Experimental drought induces short-term changes in soil functionality and microbial community structure after fire in a Mediterranean shrubland

M. B. Hinojosa¹, A. Parra¹, V. A. Laudicina², and J. M. Moreno¹

¹Departamento de Ciencias Ambientales, Universidad de Castilla-La Mancha, Campus Fábrica de Armas, 45071, Toledo, Spain
²Dipartimento Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze, Edificio 4, 90128 Palermo, Italy

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Correspondence to: M. B. Hinojosa (mariabelen.hinojosa@uclm.es)
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Abstract

Fire is a major ecosystem driver, causing significant changes in soil nutrients and microbial community structure and functionality. Post-fire soil dynamics can vary depending on rainfall patterns, although variations in response to drought are poorly known. This is particularly important in areas with poor soils and limited rainfall, like arid and semiarid ones. Furthermore, climate change projections in many such areas anticipate reduced precipitation and longer drought, together with an increase in fire severity. The effects of experimental drought and fire were studied on soils in a Mediterranean Cistus-Erica shrubland in Central Spain. A replicated \((n = 4)\) field experiment was carried out in which four levels of rainfall pattern were implemented by means of a rain-out shelters and irrigation system. The treatments were: environmental control (natural rainfall), historical control (long-term average rainfall, 2 months drought), moderate drought (25 % reduction of historical control, 5 months drought) and severe drought (45 % reduction, 7 months drought). After one growing season, the plots were burned with high fire intensity, except a set of unburned plots that served as control. Soils were collected seasonally during one year and variables related to soil nutrient availability and microbial community structure and functionality were studied. Burned soils increased nutrient availability \((P, N, K)\) with respect to unburned ones, but drought reduced such an increase in \(P\), while it further increased \(N\) and \(K\). Such changes in available soil nutrients were short-lived. Drought caused a further decrease of enzyme activities, carbon mineralization rate and microbial biomass. Fire decreased the relative abundance of fungi and actinomycetes. However, fire and drought caused a further reduction in fungi, with bacteria becoming relatively more abundant. Arguably, increasing drought and fires due to climate change will likely shift soil recovery after fire.
1 Introduction

Fire is a major ecosystem driver across many regions worldwide (Bowman et al., 2009). Severe fires have an important impact on physical, chemical and biological properties of soils (Neary et al., 1999; Certini, 2005; Caon et al., 2014). In addition, soil properties and dynamics after fire can be affected by post-fire changes in both total and patterns of precipitation (Certini, 2005). Contrasting rainfall-related extreme events, from high intensity rainfall to a prolonged drought, are relevant. The interaction between fire and torrential rainfall has been well documented, showing negative effects on soil fertility due to erosion and nutrient losses (Thomas et al., 1999; Shakesby and Doerr, 2006; Lane et al., 2008). However, a less studied issue is how post-fire soil dynamics are affected by drought. Notwithstanding, the effects of the interaction between drought and fire are poorly known, notably with regard to soil nutrient dynamics and microbial processes (Luo et al., 2008; Wu et al., 2011; Beier et al., 2012). Longer drought periods are projected to increase in extra-tropical dry areas, including the Mediterranean Basin, due to climate change (Giorgi and Lionello, 2008; Collins et al., 2013; Christensen et al., 2013). Increased temperatures and reduced precipitation, among other factors, can also increase fire activity, resulting in larger and more severe fires (Westerling et al., 2006; Bradstock et al., 2010; Bedia et al., 2014; Xistrakis et al., 2014). In addition, this mean that post-fire recovery of these ecosystems could also be affected by drought.

Drought is an important controlling factor of the microbial community (Waldrop and Firestone, 2006; Clark et al., 2009; Landesman and Dighton, 2010; Castro et al., 2010; Mauritz et al., 2014) and may limit or inhibit its activity in soils, depending on the intensity and duration of the drought event (Borken and Matzner, 2009; Sardans and Peñuelas, 2010; Schindlbacher et al., 2012). Thus, the negative effects of drought on the microbial community will in turn influence the amount of available inorganic soil nutrients (Matias et al., 2011). Moreover, drought limits the diffusion of nutrients in the soil (Papendick and Campbell, 1981). Hence, the interaction between drought, fire and subsequent soil process can be complex. The few manipulative experiments that tested
the combined effect of both fire and drought on different aspect of the belowground component of the ecosystem suggest that a drier environment will limit the extent of post-fire peak in soil nutrient availability (Potts et al., 2012; Hinojosa et al., 2012). Potts et al. (2012) found that, although resin-available nitrogen responded positively to fire in the short term, it declined with severe drought. Additionally, Hinojosa et al. (2012) found that drought effects in burned soils caused a significant decrease in the inorganic phosphate fraction; moreover, a reduction in the turnover organic P (including microbial P) and phosphatase activity was also documented.

Based on the above, we hypothesize that drought conditions after fire will reduce microbial biomass recovery and modify functionality and diversity of soil microorganisms, either survivors or colonizers after fire, which may limit or inhibit their activity in soils depending on drought magnitude. Furthermore, negative effects on microbial mineralization activity may in turn influence the amount of available inorganic soil nutrients due to the reduction of soil organic matter decomposition.

To test this hypothesis, a manipulative experiment was setup in which rainfall patterns were modified to simulate various levels of drought in a Mediterranean shrubland of central Spain before and after a high severity experimental burning (Parra et al., 2012). Changes in soil nutrient availability and microbial activity in unburned and burned soils under different levels of drought, as well as the dynamics of the soil microbial community structure, were investigated during the first year after fire.

2 Material and methods

2.1 Study area, experimental design and soil sampling

A rainfall manipulation experiment was established in a Cistus-Erica shrubland located at the Quintos de Mora Range Station (Lat. 39°25′ N, Long. 04°04′ W), Montes de Toledo mountain range (Central Spain). The study site is located in a NW facing slope; the mean annual temperature is 14.9 °C and the mean annual precipitation is 622 mm.
Soil is a Dystric Cambisol (IUSS Working Group WRB, 2007) and the parent rock is mainly quartzite. Soil texture is sandy loam (68, 18, and 14 % sand, silt and clay, respectively), with a high proportion of rock (40 %), 6.5 pH, and 11.5 C : N ratio (Laboratorio Agrario Regional de Albacete; Consejería de Agricultura de Castilla-La Mancha, Spain).

The rainfall manipulative experiment was initiated at the beginning of 2009 and different treatments were implemented based on the long-term (1948–2006) precipitation records (Figs. A1 and A2). Thus, annual precipitation was controlled by changing spring to autumn rainfall by means of a system with both automatic rain-out shelters and irrigation facilities, resulting in the following treatments: (i) environmental control (EC), having natural rainfall without any manipulation; (ii) historical control (HC, 600 mm year\(^{-1}\), with drought during July and August), resembling the average long-term rainfall in the study area; (iii) moderate drought (MD, 25 % reduction of HC, i.e. 450 mm year\(^{-1}\), 5 months drought (May to September)) and (iv) severe drought (SD; 45 % reduction of HC, i.e. 325 mm year\(^{-1}\), 7 months drought (April to October)). In September 2009, the plots were burned (+) in order to evaluate the joint effects of both drought and fire. The experimental burning was homogeneous across all plots and no significant differences in fire intensity were recorded among rainfall treatments. The mean residence time above 100 °C was 13.5 min and the average maximum temperatures was 710 °C (soil surface values measured with thermocouples) (Parra et al., 2012).

The experimental design included an additional set of unburned plots without rainfall manipulation (EC–) that were used as an unburned control. The whole set of drought-fire treatments were replicated four times in 6 m \(\times\) 6 m plots (Fig. A1). A wider description of the experimental setup was reported by Parra et al. (2012).

Soil samples were collected seasonally, from spring 2010 to spring 2011 (i.e. five sampling times). Four composite samples, each one consisting of four soil cores, were randomly collected from the top 5 cm of soil in each experimental plot. Soil samples were transported in an isothermal bag (4 °C) to the laboratory, where they were imme-
diately sieved (< 2 mm) and gravimetric water content quantified (104°C, 24 h) before further analysis.

2.2 Soil organic matter and nutrient content

Fresh soil aliquots were used to analyse exchangeable potassium by atomic absorption, according to Grant (1982), ammonium and nitrate by spectrophotometry after 2 M KCl extraction (Keeney and Nelson, 1982); and phosphate in 0.5 M NaHCO₃ (pH: 8.5) extracts (Olsen and Sommers, 1982) by the colorimetric method of John (1970). Soil organic matter was estimated by the Walkley and Black wet oxidation method (Nelson and Sommers, 1996), using 1.724 as correction factor.

2.3 Carbon mineralization rate and enzyme activities

Carbon mineralization rate was determined using the alkali-trap method described by Anderson (1982), on soil samples incubated for a 15 days at 24°C in the dark and under aerobic conditions.

Soil extracellular enzyme activity is directly linked to microbial status and soil physico-chemical properties. This makes soil enzymes excellent indicators of the soil microbial functionality, and therefore, good indicators of disturbances (Burns et al., 2013). In this study, 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 fresh soil was incubated with the corresponding substrates at 37°C for 1 h, and the product (p nitrophenol, pNP) measured at 410 nm in the supernatant after reaction was stopped with CaCl₂ and a strong alkali.
2.4 Soil microbial community structure

The potential of soil microbial communities for rapid growth and turnover makes them a very reactive component of a terrestrial ecosystem to external stress in comparison to plants and animals (Panikov, 1999). Soil microbial community structure was assessed by direct extraction of ester-linked fatty acids (ELFAs) according to Schutter and Dick (2000) method. Briefly, 3 g of soil (fresh weight) were 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 h, thus allowing the release and subsequent methylation of ELFAs. Soil pH was then neutralised by 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 h. Dried samples were re-suspended in 100 µL hexane to be analysed by a gas chromatograph (GC) on a Thermo Scientific FOCUS™ GC, 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 GC temperature progression was: 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 to 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 internal standard. The fatty acid nomenclature was that described by Hinojosa et al. (2005). ELFAs reported as typical of 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), and actinomycetes (10Me18:0) were used as signature biomarkers for these microbial groups (Bossio and Scow, 1998; Zelles et al., 1999). The summed mass of all the extracted ELFAs (nmol fatty acid g⁻¹ soil) was used as indicator of soil microbial biomass.
2.5 Statistical analyses

Time-course measurements (seasonal sampling) were analysed using repeated measures analysis of variance (ANOVA) to determine the effect of both “burning treatments under natural rainfall” (test EC−/EC+) and “rainfall treatments in burned plots” (test EC+/HC+/MD+/SD+) over the investigated variables (n = 4). In addition, one-way ANOVA was used to test the effect of both burning and rainfall pattern after burning per each sampling time, followed by a post-hoc SHD Tukey test. Before performing parametric statistical analyses, data were tested for normality and homoscedasticity and transformed if necessary (logarithmic transformation was enough in all cases). These analyses were carried out using STATISTICA 7 (StatSoft, Inc. 2004).

The effects of treatments on soil microbial community structure were assessed using PERMANOVA (permutational multivariate analysis of variance) for the relative abundance of the whole set of fatty acids present in the soil samples (Anderson, 2001), for spring 2010 and spring 2011 samples separately. This analysis was carried out with PERMANOVA (Anderson, 2005) using 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 (NMS) analysis was performed. NMS analysis was based on the Sørensen’s distance and the “slow and thorough” autopilot mode of NMS in PC-ORD (McCune and 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. number of iterations. In addition, NMS axes were correlated to the main groups of fatty acids included in the ordination by the Spearman correlation coefficients.
3 Results

3.1 Soil water content

Soil water content was not significantly different between burned and unburned plots under natural rainfall treatments ($F = 3.34; P > 0.05$). However, time effect was significant ($F = 75.63; P < 0.0001$), with higher values in winter and lower values in summer. In this repeated measures ANOVA model, the interaction term was not significant ($F = 1.33; P > 0.05$) (Fig. 1a).

On the other hand, rainfall treatments ($F = 9.15, P < 0.01$), time ($F = 113.43, P < 0.001$) and their interaction ($F = 2.66, P < 0.01$) significantly affected soil water content in burned plots under different rainfall patterns. A significant decrease in soil water content occurred in spring and autumn as a consequence of the implemented drought treatments. The lowest and the highest soil water content were found in summer and winter, respectively, and no significant differences were observed among rainfall treatments in these seasons (Fig. 1b).

3.2 Soil nutrients

Soil ammonium concentration significantly decreased as a consequence of fire under natural rainfall (Table 1), being this reduction mainly visible in both spring and winter 2010 (Fig. 2a). However, no significant effect of rainfall manipulation was observed in ammonium concentration of burned soils, except a slight reduction in summer (Fig. 2b).

Under natural rainfall, burned soils had a nitrate concentration 6-fold significantly higher than unburned ones in spring and summer 2010, whereas from autumn no significant differences occurred between burned and unburned soils (Fig. 2a, Table 1). Burned soils under drought treatments (MD+ and SD+) showed, in average, 8.5, 3.5 and 5.5-fold more nitrate than the historical control treatment (HC+) in spring, summer and autumn, respectively. Nevertheless, these effects of drought on soil nitrate concentration were reverted by winter (Fig. 2b, Table 1).
Phosphate concentration also increased 2, 3.5 and 3 fold in burned soils compared to unburned ones in spring, summer and autumn 2010, respectively. Subsequently, from winter onwards, soil phosphate concentration was similar in burned and unburned plots (Fig. 2a, Table 1). On the other hand, only in the first spring after fire burned soils under severe drought (SD+) treatments showed a lower phosphate concentration than the historical control treatment (HC+) (Fig. 2b).

In treatments under natural rainfall, the concentration of soil exchangeable potassium decreased as a consequence of fire at all sampling times (Fig. 2a, Table 1). However, in burned treatments soil potassium concentration increased 1.2 and 1.3 folds in spring and autumn 2010, respectively, as a consequence of drought (MD+ and SD+ treatments), compared to the historical control (HC+) treatment (Fig. 2b).

### 3.3 Soil organic matter and carbon mineralization rate

Soil organic matter was significantly lower in burned soils (7.43 ± 0.61 %) than in the unburned ones (9.75 ± 0.55 %) under natural rainfall (pooling all seasons together), with higher differences detected in spring 2010 and 2011 (Fig. 3a, Table 1). Soil organic matter of burned soils did not show significant differences among different rainfall treatments, with the exception of spring 2011, where EC+ treatment had significantly lower values (Fig. 3b, Table 1).

Burning treatment halved soil carbon mineralization rate in comparison to unburned soils. These differences were significant in spring, autumn and winter 2010. In spring 2011, both burned and unburned soils under natural rainfall showed similar values of carbon mineralization rates (Fig. 3a, Table 1). The amount of mineralised carbon was even more reduced due to drought treatments (Fig. 3b), and such reduction was still significant in the second spring after fire (2011), with both drought treatments (MD+ and SD+) showing less than half the values of the HC+ treatment (Fig. 3b, Table 1). In all cases the lowest values of soil carbon mineralization rate were observed in summer, when significant differences between treatments were not detected.
3.4 Soil enzyme activities

With the exception of alkaline phosphatase, soil enzyme activities showed significantly lower values in burned soils than in unburned ones under natural rainfall, with acid phosphatase, β-glucosidase and arilsulfatase diminishing a 30, 42 and 54 %, respectively (pooling all seasons together). These enzyme activities were not recovered after fire, at least during the studied period (Fig. 4a, Table 1).

Comparing burned soils under different rainfall treatments, the drought treatments (MD+ and SD+) showed significantly lower enzyme activities than the historical control (HC+), being this fact especially clear in spring (Fig. 4b, Table 1). Thus, in spring 2011 the activity of acid phosphatase, alkaline phosphatase, β-glucosidase and arylsulfatase was decreased about a 56, 65, 51 and 84 %, respectively, in burned soils under drought treatments in relation to the historical control (HC+).

3.5 Soil microbial community structure

Under natural rainfall, soil microbial biomass was significantly lower in burned soils (EC−) than in the unburned ones (EC+) (P < 0.05), showing a reduction of 25 and 40 % in spring 2010 and 2011, respectively (Fig. 5). Soil microbial biomass was further reduced in the burned soils as a consequence of drought (P < 0.05), with no significant differences between MD+ and SD+ treatments, being this decrease of 27 and 35 % in spring 2010 and 2011, respectively, with respect to the historical control (HC+) (Fig. 5).

Both burning and rainfall manipulation treatments significantly affected soil microbial community structure (Tables 2 and 3). Under natural rainfall, a significant decrease of fungi and actinomycetes markers was observed in both sampling times (spring 2010 and 2011), as a consequence of fire. On the other hand, in the burned soils, the moderate and severe drought treatments (MD+ and SD+) decreased the relative abundance of fungi, bacteria and Gram-positive markers, as well as the fungi to bacteria ratio, and increased the relative abundance of actinomycetes marker (Tables 2 and 3).
PERMANOVA results indicate that ELFA profiles showed significant differences between burned and unburned soils at both sampling springs (2010, 2011) ($F = 2.30$, $P < 0.05$ and $F = 2.33$, $P < 0.05$ for spring 2010 and 2011, respectively) (Table A1). On the other hand, although ELFA profiles of burned soils showed no differences under different rainfall treatments in spring 2010 ($F = 1.49$, $P > 0.05$), significant differences were found in spring 2011 as a consequence of the different rainfall treatments ($F = 2.32$, $P < 0.05$) (Table A1).

NMS analysis using the whole set of ELFAs confirmed the PERMANOVA results and clearly differentially ordinated the microbial communities in burned and unburned soils. Axis 2 separated burned and unburned soils, with burned soil showing a lower relative abundance of fungi and gram-positive markers and monounsaturated fatty acids and a higher relative abundance of actinomycetes marker and C16:1ω7c and C17:0cy fatty acids (Fig. 6, Table A2). No changes in microbial community structure due to drought were observed shortly after fire (spring 2010). Nevertheless, one year later (spring 2011), the implemented rainfall treatments in burned soils resulted in a differential microbial structure, mainly due to a further reduction of fungi marker and monosaturated fatty acids (Fig. 6, Table A2).

4 Discussion

4.1 Effect of fire under natural rainfall

Our results document an increase in soil phosphate concentration after fire, which could be mainly due to direct pyromineralization, as reported by Hinojosa et al. (2012). Nevertheless, such an increase was transient, as inorganic phosphate soon declined, suggesting that it entered into the organic phosphorus pool (by plant and/or microbial uptake), or was sorbed onto secondary mineral surfaces interacting with free cations as Al, Fe and Mn (Certini et al., 2005). Regarding soil mineral nitrogen, the concentration of both nitrate and ammonium significantly increased immediately after fire, reaching
effects of experimental drought and fire on soil

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Burning significantly reduced both soil organic matter and carbon mineralization rate in comparison with unburned soils. In fact, the peak temperatures reached in our experiment exceeded those required for killing living soil microorganisms at the soil surface (DeBano et al., 1998). Heat also could indirectly affect survival and recolonization of soil microorganisms through reduction and modification of organic substrates (González-Pérez et al., 2004; Certini, 2005). In our study, fire altered both soil microbial biomass and the specific composition of the soil microbial community. The total amount of ELFAs, a proxy of total microbial biomass, was reduced 25% in burned soils relative to unburned ones, with no recovery along the studied period. Bárcenas-Moreno and Bååth (2009) suggested that a decrease in soil fatty acids in burned soils could be due to the direct destruction by high temperatures reached during the fire and to a subsequent degradation of PLFAs from dead organisms that were not immediately destroyed by heating.

Our study documents that burned soils had a more bacteria-dominated microbial community, supporting earlier findings (Vázquez et al., 1993; Bååth et al., 1995; Bárcenas-Moreno et al., 2011). This could be due to differences in sensitivity to high temperature, as fungi are more sensitive than bacteria (Dunn et al., 1985; D’Ascoli...
et al., 2005) or to differences in the capacity of the inoculum to recolonize the burned soils. Bárcenas-Moreno and Bååth (2009) described that bacterial biomass recovered rapidly after experimental heating, while fungi took longer and did not recover as well. This is in agreement with the increase of FAs 16:1ω7c and cy17:0 (Gram-negative bacteria markers) that we found, often associated to bacterial growth (Söderberg et al., 2004).

Traditionally, it has been reported that soil actinomycetes and bacteria behave similarly in response to fire (Ahlgren, 1974). However, we show that burned soils had a higher relative amount of actinomycetes marker, which supports results by Bárcenas-Moreno et al. (2011) and could be related to the ability of actinomycetes (as well as many bacteria) to form spores making them more heat resistant, and consequently less affected by fire.

Changes in soil microbial biomass and community structure as a consequence of fire were linked to a reduction of the activity of soil extracellular enzymes. Such reduction was particularly consistent for acid phosphatase, β-glucosidase and arilsulfatase enzymes, which did not recover along the studied period. Other previous studies also have reported reduction of these enzyme activities after fire under different types of ecosystems (Saa et al., 1993; Boerner et al., 2005; Holden et al., 2013). The reduction of soil enzyme activity after fire could be initially due to the high temperatures reached in our experimental fires, which are known to degrade or inactivate enzymes secreted by soil microorganisms (Tiwari et al., 1988). However, other factor that could have extended along time this negative effect of fire on enzyme activity could be the decrease of soil microbial and plant biomass.

4.2 Effect of fire under manipulated rainfall patterns

Our results documented that changes in soil water content in the burned soil altered the availability of soil nutrients. The initial higher concentration of nitrate and potassium in the burned soils under severe and moderate drought treatments could be due to lower losses of these nutrients by lixiviation (Jonhson et al., 2002, 2008); but a lower plant
and microbial uptake as a consequence of their inhibited diffusion under drought conditions can not be excluded (Manzoni et al., 2012; Rouphael et al., 2012). In any case, these effects were transient and, after the autumn-winter rainfall period, soil nitrate and potassium concentration under rainfall manipulation treatments became similar to the one observed in the historical control treatment.

In relation to soil phosphate, these and previous results (Hinojosa et al., 2012) confirmed that, under dry conditions, the mineralizing effect of fire was partially or completely offset, limiting the extent of post-fire peak in soil P availability.

Soil moisture is one of the most important environmental factors affecting soil carbon mineralization (Rustad et al., 2000; Grünzweig et al., 2009). In Mediterranean-type ecosystems, responses of soil respiration to rainfall manipulations vary among seasons and years, depending on respective rainfall amounts (Asencio et al., 2007). Thus, in general, experimental rainfall exclusions decrease soil carbon mineralization rate (de Dato et al., 2010; Talmon et al., 2010; Sherman et al., 2012; Maestre et al., 2013). We also found a reduction in carbon mineralisation rate with drought after burning. This could be explained by (i) a further reduction in the activity of organisms present in the burned soils owing to osmotic regulation allocations and limited diffusive transport (Schimel et al., 2007; Voroney, 2007), (ii) a reduction in the amount and quality of substrate for carbon mineralization (Vargas et al., 2012), and (iii) the changes observed in the composition of the microbial community after fire due to drought (Goberna et al., 2012).

No major changes in microbial community structure due to drought were observed shortly after fire (spring 2010). However, one year later (spring 2011), the implemented drought treatments resulted in a differential microbial structure, preferentially affecting the fungal community rather than the bacterial one. Previous results of Six (2012) suggested that increasing drought conditions in unburned ecosystems appear to favour a fungal-rich microbial community, as fungi were able to better overcome the disadvantages of drier conditions than bacteria. However, we found that fungal abundance significantly decreased with fire, and it was even more reduced as a consequence of
drought. It is likely that once the bacteria had recolonized the soil after fire, they could be antagonistic towards fungi, as suggested by Bárcenas-Moreno and Bååth (2009), delaying even further the recovery of fungi as a consequence of drought. In a similar way, actinomycetes, which increased after fire, were even more abundant under the drought treatments.

Our results raise the question of to what extent burned Mediterranean ecosystems will shift from fungal-dominated to bacterial-dominated communities, due to the drier conditions brought by climate change, and whether these changes might later affect soil nutrient cycling. This is important because bacterial-dominated communities sequester less C than fungal-dominated communities (Bailey et al., 2002; Six et al., 2006; Treseder and Holden, 2013). In addition, our results showed a significant reduction of enzyme activities related to nutrient cycling owing to the drought treatments. The lowest soil water content in summer resulted in a generalized lower enzyme activity, with no differences among rainfall treatments, which supports previous results of rainfall interception experiment in Mediterranean ecosystems (Sardans et al., 2006, 2008). Yet, the reduction of soil enzyme activities in response to manipulated rainfall reductions was widely observed in spring, when soil moisture and temperature are optimal for plant and microbial activity (García et al., 2002).

Soil enzyme activities are an integrative indicator of a change in the biology and biogeochemistry of the soil. Our results suggest that, in soils of the Mediterranean Basin, reduced rainfall could decrease the potential transformation rate of organic matter and, consequently, influence the long-term availability of soil inorganic nutrients. Thus, we argue that our results support that drought could in turn shift the recovery of soil functionality in Mediterranean shrublands after fire owing to the reduction of microbial metabolism. Consequently, although this study showed minimal and/or transitory effects of changes in precipitation on nutrient concentrations in burned soils, longer term changes could be expected as a consequence of the significant impact of drought on the soil microbial community structure and functionality. Long-term observations, as well as studies in other ecosystem types, are needed to clearly elucidate how drought
affects soil biogeochemical processes in burned ecosystems and influence their recovery dynamics, including soil-plant interactions (Beier et al., 2012; Thompson et al., 2013).

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Table 1. $F$ coefficient of the repeated measures ANOVA, testing differences in soil nutrient content, soil organic matter (SOM), carbon mineralization rate (C min) soil enzyme activities (Acid P-ase; Alk P-ase, $\beta$ Gl-ase, Aril-ase) for both (a) burning treatments under natural rainfall, i.e. EC− and EC+ and (b) rainfall treatments in burned plots, i.e. EC+, CH+, MD+ and SD+.

(a) Burning treatments under natural rainfall

<table>
<thead>
<tr>
<th></th>
<th>N-NH$_4^+$</th>
<th>N-NO$_3^-$</th>
<th>P-PO$_4^{3-}$</th>
<th>K extr</th>
<th>SOM</th>
<th>C min</th>
<th>Acid P-ase</th>
<th>Alk P-ase</th>
<th>$\beta$ Gl-ase</th>
<th>Aril-ase</th>
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<tbody>
<tr>
<td>Burning ($B$)</td>
<td>11.49$^a$</td>
<td>6.21$^b$</td>
<td>17.38$^b$</td>
<td>20.37$^b$</td>
<td>6.54$^a$</td>
<td>18.10$^b$</td>
<td>32.72$^b$</td>
<td>0.01$^c$</td>
<td>80.50$^c$</td>
<td>18.77$^b$</td>
</tr>
<tr>
<td>Time ($T$)</td>
<td>46.42$^c$</td>
<td>5.85$^b$</td>
<td>8.34$^c$</td>
<td>2.32$^b$</td>
<td>11.74$^c$</td>
<td>24.64$^c$</td>
<td>54.29$^c$</td>
<td>84.37$^c$</td>
<td>13.80$^c$</td>
<td>7.33$^c$</td>
</tr>
<tr>
<td>$B \times T$</td>
<td>4.65$^b$</td>
<td>2.30$^b$</td>
<td>1.21</td>
<td>2.37$^b$</td>
<td>0.41</td>
<td>2.72$^b$</td>
<td>0.08</td>
<td>0.58</td>
<td>2.39</td>
<td>2.19$^b$</td>
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(b) Rainfall treatments in burned plots

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<tr>
<th></th>
<th>N-NH$_4^+$</th>
<th>N-NO$_3^-$</th>
<th>P-PO$_4^{3-}$</th>
<th>K extr</th>
<th>SOM</th>
<th>C min</th>
<th>Acid P-ase</th>
<th>Alk P-ase</th>
<th>$\beta$ Gl-ase</th>
<th>Aril-ase</th>
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<tbody>
<tr>
<td>Rainfall ($R$)</td>
<td>2.54$^a$</td>
<td>35.23$^c$</td>
<td>2.36</td>
<td>2.28</td>
<td>1.90</td>
<td>5.83$^a$</td>
<td>16.46$^c$</td>
<td>8.18$^b$</td>
<td>6.41$^b$</td>
<td>2.94$^a$</td>
</tr>
<tr>
<td>Time ($T$)</td>
<td>87.14$^c$</td>
<td>13.36$^c$</td>
<td>29.62$^c$</td>
<td>4.02$^b$</td>
<td>13.31$^c$</td>
<td>29.50$^c$</td>
<td>115.94$^c$</td>
<td>293.44$^c$</td>
<td>9.34$^c$</td>
<td>5.23$^b$</td>
</tr>
<tr>
<td>$R \times T$</td>
<td>1.98$^a$</td>
<td>4.85$^c$</td>
<td>1.25</td>
<td>1.19</td>
<td>1.42</td>
<td>2.07$^a$</td>
<td>2.88$^b$</td>
<td>2.05$^a$</td>
<td>3.36$^b$</td>
<td>0.61$^c$</td>
</tr>
</tbody>
</table>

$^a$ $P < 0.05$

$^b$ $P < 0.01$

$^c$ $P < 0.001$
**Table 2.** Relative abundance of fatty acid groups (markers) averaged per treatments in 2010 and 2011 samplings. Standard error of the mean is noted between brackets. Different uppercase or lowercase letters indicate a significant difference ($P < 0.05$, $n = 4$) between burned and unburned treatments (EC− vs. EC+) and among different rainfall patterns in burned plots (EC+, HC+, MD+, SD+), respectively.

<table>
<thead>
<tr>
<th></th>
<th>Fungi</th>
<th>Bacteria</th>
<th>Gram+</th>
<th>Gram−</th>
<th>Actinomycetes</th>
<th>G+/G−</th>
<th>Fungi/bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spring 2010</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC−</td>
<td>0.053 (0.005) A</td>
<td>0.104 (0.005)</td>
<td>0.081 (0.005)</td>
<td>0.022 (0.000)</td>
<td>0.003 (0.000) A</td>
<td>3.668 (0.083)</td>
<td>0.406 (0.066)</td>
</tr>
<tr>
<td>EC+</td>
<td>0.036 (0.004) B</td>
<td>0.096 (0.005)</td>
<td>0.075 (0.004)</td>
<td>0.021 (0.001) a</td>
<td>0.004 (0.000) B</td>
<td>3.578 (0.078)</td>
<td>0.388 (0.060)</td>
</tr>
<tr>
<td>HC+</td>
<td>0.027 (0.003)</td>
<td>0.112 (0.002)</td>
<td>0.090 (0.001)</td>
<td>0.021 (0.002) a</td>
<td>0.004 (0.000)</td>
<td>4.486 (0.730)</td>
<td>0.239 (0.023)</td>
</tr>
<tr>
<td>MD+</td>
<td>0.027 (0.002)</td>
<td>0.114 (0.004)</td>
<td>0.088 (0.005)</td>
<td>0.026 (0.001) b</td>
<td>0.004 (0.000)</td>
<td>3.365 (0.177)</td>
<td>0.235 (0.018)</td>
</tr>
<tr>
<td>SD+</td>
<td>0.025 (0.002)</td>
<td>0.107 (0.002)</td>
<td>0.080 (0.002)</td>
<td>0.026 (0.001) b</td>
<td>0.005 (0.000)</td>
<td>3.063 (0.110)</td>
<td>0.238 (0.014)</td>
</tr>
<tr>
<td><strong>Spring 2011</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC−</td>
<td>0.067 (0.005) A</td>
<td>0.106 (0.005)</td>
<td>0.086 (0.005)</td>
<td>0.020 (0.001)</td>
<td>0.003 (0.000) A</td>
<td>4.317 (0.293)</td>
<td>0.641 (0.058) A</td>
</tr>
<tr>
<td>EC+</td>
<td>0.053 (0.006) Ba</td>
<td>0.109 (0.002) a</td>
<td>0.085 (0.001) a</td>
<td>0.023 (0.002)</td>
<td>0.004 (0.000) Ba</td>
<td>3.794 (0.271)</td>
<td>0.486 (0.061) Ba</td>
</tr>
<tr>
<td>HC+</td>
<td>0.048 (0.004) a</td>
<td>0.120 (0.002) b</td>
<td>0.095 (0.003) b</td>
<td>0.026 (0.001)</td>
<td>0.004 (0.001) a</td>
<td>3.722 (0.122)</td>
<td>0.406 (0.045) a</td>
</tr>
<tr>
<td>MD+</td>
<td>0.036 (0.002) b</td>
<td>0.118 (0.001) b</td>
<td>0.093 (0.002) b</td>
<td>0.025 (0.000)</td>
<td>0.004 (0.000) a</td>
<td>3.661 (0.055)</td>
<td>0.305 (0.011) b</td>
</tr>
<tr>
<td>SD+</td>
<td>0.028 (0.004) b</td>
<td>0.112 (0.002) a</td>
<td>0.090 (0.002) a</td>
<td>0.021 (0.005)</td>
<td>0.007 (0.001) b</td>
<td>4.333 (0.042)</td>
<td>0.242 (0.040) b</td>
</tr>
</tbody>
</table>
Table 3. $F$ coefficient of the repeated measures ANOVA, testing differences in relative abundance of soil fatty acid marker for both (a) burning treatments under natural rainfall, i.e. EC− and EC+ and (b) rainfall treatments in burned plots, i.e. EC+, HC+, MD+ and SD+.

<table>
<thead>
<tr>
<th></th>
<th>Fungi</th>
<th>Bacteria</th>
<th>Gram+</th>
<th>Gram−</th>
<th>Actinomycetes</th>
<th>G+/G−</th>
<th>Fungi/bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Burning treatments under natural rainfall</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burning ($B$)</td>
<td>1.64</td>
<td>0.04</td>
<td>0.02</td>
<td>0.41</td>
<td>0.77</td>
<td>0.57</td>
<td>1.57</td>
</tr>
<tr>
<td>Time ($T$)</td>
<td>2.99</td>
<td>0.59</td>
<td>0.70</td>
<td>0.05</td>
<td>2.13</td>
<td>0.15</td>
<td>4.05</td>
</tr>
<tr>
<td>$B \times T$</td>
<td>0.05</td>
<td>2.26</td>
<td>1.86</td>
<td>7.89$^a$</td>
<td>0.64</td>
<td>1.24</td>
<td>0.59</td>
</tr>
<tr>
<td>(b) Rainfall treatments in burned plots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rainfall ($R$)</td>
<td>13.72$^c$</td>
<td>3.78$^a$</td>
<td>7.46$^b$</td>
<td>0.93</td>
<td>5.89$^a$</td>
<td>0.53</td>
<td>14.03$^c$</td>
</tr>
<tr>
<td>Time ($T$)</td>
<td>9.51$^a$</td>
<td>15.24$^b$</td>
<td>21.60$^c$</td>
<td>0.27</td>
<td>0.39</td>
<td>0.94</td>
<td>5.68$^a$</td>
</tr>
<tr>
<td>$R \times T$</td>
<td>1.16</td>
<td>0.22</td>
<td>0.71</td>
<td>1.42</td>
<td>1.43</td>
<td>1.85</td>
<td>1.01</td>
</tr>
</tbody>
</table>

$^a P < 0.05$
$^b P < 0.01$
$^c P < 0.001$
Table A1. PERMANOVA results of the relative abundances of ELFA profiles for spring 2010 and spring 2011 in response to fire under natural rainfall (EC− and EC+) and in response to different rainfall pattern in burned soils (EC+, HC+, MD+ and SD+). P (perm) values < 0.05 are shown in boldface.

<table>
<thead>
<tr>
<th></th>
<th>2010 F</th>
<th>P (perm)</th>
<th>2011 F</th>
<th>P (perm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fire</td>
<td>2.30</td>
<td>0.02</td>
<td>2.33</td>
<td>0.02</td>
</tr>
<tr>
<td>Rainfall</td>
<td>1.49</td>
<td>0.14</td>
<td>2.32</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Pair-wise a posteriori comparisons (2011 rainfall pattern)

<table>
<thead>
<tr>
<th></th>
<th>t</th>
<th>P (perm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC+ HC+</td>
<td>1.10</td>
<td>0.255</td>
</tr>
<tr>
<td>EC+ MD+</td>
<td>2.16</td>
<td>0.028</td>
</tr>
<tr>
<td>EC+ SD+</td>
<td>2.25</td>
<td>0.028</td>
</tr>
<tr>
<td>HC+ MD+</td>
<td>0.88</td>
<td>0.632</td>
</tr>
<tr>
<td>HC+ SD+</td>
<td>2.16</td>
<td>0.026</td>
</tr>
<tr>
<td>MD+ SD+</td>
<td>1.64</td>
<td>0.196</td>
</tr>
</tbody>
</table>
Table A2. Spearman correlation coefficients between samples coordinates on the ordination axes resulting from NMS analysis and the original variables (relative amount of soil ELFAs). Values with $P < 0.05$ are shown in boldface.

<table>
<thead>
<tr>
<th>ELFAs</th>
<th>Main groups</th>
<th>Axis 1</th>
<th>Axis 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>unsaturated</td>
<td>−0.340</td>
<td>0.288</td>
</tr>
<tr>
<td>C14:0</td>
<td>unsaturated</td>
<td>−0.767</td>
<td>−0.062</td>
</tr>
<tr>
<td>C15:0</td>
<td>unsaturated</td>
<td>−0.069</td>
<td>−0.306</td>
</tr>
<tr>
<td>C15:0a</td>
<td>Gram+ marker</td>
<td>0.173</td>
<td>−0.552</td>
</tr>
<tr>
<td>C15:0i</td>
<td>Gram+ marker</td>
<td>0.484</td>
<td>−0.161</td>
</tr>
<tr>
<td>C16:0</td>
<td>unsaturated</td>
<td>−0.696</td>
<td>0.035</td>
</tr>
<tr>
<td>C16:0i</td>
<td>Gram+ marker</td>
<td>0.291</td>
<td>−0.032</td>
</tr>
<tr>
<td>C16:1ω5c</td>
<td>monosaturated</td>
<td>0.545</td>
<td>−0.179</td>
</tr>
<tr>
<td>C16:1ω7c</td>
<td>monosaturated</td>
<td>0.276</td>
<td>−0.745</td>
</tr>
<tr>
<td>C17:0</td>
<td>unsaturated</td>
<td>0.253</td>
<td>0.075</td>
</tr>
<tr>
<td>C17:0cy</td>
<td>Gram− marker</td>
<td>−0.079</td>
<td>−0.579</td>
</tr>
<tr>
<td>C17:0i</td>
<td>Gram+ marker</td>
<td>0.634</td>
<td>0.235</td>
</tr>
<tr>
<td>C17:1ω7c</td>
<td>monosaturated</td>
<td>0.536</td>
<td>0.012</td>
</tr>
<tr>
<td>C18:0</td>
<td>unsaturated</td>
<td>−0.498</td>
<td>0.306</td>
</tr>
<tr>
<td>C18:0Me</td>
<td>Actinomyces marker</td>
<td>−0.321</td>
<td>−0.495</td>
</tr>
<tr>
<td>C18:1ω5c</td>
<td>Gram− marker</td>
<td>−0.213</td>
<td>−0.004</td>
</tr>
<tr>
<td>C18:1ω7c</td>
<td>monosaturated</td>
<td>0.469</td>
<td>0.466</td>
</tr>
<tr>
<td>C18:1ω9c</td>
<td>monosaturated</td>
<td>0.625</td>
<td>0.734</td>
</tr>
<tr>
<td>C18:1ω9t</td>
<td>monosaturated</td>
<td>0.005</td>
<td>−0.082</td>
</tr>
<tr>
<td>C18:2ω6,9c</td>
<td>Fungi marker</td>
<td>0.621</td>
<td>0.742</td>
</tr>
<tr>
<td>C18:3ω3c</td>
<td>poliunsaturated</td>
<td>0.418</td>
<td>0.127</td>
</tr>
<tr>
<td>C19:0</td>
<td>unsaturated</td>
<td>0.172</td>
<td>−0.356</td>
</tr>
<tr>
<td>C19:0cy</td>
<td>Gram− marker</td>
<td>0.251</td>
<td>−0.221</td>
</tr>
<tr>
<td>C20:0</td>
<td>unsaturated</td>
<td>−0.668</td>
<td>−0.067</td>
</tr>
</tbody>
</table>
Figure 1. Soil water content (%) at the sampling times from spring 2010 until spring 2011. Means ± standard error (SE) are given. * indicates significant differences (a) between burning treatments under natural rainfall (i.e. EC− vs. EC+) or (b) among different rainfall treatments in burned plots (i.e. EC+, HC+, MD+, SD+) within each sampling time (P < 0.05, one-way ANOVA).
**Figure 2.** Soil concentration of ammonium, nitrate, inorganic phosphate and exchangeable potassium at the sampling times from spring 2010 until spring 2011. Means ± SE are given. * indicates significant differences (a) between burning treatments under natural rainfall (i.e. EC− vs. EC+) or (b) among different rainfall treatments in burned plots (i.e. EC+, HC+, MD+, SD+) within each sampling time ($P < 0.05$, one-way ANOVA).
Figure 3. Soil organic matter content and carbon mineralization rate at the sampling times from spring 2010 until spring 2011. Means ± SE are given. * indicates significant differences (a) between burning treatments under natural rainfall (i.e. EC− vs. EC+) or (b) among different rainfall treatments in burned plots (i.e. EC+, HC+, MD+, SD+) within each sampling time (P < 0.05, one-way ANOVA).
Figure 4. Soil enzyme activities at the sampling times from spring 2010 until spring 2011. Means ± SE are given. * indicates significant differences (a) between burning treatments under natural rainfall (i.e. EC− vs. EC+) or (b) among different rainfall treatments in burned plots (i.e. EC+, HC+, MD+, SD+) ($P < 0.05$, one-way ANOVA).
Figure 5. Total fatty acid content in soil samples from the burning and rainfall treatments considered in this study. Means ± SE are given. Different uppercase and low case letters represent significant differences among treatments in spring 2010 and 2011, respectively ($P < 0.05$, one-way ANOVA).
Figure 6. Non-metric multidimensional scaling analysis of fatty acid profiles for soil representative of the burning and rainfall treatments considered in this study, sampled in spring 2010 and 2011. The data within different treatments were pooled; values represent means ± SE. Significant ($P < 0.05$) Spearman correlations between main microbial markers derived from ELFA and the ordination axes are shown next to them.
Figure A1. Layout of the rainfall and fire manipulation experiment at Quintos de Mora (Toledo, Spain).
Figure A2. Seasonal precipitation (represented on left axis) and mean temperature (on the right axes) recorded for the rainfall treatments applied in the study before and after fire.