisner et al., 2015; Monger, Rachal, Driese, & Nordt, 2013). Legacy effects of drought can be sufficiently important to be considered when modeling rates of biogeochemical processes in burned soils.

4.3 | Effect of fire

Our experiment enabled us to evaluate soil changes due to fire under natural rainfall conditions over a 6-year period. Such a long period of study is uncommon, since the majority of studies tend to contemplate the short-term post-fire monitoring of soil properties or consist of isolated long-term research, without any temporal evaluation of the dynamics of soil properties. In general, the effect of fire on soil nutrients was short lived, and changes produced by fire in soil microbial community structure typically disappeared with time. However, some soil functions (soil respiration and enzyme activities) did not recover 6 years after the fire. Consequently, a significant increase in soil nitrate, ammonium, and phosphate has been reported to occur immediately after fire, mainly as an effect of pyromineralization (Dannenmann et al., 2018; Hinojosa et al., 2016, 2012; Karhu et al., 2015). However, results obtained here suggest that such changes were transient since they disappeared in the second spring after fire.

The lower concentration of exchangeable potassium in burned soils under natural rainfall conditions compared to unburned ones does not coincide with previous studies (Caon, Vallejo, Ritsema, & Geissen, 2014; Certini, 2005), which reported an ephemeral increase in potassium after fire. Such contrasting results may be due to the fact that, as our first soil sampling was carried out 8 months after fire, leaching or surface runoff may have occurred in the period between fire and sampling (Cancelo-González, Rial-Rivas, & Díaz-Fierros, 2013).

The temperatures reached during burning were sufficiently high (Parra et al., 2012) in order to reduce soil organic matter and microbial biomass in the surface layers (DeBano, Neary, & Ffolliott, 1998). Thus, the low soil microbial activity (respiration rate and enzyme activities) observed in the burned soils shortly after fire (first post-fire spring) could be due to the slow recovery of soil microbial biomass through the reduction and modification of soil organic matter (Certini, 2005; González-Pérez, González-Vila, Almendros, & Knicker, 2004). Furthermore, it could also be associated with a lower plant cover observed the first 2 years after fire (Figure S3) (Parra & Moreno, 2018). Mataix-Solera, Guerrero, García-Orenes, Bárcenas and Torres (2009) reported that when the vegetation recovers, and the rates of organic inputs to soil are re-established, a trend toward pre-fire values is expected. Our finding, however, suggests that, with time, a decoupling between the post-fire recovery of plant cover and both soil organic matter and microbial activity and biomass occurred. Although plant cover was highly recovered 4 years after fire (Parra & Moreno, 2018), soil organic matter and enzyme activity (mainly, arylsulfatase, acid phosphatase and β-glucosidase) were still significantly lower in burned soils than in unburned ones under natural rainfall

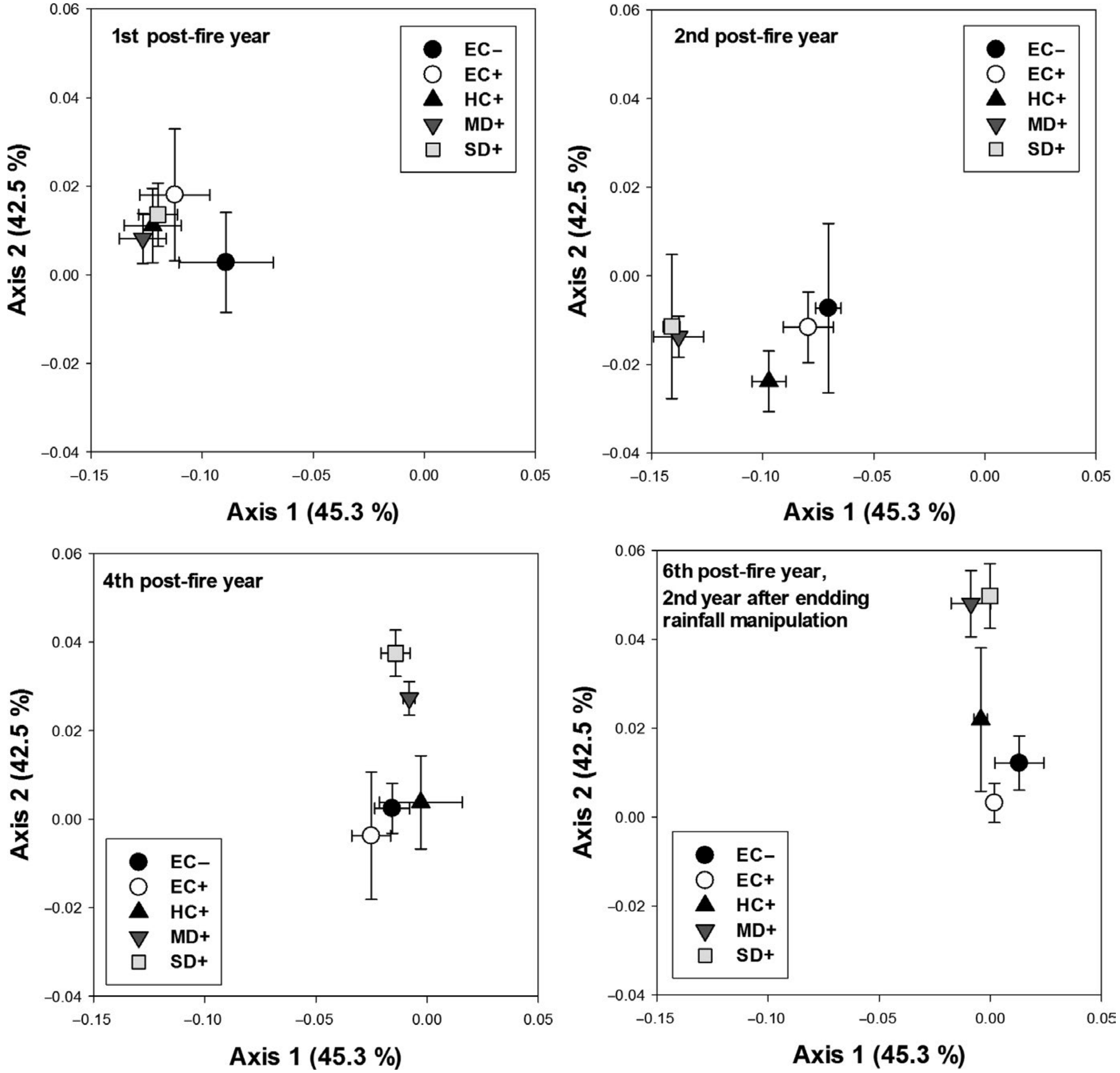

FIGURE 6 Non-metric multidimensional scaling (NMDS) analysis of fatty acid profiles for soils in all study treatments. A single NMDS analysis was carried out, but the results are displayed in four separate graphs to illustrate the differences and similarities across the four sampling years. Mean values ± *SE* are shown. The proportion of variance explained by each axis is reported in brackets

condition, even 6 years after fire. This long-lasting effect of fire on soil enzyme activity supports the claims of previous studies (Brooks, Twieg, Grayston, & Jones, 2013; López-Poma & Bautista, 2014; Miesel, Boerner, & Skinner, 2011; Staddon, Duchesne, & Trevors, 1998). Soil fatty acid profile data indicated a significant effect of fire under natural rainfall condition on soil microbial biomass and community structure. In the short term, fire significantly reduced soil microbial biomass, supporting earlier findings (Certini, 2005; Neary, Klopatek, DeBano, & Ffolliott, 1999). Such reduction was long lasting, and significant, even 6 years after burning. However, the observed changes in the soil microbial community structure were less persistent over the time. Since the first sampling, bacterial markers showed no significant changes, probably due to their fast post-fire recovery, since post-fire conditions are beneficial for their proliferation (Choromanska & DeLuca, 2002; Jokinen, Kiikkilä, & Fritze, 2006). The significant increase of gram-negative bacteria markers during the first post-fire years could be associated with bacterial growth (Söderberg, Probanza, Jumpponen, & Bååth, 2004). At the same time, the higher relative abundance of actinomyces could be related to their ability (as well as many others bacteria) to form spores which are highly resistant to the heat of fire (Bárcenas-Moreno, Rousk, & Bååth, 2011).

Nevertheless, fungal markers were significantly lower after fire. As fungi, in general, are killed at lower temperatures than bacteria, they could have suffered a significant decrease in the length of the mycelium as a consequence of fire (Mataix-Solera et al., 2009; Vázquez, Acea, & Carballas, 1993). In addition, fungi may be also sensitive to some toxic compounds present in burned soils (Fritze, Pennanen, & Kitunen, 1998; Widden & Parkinson, 1975). Six years after fire, all these effects disappeared with time and microbial community structure tended to be similar to that of unburned soils.

In summary, although some soil properties, such as soil nutrient availability and microbial community structure, recovered 6 years after fire under natural rainfall conditions, variables related to soil functionality (soil enzyme activities and soil respiration) did not, thereby confirming the long-lasting effect of fire. This slow recovery of soil functionality appears to be related to a slow recovery of soil microbial biomass and soil organic matter content. These results indicate that the resilience of some soil properties affected by fire is not as high as it might be assumed, which should be considered in the post-fire management of such ecosystems. In fact, the capacity of soil properties to recover from fire can be affected by changes in fire regime characteristics (i.e., fire intensity and/or fire frequency), among other factors (Pereira, Francos, Brevik, Ubeda, & Bogunovic, 2018). In this sense, changes in fire intensity or frequency, which are projected to occur under unabated climate change conditions (Amatulli, Camia, & San-Miguel-Ayanz, 2013; Bedia et al., 2015; Turco et al., 2018), must receive special attention due to their important effects on the ecosystem (Safford et al. 2009; Zedler, Gautier, & McMaster, 1983). These changes in fire regime, together with extreme weather events, such as drought, may lead to complex interactions and unpredictable effects, including tipping points (Seidl et al., 2017).

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ORCID

María Belén Hinojosa https://orcid.org/0000-0002-1241-8637

Vito Armando Laudicina https://orcid.org/0000-0002-5531-0669

Antonio Parra https://orcid.org/0000-0002-0327-2875

José Manuel Moreno https://orcid.org/0000-0002-3729-9523

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