Effects of drought on soil phosphorus availability and fluxes in a burned Mediterranean shrubland

M. Belén Hinojosa, Antonio Parra, David A. Ramírez, José A. Carreira, Roberto García-Ruiz, José M. Moreno

ABSTRACT

Little research has been done to study the combined fire-drought impacts on the structure and functioning of Mediterranean fire-prone ecosystems. In this study we have evaluated post-fire patterns of soil phosphorus (P) availability and transformation rates in a Mediterranean shrubland subjected to different drought treatments. Precipitation was manipulated in various treatments to evaluate the combined effects of fire and moisture in the soil. The following treatments were included: long-term historical average precipitation, or historical control (HC); moderate drought (MD, 25% reduction from HC); severe drought (SD, 45% reduction from HC); and environmental control without rainfall manipulation (EC). In late summer, these plots were burned in order to evaluate the joint effects of drought and fire. In order to compare burned and non-burned scenarios, unburned plots were maintained without rainfall manipulation. Post-fire soil samples, collected in the spring, were analyzed for P fractions, P transformation rates (net solubilization and immobilization, gross mineralization and phosphatase activity), mid- to high-intensity fire caused a short-term increase of rapid-to-mid turnover inorganic P pools in the soil, as well as a decline of rapid turnover organic P pools (including microbial P) and acid phosphatase activity. Fire also induced an overall rise in the flux among P pools, including gross P mineralization, net microbial immobilization and net geochemical solubilization, with the latter being the most affected. Moreover, under drier than average conditions, this ‘mineralizing’ effect of fire was partially (rapid turn over inorganic P, total P flux among pools and net solubilisation) or completely (net immobilization) offset. Thus, our results indicate that a drier environment will limit the extent of post-fire peak in soil P availability. This effect may act synergistically with increased water stress to further inhibit vegetation recovery after fire, possibility which is particularly relevant for fire-prone Mediterranean shrublands where P limitation is widespread.

1. Introduction

Water deficit and fire disturbance regimes are the two main environmental factors controlling the structure and function of Mediterranean-type ecosystems (Di Castri and Mooney, 1973). Water constraint is the prevalent limiting factor affecting primary production, species richness and soil genesis in Mediterranean-type regions (Yaalon, 1997). Fire regimes play key evolutionary and disturbance-recovery roles in shaping plant communities and landscapes in these regions (Rundell, 1998a). Fire also has a strong influence on the biogeochemical cycles of several key elements in Mediterranean ecosystems (DeBano et al., 1998; Moreno and Oechel, 1994; Rundell, 1983). Fire has long been recognized as a major, although poorly conservative, mineralizing agent which strongly impacts nutrient availability in Mediterranean soils (St. John and Rundell, 1976; Stock and Lewis, 1986). In turn, nutrient supply is often the key factor affecting plant species, functional types, and the structure, dynamics and distribution of plant communities in these regions (Carreira and Niell, 1992; Henkin et al., 1998; Specht, 1973). In this sense, phosphorus (P) limitation is especially widespread (Cramer, 2010; Henkin et al., 2006; Mayor and Rodà, 1994), since total P in Mediterranean soils is commonly limited, and even more so the proportion of readily available and labile P forms (Carreira et al., 1997; Sardans et al., 2005).

Both soil water and fire disturbance regimes are very sensitive to drivers of climate change (sensu IPCC, 2007). Mediterranean-type ecosystems may be particularly vulnerable to climate change drivers (Moreno et al., 2010; Sala et al., 2000; Schröter et al., 2005). Climate models project reductions in soil water content and increase in air...
temperature for the Mediterranean Basin (Christensen et al., 2007; Schröter et al., 2005). The way in which rainfall pattern changes will alter soil nutrient availability has been well explored in temperate and boreal biomes (e.g., Allison and Treseder, 2008; Emmett et al., 2004; Johnson et al., 2008), and are also being addressed in the Mediterranean context. Studies generally agree that, in Mediterranean systems, under drier conditions, soil labile inorganic P (Pi) and phosphatase activities decline, accompanied by an increase in soil organic P forms that are not directly available to plants (García-Fayos and Bochet, 2009; Sardans and Penuelas, 2004, 2005, 2007, 2010; Sardans et al., 2006).

There is a relatively large body of research analyzing short and long term fire effects on nitrogen (N) and P availability in Mediterranean soils (Carreira et al., 1994, 1997; Debano and Conrad, 1978; Marion et al., 1991; Rundell, 1983). The role of fire on nutrient cycles is exerted through direct (e.g. volatilization, ash deposition, chemical transformation of charred litter and plant materials) and indirect (e.g. post-fire erosion and changed conditions for soil microbial activity) effects. In relation to fire, the effects on P cycle have received less attention than the effects on C or N cycle. In contrast to N, loss of P from plant biomass through volatilization during fire is low, and the resulting ash provides a high P inputs to the soil in readily available inorganic forms (Arianoutsou and Margaris, 1981; Carreira and Niell, 1995). Heat during a fire can also affect the topsoil by converting relatively non-labile forms of P, such as organic P (Po) (e.g., phosphate mono- and di-esters) to labile forms of inorganic P (Pi) that are soluble or weakly surface-adsorbed, such as POI$^-$ (Cade-Menun et al., 2000; Galang et al., 2010; Giovannini et al., 1990). As a result, there is frequently a short-term, post-fire peak in Pi which quickly declines (Certini, 2005). While some studies have shown no change in P availability after burning (e.g., Binkley et al., 1992; Boyer and Miller, 1994), these apparent discrepancies can be attributed to differences in intensity and timing of fire, in pre-fire soil P levels, and/or in the selected P availability index. Other short-term effects, commonly found after fire, are the decrease of microbial biomass and phosphatase activity of surface soils (Boerner and Brinkman, 2003; Eivazi and Bayan, 1996; Saá et al., 1998). However, microbial activity tends to recover shortly thereafter due to increased substrate availability and altered soil temperature and moisture conditions. Recovery of microbial P and phosphatase activity has been reported following the initial post-fire decline (Arévalo et al., 1994; Serrasolsas and Khanna, 1995).

According to the findings reported above, P supply in Mediterranean soils may depend on a trade-off between the direct effects of drought conditions which could depress mineralization, and an indirect impact of fire disturbance and its role as a mineralizing agent. However, no previous studies have attempted to quantitatively distinguish between the joint effects of both drought and fire on soil nutrient cycling and availability. In this study, we have assessed how the occurrence of a fire in a Mediterranean shrubland affects the short-term pattern of change in soil P cycling and availability, depending on the particular drought conditions. A better understanding of the role of fire in Mediterranean ecosystems under scenarios of drought is relevant to calibrate mechanistic models of succession and management of burned areas.

We hypothesized that increasing drought in post-fire environments will limit the extent of the short-term peaks in soil P availability following fires, primarily because of the effect of drier conditions on: i) decreasing biological and biochemical mineralization, and ii) increasing chemical precipitation of soluble inorganic P forms due to the higher ionic strength of the soil solution in reduced moisture soils. To test this hypothesis, we studied the effects of fire on the patterns of P fractions, P transformation rates (net solubilization and immobilization, gross mineralization) and phosphatase activity in soils from a Mediterranean shrubland under various drought treatments.

2. Material and methods

2.1. Study area, experimental design and soil sampling

The study was conducted in a Cistus-Erica Mediterranean shrubland of central Spain located in the Coto Nacional de Quintos de Mora (Lat. 39° 25′ N; Long 04° 04′ W), in Montes de Toledo mountains. The study site (900–925 m altitude, 20% slope and a northwest aspect) has a typical Mediterranean climate with a mean annual temperature of 14.9 °C and mean annual precipitation of 622 mm (only 7% occurs during the summer) (Parra et al., 2012). Soil is a Dystric Cambisol (FAO soil classification) and the parent rock consists mainly in quartzite.

A rainfall manipulative experiment was initiated in April 2009. Rain manipulation treatments were defined based on long-term (1948–2006) precipitation records from a nearby meteorological station (‘Los Cortijos’ meteorological station; 39°19′ N, 4°04′ W; AEMET, Ministerio de Medio Ambiente, Rural y Marino, Spain). First of all, the long-term average precipitation for each two-week period during the year was calculated, excluding extreme values of the data series (99th percentile) for each of the time period. Secondly, a biweekly rainfall schedule for each experimental treatment was designed by modifying the historical long-term rainfall pattern as follows (Fig. 1): i) environmental control (EC) without manipulation and no changes in the natural rainfall pattern (540 mm fell during the hydrological year of experimentation); ii) historical control (HC) simulating long-term historical average precipitation (600 mm y$^{-1}$ and drought during July and August); iii) moderate drought (MD), 25% decrease in rainfall relative to HC treatment (450 mm y$^{-1}$ and drought from May to September; and iv) severe drought (SD), 45% decrease in rainfall relative to HC treatment (325 mm y$^{-1}$ with drought from April to October).

The simulated rainfall patterns were consistent with projections for the Mediterranean region (Christensen et al., 2007), i.e., lengthened summer drought and rainfall concentrated in winter.

The target rainfall for each treatment was reached by excluding rainfall using automatic rainout shelters or watering plots by sprinkler irrigation at the end of each biweekly period; depending if natural rainfall had been higher or lower, respectively, than the average of the long-term rainfall assigned for the treatment on this period. A more complete description of the mechanical system used to manipulate rainfall in this experiment can be read at Parra et al., 2012). This approach was chosen to maximize the natural rainfall input, and minimize rainout shelter effects on the microenvironment of the plots. The four different treatments were applied to replicate 6 × 6 m plots ($n=4$) (Fig. 1).

At the end of the 2009 dry season (late September) these plots were burned (hereafter denoted by adding ‘+’ to the corresponding rainfall treatment reference) in order to assess the joint effects of drought and fire. Experimental fire was homogeneous across all plots; significant differences in fire intensity were not recorded among rainfall treatments. The mean residence time above 100 °C was 13.76 min, and the mean maximum temperature was 710 °C (Parra et al., 2012). In order to compare burned and non-burned treatments, an additional set of four plots without rainfall manipulation was left unburned (EC−), resulting in a total of five replicate treatments: EC−, EC+, HC+, MD+ and SD+. Detailed information about the experimental design, rainfall manipulations and fire is provided in Parra et al. (2012).

Soil samples were collected in May 2010, eight months after burning. We expected that this mid-spring sampling date would provide the greatest soil moisture differences among drought treatments, together with optimum temperature for soil microbial activity. Four composite samples, each one consisting of four soil cores, were collected from the top 5 cm of mineral soil in each experimental plot. Soil samples were transported in an isothermic bag (4 °C) to the lab.
laboratory, where they were sieved (<2 mm) before further analysis. The collected soil was determined to be sandy loam (74% sand, 20% silt and 6% clay) with 3.7% carbonate content (Laboratorio Agroalimentario Regional de Albacete).

2.2. General physico-chemical soil properties

Soil water content was estimated gravimetrically. Soil pH and conductivitity were determined in a slurry (1:2.5; soil:H₂O) according to McLean (1982). Particle size distribution was determined using a densimeter according to the Bouyoucos method (Gee and Bauder, 1986). Total nitrogen was analyzed according to the Kjeldahl method. 

2.3. Soil phosphorus fractionation

Field-moist soil samples were analyzed for soil P fractions using a variation of the P fractionation scheme of Hedley et al. (1982), to sequentially extract inorganic and organic P held in a variety of bioavailable and poorly-available pools. Briefly, 3.5 g of each soil sample were placed in a 50-ml centrifuge tube containing 30 ml of distilled water and a 5 × 2.5 cm² section of an anion-exchange membrane (type 204-U-386 Ionics Inc., Watertown, MA, USA), equivalent to 1 g of dry resin (Cooperband and Logan, 1994), previously saturated with Cl⁻⁻. After being shaken for 16 h, the membrane was removed and treated with 0.5 M HCl to elute the PO₄³⁻ ions sorbed onto it. This procedure provides the membrane-extractable inorganic P (Pi) fraction (hereafter, memb-Pi), which represents the most readily bioavailable P and includes soil solution Pi as well as the most labile adsorbed Pi (Sibbesen, 1978). The soil remaining in the tube was recovered by centrifugation (3500 rpm for 10 min.) and extracted with 0.5 M NaHCO₃ at pH 8.5 to obtain the rest of the labile P pool. This pool is considered a good indicator of short- to mid-term plant available-P and includes both inorganic (bic-Pi) and organic (bic-Po) forms of P, reversibly sorbed onto soil colloids and surfaces (Bowman and Cole, 1978). Following additional centrifugation steps, the moderately to poorly labile pool was sequentially extracted from the remaining soil with 0.5 M NaOH followed by 1.0 M HCl. The NaOH-extractable fraction includes non-occluded, inorganic P (NaOH–Pi) and organic P (NaOH–Po) forms bound to Fe and Al minerals (Ryden et al., 1977). The HCl-extractable fraction includes both inorganic and organic Ca-bound forms of P (Williams et al., 1971), although in the current investigation, only the inorganic fraction was quantified (HCl-Pi).

All the supernatants obtained during the sequential extraction were filtered, neutralized, and then directly analyzed for phosphate by the ascorbic acid-molybdenum blue method (John, 1970). This process allows the calculation of inorganic P concentrations in the different fractions. Additionally, aliquots of the bicarbonate and NaOH extracts were subjected to a sulfuric acid-potassium persulfate digestion (D’Elia et al., 1977). The concentration of organic P in these two fractions was estimated by subtracting inorganic P from the total P, determined in the corresponding digested extract.

Microbial P (micr-P) was estimated as the difference in bicarbonate-extractable P between chloroform-fumigated and non-fumigated pairs of replicated soil samples, using a conversion factor of K₀ = 0.4 (Brookes et al., 1985; Hedley and Stewart, 1982). Finally, total soil P was determined on separate soil subsamples (0.2 g DW) after nitric-perchloric acid (5:3 v:v) digestion (Sommer and Nelson, 1972). Residual P was calculated as the difference between total P and the sum of all P fractions obtained in the sequential extraction scheme.

All data are expressed on dry weight basis.

2.4. Phosphorus transformation rates and phosphatase activity

Soil P transformation rates were evaluated using a modification of the radiation-autoclaving-incubation procedure of Zou et al. (1992). Three subsets were obtained by sub-sampling each field-moist compost soil sample. One subset was used as a control and the other two were sterilized, using HgCl₂-addition at a rate of 2500 mg kg⁻¹ of dry soil (Wolf and Skipper, 1994), instead of γ-radiation (Viñegla et al., 2006). To apply the HgCl₂-treatment, the corresponding samples were evenly mixed with the appropriate amount of HgCl₂. All samples were then incubated for 12 d, adding water when needed to maintain the original soil moisture. Soil respiration rates were measured during incubation, using the aqueous alkali trapping method (Zibilske, 1994) to check for the degree of microbial sterilization achieved by the HgCl₂ treatment, and we found that soil respiration was reduced by 92% after this treatment. One of the two HgCl₂-treated subsets was then autoclaved (120 °C for 30 min) three times to denature soil enzymes. As a result, three independent treatments were created for analysis: (i) control, (ii) HgCl₂-addition, and (iii) HgCl₂-addition plus autoclaving.

Immediately after treatment, all samples of the three soil subsets were examined for membrane-extractable P during a 48 h incubation period. This was achieved by shaking 2.5 g of soil with 30 ml deionized water and a 12.5 cm² section of an anion-exchange membrane (type 204-U-386 Ionics Inc., Watertown, MA, USA) in 50 ml centrifuge tubes in a reciprocal shaker (200 strokes min⁻¹). To ensure complete soil sterilization in the HgCl₂ and the HgCl₂ plus autoclaving treatments, and to maintain sterile conditions during incubation with membranes, additional HgCl₂ (2500 mg kg⁻¹ of dry soil) was added, along with the deionized water (30 ml), to the corresponding centrifuge tubes. Following incubation, membranes were extracted with 30 ml 0.5 M HCl on a reciprocal shaker for 1 h. Phosphate
concentration in the solutions was determined by the ascorbic acid–molybdenum blue method of John (1970).

By extracting P with membranes from the control soils, the net balance between solubilization of inorganic P, mineralization of organic P and immobilization of solution P can be determined. Membrane-extractable P from the HgCl2-treated samples is an estimate of the sum of solubilized P and mineralized P (HgCl2-sterilization avoids microbial immobilization of solution P). Phosphorus extracted from the HgCl2-treated plus autoclaved soils is derived only from the solubilization of inorganic P (autoclaving of HgCl2-treated soils additionally avoids P mineralization by phytase activity), and gives an estimate of the net P solubilization rate. Therefore, the difference in the concentrations of membrane-extractable P between the HgCl2-addition and the HgCl2-addition plus autoclaved treatments is an estimate of the gross P mineralization rate. The difference between the HgCl2-addition and control treatments is an estimate of the P immobilization rate.

To check for the direct effect of HgCl2-addition and autoclaving on membrane-extractable P, three subsamples of 2.5 g moist soil from each of the control, HgCl2-addition, and HgCl2-addition plus autoclaving treatments were extracted with anion exchange membranes as above, except that extraction time was only 1 h. If differences were found in membrane-P concentrations before and after HgCl2-addition and HgCl2-addition plus autoclaving, correction factors were applied in the calculation of P transformation rates, to allow for direct treatment effects (Zou et al., 1992).

Effects of fire and rainfall manipulation treatments on phosphatase activity were also tested. Both acid and alkaline phosphomonoesterase (PMEase) activities were measured on soil samples following the method developed by Tabatabai (1982), using p-nitrophenyl phosphate (pNPP) as enzyme substrate.

2.5. Statistical analysis

A one-way ANOVA was performed to test the effects of fire (comparison of burned and unburned soils), and to test the effect of rainfall manipulation treatments on burned soils. ANOVA requirements of normality (Kolmogorov–Smirnov test) and homogeneity of variance (Bartlett–Box F test) were checked at α = 0.01. For variables that did not meet such requirements, log (x + 1) transformation was sufficient to achieve normality and homocedasticity. Statistical differences were determined at a significance level of α = 0.05, n = 4. Differences among levels of a treatment were tested using the Fisher Least Significant Difference (LSD) test.

3. Results

3.1. General soil properties

At sampling time (mid spring), the soil water content was not significantly different between burned and unburned plots under environmental control conditions (no rainfall manipulation) (Table 1). As expected, the drought treatments applied to burned plots strongly decreased soil water content (40% and 70% reductions in the MD+ and SD+ treatments, respectively, compared to the long-term historical average precipitation treatment, HC+). Soil electrical conductivity showed no effect due to fire, but it increased significantly with the drought applied to burned soils (Table 1). Conversely, soil pH was not significantly affected by rainfall reduction, although it was higher in burned plots than in unburned plots, under environmental control conditions. Fire did not significantly change soil organic matter and total N. The decrease in total C was not statistically significant but provoked a significant change in C/N ratio. Rainfall treatments had no significant effect on total C or organic matter content of burned soils. However, soil total N was significantly higher in HC+ than in the SD+ treatment.

3.2. Soil phosphorus fractionation

Neither the burning nor the rainfall manipulation treatments showed significant effects on soil total phosphorus concentrations (Table 2). However, substantial differences among treatments were found for the various analyzed P fractions (Table 2). Mean concentrations of the most biologically available inorganic P fraction (memb-P) ranged from 5 to 15 μg P g−1, and accounted for just 3.0% and 1.6% of the total P in burned and unburned soils, respectively. Fire significantly raised the concentration of memb-Pi on plots under normal environmental conditions. A moderate rainfall reduction (MD+ treatment) also increased memb-Pi in burned plots, although this effect was not observed under more severe drought conditions (SD+ treatment). Labile inorganic P levels (bic-Pi) in burned soils were twice those in unburned soils, accounting for 3.4% and 1.7% of total P, respectively. Rainfall reduction on burned soils caused a significant decrease in the bic-Pi fraction (from 19.4±3.9 μg P g−1 in the HC+ treatment to 9.0±1.1 μg P g−1 in the SD+ treatment). Labile organic P (bic-PO), which accounted for 5% (on average) of total P, was not significantly affected by burning alone or the joint effect of burning and altered rainfall. The microbial P fraction (micr-P) was also non-responsive to changes in rainfall on burned soils; however, it was reduced by a factor of 1.5 in burned plots under environmental control conditions in comparison to values of unburned soils. This fraction represented 14.6% of total soil P in EC- plots and 9.7% in EC+ plots. Considerably higher concentrations of inorganic, but especially organic, P were extracted from all soils when using a strong base (sodium hydroxide) instead of a weak base (bicarbonate). The concentration of organic P forms always exceeded the concentration of inorganic P in the NaOH-extractable P pool. Excluding residual-P, the NaOH-Pi plus NaOH-Po fractions made the greatest contribution (33% to 41%) to total P. The NaOH-Pi concentration increased significantly after fire, but it decreased on burned soil as a consequence of severe drought. The NaOH-PO fraction was significantly reduced in both the burn and drought treatments. Effects on the Ca-bound
Phosphorus fractions (μg P g⁻¹ DW) in the upper (0–5 cm) mineral soil layer of study plots. Burned (EC+) and unburned (EC−) plots under environmental conditions, and burned plot with different rainfall (HC+: long-term historical average precipitation; MD+: moderate drought; SD+: severe drought. Data represent the mean (standard deviation). Different uppercase letters within each column indicate significant differences between burned and unburned soils. Different lowercase letters within each column denote significant differences among rainfall treatments in burned soils (p<0.05, n=4).

### Table 2

<table>
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<th>Treatments</th>
<th>Phosphorus fractions</th>
<th>Rapid turnover Po</th>
<th>Mid to slow turnover P</th>
<th>Total P</th>
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<td>memb-Pi</td>
<td>bic-Pi</td>
<td>NaOH-Po</td>
<td>residual-P</td>
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<td>EC−</td>
<td>5.3 (1.5) A</td>
<td>5.8 (1.8) A</td>
<td>49.0 (8.7) B</td>
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<td>sum pool: 1417.3 (30) A</td>
<td>sum pool: 1402.2 (126) A</td>
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<td>11.2 (3.1) B</td>
<td>31.8 (7.0) B</td>
<td>21.8 (2.5) B</td>
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<td>sum pool: 134.4 (42) A</td>
<td>sum pool: 161.7 (30) A</td>
<td>sum pool: 163.0 (126) A</td>
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**Comparison factor:** unburned vs burned plots under environmental conditions

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<th>Total P</th>
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<td>EC−</td>
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<tr>
<td>EC+</td>
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<td>sum pool: 21.3 (2.0) B</td>
<td>sum pool: 134.4 (42) A</td>
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**Comparison factor:** levels of rainfall manipulation in burned plots

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<th>Rapid turnover Pi</th>
<th>Total P</th>
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<tr>
<td>HC+</td>
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<td>19.4 (3.9) c</td>
<td>34.2 (5.6) b</td>
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<td>146.0 (38.4) a</td>
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<td>R1+</td>
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<td>160.2 (28.5) a</td>
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### 3.3. Soil phosphorus transformation rates

Total P flux through the soil solution was significantly higher in burned (4.6 ± 0.1 μg P g⁻¹ d⁻¹) than in unburned soil (0.8 ± 0.1 μg P g⁻¹ d⁻¹) in plots under environmental control conditions (Fig. 2). Drought superimposed on burned soils caused a reduction in the total P flux, from a mean of 6.7 ± 0.6 μg P g⁻¹ d⁻¹ in the plots subjected to historical mean rainfall amounts, to 3.1 ± 0.4 μg P g⁻¹ d⁻¹ when this rainfall was reduced by 45%. The increasing effect of fire on total P flux was due mainly to a five-fold increase in net chemical solubilization rates; gross mineralization rates and net microbial immobilization rates were unaffected. These results are consistent with the fact that burning had no significant effect on the net change in the size of the soil organic P pool during incubation,

Fig. 2. Net solubilization, gross mineralization, and immobilization rates of P in burned soils under different rainfall treatments and in unburned soils. Circles represent P pools; inner numbers denote the net change in the pool size during incubation (μg P g⁻¹ soil). Processes are represented by arrows and the corresponding P transformation rate (μg P g⁻¹ d⁻¹). Uppercase letters represent the same process (italicized for solubilization, regular type for mineralization, with an apostrophe for immobilization) indicate significant differences among burn treatments; lowercase letters reflect significant differences among rainfall treatments (p<0.05, n=4).
Despite a significant, three-fold net increase in the soluble P pool (Fig. 2), regarding the effects of different rainfall treatments applied to burned soils, the observed decrease in total P flux upon soil incubation relied on significant and similar reductions in both net chemical solubilization and microbial immobilization rates. Thus, the size of the soluble P pool was unchanged upon soil incubation. The net change in the organic P pool significantly shifted from positive in HC+ soils to negative in MD+ and SD+ soils, which is explained by increasing (though not significant) gross mineralization in response to elevated drought intensity.

Potential phosphomonoesterase (PME) activity was between 200 and 400 μg PNP g⁻¹ dw soil h⁻¹ (Fig. 3). No significant correlation was found between PME activity and net P mineralization rates (data not shown). Burning of plots under environmental control conditions, while having no effect on soil alkaline PME, showed a significant reduction in soil acid PME activity, relative to unburned plots. In addition, the activity of both enzymes in burned soils was significantly reduced as a consequence of drought, with activity showing a significant positive correlation with soil water content (r = 0.798 for acid PME and r = 0.772 for alkaline PME, p < 0.01).

4. Discussion

Our study site is representative of the type of nutrient-poor Mediterranean heathlands and shrublands that demonstrate widespread P limitation, due to highly weathered soils developed on old Palaeozoic materials in the core of tectonic plates (Specht and Moll, 1983). The study site, located in the center of the Hercynian Iberian Belt, is characterized by nutrient-poor soils and reduced P availability. Mean total P (333.6 μg P g⁻¹) falls within the mid-range for Mediterranean soils worldwide (Rundell, 1998b). However, the most readily available P fraction (memb-Pi) represented just 1.6% of the total soil P pool in the environmental control, whereas occluded and recalcitrant forms (residual-P) accounted for more than 1/3 of total P (Table 2). This proportion rose to more than 75% of the total P if the mid to slow turnover fractions (NaOH, HCl and residual) are pooled together. The very low measured concentration of primary, apatite-like, Ca-P minerals (see HCl-Pi in EC-plots, Table 2), together with the high proportion of P bound to secondary Fe and Al minerals (NaOH-fractions), are indicative of highly weathered P-deficient soils (Cross and Schlesinger, 1995; Walker and Syers, 1976). Moreover, the importance of the microbial P fraction (15% of total P in EC-soils) and a Po/Pi ratio as high as 16.4 in the rapid turnover P pool, suggest that phosphate availability in the tested soils rely to a large degree on P from the organic subcycle. Such features are common in soils and ecological successional stages of fire-prone shrublands where symptoms of P-limitation have been reported (Carreira et al., 1997; Vitousek and Farrington, 1997). Thus, our study site represents a context in which an effect of drier conditions, even if only marginal, on phosphorus availability will be of particular significance for vegetation recovery after fire, since P shortage might mean an additional constraint to that of water stress.

By releasing P previously held in senescent vegetation, and by fostering the build-up of rapid turn-over soil P, natural fire-regimes act as mineralizing agents in P-poor Mediterranean ecosystems (St. John and Rundel, 1976; Stock and Lewis, 1986). Indeed, we noted a short-term doubling of memb-Pi and bic-Pi concentrations after burning plots under environmental control conditions (Table 2). Post-fire peaks in soil P availability may result from: i) total and partial pyromineralization of P compounds in plant tissues, litter and the O horizon, and subsequent deposition as ash and charred material onto the A horizon (Galang et al., 2010); ii) heating of the A horizon that results in partial oxidation of soil Po (Giardina et al., 2000); and iii) enhanced P mineralization after fire due to increased substrate availability (easily mineralizable P derived from the former processses (Turrion et al., 2010)), and/or to improved process conditions in the soil. Data from our study indicate that direct pyromineralization and heating effects, as well as chemical speciation of the released phosphate, are the main factors explaining the post-fire peak in P availability. First, all the individual inorganic P fractions (including NaOH-P and HCl-Pi) significantly increased after fire exposure (Table 2). Fire also induced a soil pH shift from slightly acidic (5.5) to near neutral (6.6) (Table 1). At pH around 6.5, soils commonly show minimum phosphate fixing capacity (McDowell and Condron, 2001). Second, the organic and microbial fractions, as well as phosphatase activities, remained unchanged (bic-Po, alkaline phosphatase) or diminished (NaOH-Po, micr-P, acid phosphatase) (Table 2, Fig. 3). Finally, geochemical mechanisms (net solubilization) explain a greater proportion of the effect of fire on total P flux and the size of the soluble P pool upon soil incubation, than do biologically-mediated mechanisms (mineralization/immobilization balance) (Fig. 2). In summary, our results support the significant role of fire as a mineralizing agent and the occurrence of a post-fire peak in soil P availability. Even though loss of P in runoff and erosion were not measured in our study, as they were minimized in our experimental setup, they should be considered also as potential factors affecting total P in soil after a fire.

The effects of reduced water availability on P cycling and availability have recently been assessed for Mediterranean-type climates. Drought treatments applied to an evergreen forest in NW Spain led to the build up of soil organic P that was not directly available to plants, and a subsequent decline in standing biomass P (Sardans and Peñuelas, 2004). In the same long-term experiment, soil enzyme...
activity decreased (Sardans and Peñuelas, 2005, 2010), and the inorganic P (Pi) to organic P (Po) ratio in the soil solution declined (Sardans and Peñuelas, 2007). Similar responses to increased drought have been reported in an Erica-dominated shrubland (Sardans et al., 2006). García-Fayos and Bochet (2009) found that labile Pi decreased under dry and warm conditions in semi-arid Mediterranean shrublands. Thus, generally the literature appears to be consistent in its demonstration of decreasing mineralization under water-limited conditions in Mediterranean ecosystems.

The results of our study, which explored how fire and soil moisture interactions affect the short-term pattern of soil P cycling and availability, distinguished two main effect groups (Fig. 4): i) fire effects reduced by drought and ii) fire effects enhanced by drought.

i) Fire effects reduced by drought

The pool of rapid turnover Pi (primarily the bic-Pi fraction), which doubled in response to fire on non-manipulated rainfall plots, was significantly reduced under severe drought conditions in comparison to burned soil with historical rainfall (HC). However, this pool was still significantly higher in burned soil under severe drought than in unburned soils. A similar tendency was observed for Pi-NaOH (a fraction of mid-turnover Pi). Thus, drought partially offset the increase of low- and mid-turnover Pi pools induced by fire. Similar results have been reported elsewhere on unburned soils (García-Fayos and Bochet, 2009; Johnson et al., 2008). While fire effects are dominated by the geochemical controls on P availability, the net balance of soluble P pools under different soil moisture conditions may be defined by both, geochemical and biologically-mediated processes. Therefore, in the drought plus fire treatments, the observed post-incubation decrease in total P flux was due to both significant reductions in net chemical solubilization and net microbial immobilization rates. The reduction in net chemical solubilization could be due to increased ionic strength of the soil solution induced by drought (thus, higher ion concentrations due to less dilution, which favor precipitation of phosphate minerals). This hypothesis is supported by a significant increase on soil electrical conductivity of burned soils due to drought, which suppose to be strongly related to ionic strength of soil solution (Alva et al., 1991; Jallah and Smyth, 1995). On the other hand, the reduction in net microbial immobilization may be in response to a reduction of microbial biomass as a consequence of low soil moisture. This possibility is supported by our study data which show a decrease (though non-significant) in microbial P under drought conditions. However, the reduction of net P immobilization under drought conditions may be affected also by the differential response of various groups of soil microorganisms under water stress (Killham, 1994), in relation to i) their tolerance to water stress, ii) P flux rates associated with osmoregulatory strategy, and/or iii) in the extent to which P flux changes with cell death as a consequence of dehydration.

ii) Fire effects enhanced by drought

Fire caused a moderate drop of the rapid- to mid-turnover organic P (including microbial P) and phosphatase activity in the soil. All these fire effects were magnified under drier conditions. Our study showed that the moderate decrease of rapid- to mid-turnover Po that occurred after fire was exacerbated by drought. In addition, phosphatase enzyme activity, previously decreased as a consequence of fire, was also significantly reduced as a consequence of drought. Thus, our results agree with previous investigations on unburned soil showing a clear decrease of potential phosphatase activity under drier climatic conditions (Li and Sarah, 2003; Sardans and Peñuelas, 2005). In this sense, phosphatase enzyme activity has shown to be once more a sensitive and earlier indicator of ecosystem P limitation (Aon et al., 2001; Arévalo et al., 1994).

5. Conclusions

The assessment of drought impacts on phosphorus biogeochemistry in Mediterranean systems should take into account post-fire environments, since the interactive effects of these two factors may enhance, mask or ameliorate any particular singular effect. Increasing water stress had the effect of limiting the extent of the peak in rapid turnover inorganic P pools caused by fire. Drought also constrained the fire-induced rise of the overall flux between soil P pools. In contrast, drier environmental conditions enhanced the moderate drop of the rapid-to-mid turnover of organic P (including microbial P) and phosphatase activity in the soil caused by fire. Overall, our results indicate that a drier environment will limit the extent of the post-fire peak in soil P availability. In P-deficient ecosystems, this effect may act synergistically with increased water stress to further inhibit vegetation recovery after fire. However longer-term studies are needed to determine if these effects persist over time.

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Fig. 4. Changes induced by rainfall manipulation treatments on the short-term effects of fire in process rates (A) and P pools (B) of a Mediterranean shrubland soil. Data are expressed as the relative change (%) with respect to unburned plots without rainfall manipulation. Pir: rapid turnover inorganic P pool; Por: rapid turnover organic P pool; Ps: slow turnover P pool. See Section 2.4 and Fig. 2 for details regarding “total P flux” and the displayed P transformation rates. Asterisks indicate significant differences (p<0.05, n = 4) for modulation exerted by rainfall treatments on the effect of fire.
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