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Effects of an experimental fire and post-fire stabilization treatments on soil microbial communities

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ABSTRACT

Wildfire is the major type of disturbance in forest and shrubland ecosystems in Galicia (NW Spain). Soil stabilization and rehabilitation techniques are frequently used to minimize the impact of fire on the ecosystems affected. However, information concerning the specific effects of these post-fire practices on soil microbiota is particularly scarce. In the present study we assessed the effect of an experimental fire of low severity, alone and combined with one of two post-fire stabilization treatments (seeding and mulching), on soil microbial communities in a shrubland area in the region. Measurements of soil microorganism biomass (microbial C determined by both the fumigation-extraction and the substrate induced respiration techniques), activity (respiration, β-glucosidase, urease and phosphatase) and diversity (community level physiological profiles by Biolog Ecoplates) were made at different times (1, 90, 180 and 365 days) after the fire and application of the stabilization treatments, and compared with the same measurements made in the respective unburned control soil. Microbial biomass and activity were generally reduced by fire, whereas the microbial diversity was increased by fire. However, the fire-induced changes in microbial communities were relatively small compared with the marked temporal variations in the microbial parameters analyzed, suggesting that this type of fire does not substantially change the soil functioning. This response can be partly explained by the relatively low temperature that the soil reached during the experimental fire. Mulching and seeding treatments did not have any effect on biomass, activity and diversity of soil microorganisms. The implications of these results for management practices are discussed.

1. Introduction

Wildfire is the major type of disturbance in forest and shrubland ecosystems in Galicia (NW Spain). In fact, Galicia and the North of Portugal are the areas of Europe most affected by forest wildfires, and worldwide they are amongst the areas with the greatest number of fires per hectare and inhabitant (Carballas et al., 2009; Catry et al., 2010). Approximately half of the wildfires in Spain occur in the temperate humid zone; the number of fires is around 9000 per year on average, and the area burned was on average about 40,000 ha in each of the last ten years (Ministerio de Medio Ambiente, Medio Rural y Marino, 2010). Most of the wildfires occur in shrubland areas (Xunta de Galicia, 2011), which are often located on sloping terrain, in soils with moderate erodibility due to rainfall. All of these factors and post-fire meteorological conditions (abundant high-intensity rainfall events in the autumn period immediately after wildfires) tend to increase runoff and erosion processes in the surface soil horizon (Díaz-Fierros et al., 1982, 1990; Fernández et al., 2008; Vega et al., 2005).

Soil microorganisms are the main agents responsible for the long-term sustainability of soil ecosystems because they control the breakdown of organic matter and the net fluxes and amounts of soil carbon and nutrients via decomposition, mineralization, and immobilization processes (Nannipieri et al., 2003). Furthermore, as microorganisms reflect environmental conditions, they can be used as early indicators of changes in soil quality due to soil perturbation, land use or soil management, before such changes can be easily detected in other soil properties. Hence, analysis of microorganisms is important to elucidate the role of fire in forest ecosystems and to determine the contribution of microbes to ecosystem recovery after fire. Previous studies have shown that several aspects of microbial presence, including number, biomass, activity and composition may be affected by wildfire and that these effects may vary widely depending on fire severity, changes in some soil properties and post-fire environmental conditions (see reviews by Certini, 2005; DeBano et al., 1998; Mataix-Solera et al., 2009; Neary et al., 2005). In general, these studies have shown that fire directly alters the composition of the soil microbial community and activity (in the short-term)

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through selective heat-induced microbial mortality, whereas medium- and long-term responses of soil microorganisms to fire may due to indirect effects through modification of their environment (physical and chemical soil properties, alterations in plant community composition and production).

Most investigations concerning the effects of fire on soil microbiota have focused on changes in total microbial biomass and activity, but the effects on the composition of these populations and the consequences of altering the microbial structure have received less attention, especially when considered simultaneously with microbial biomass and activity-related microbial parameters (Bergner et al., 2004; D'Ascoli et al., 2005; Giai and Boerner, 2007; Guénon et al., in press). Knowledge of all these soil microbial characteristics (biomass, activity, and diversity) may improve our understanding of the role played by the soil microbial community in the post-fire recovery of the soil ecosystem, especially when this information is linked to changes in key physical and chemical soil properties, vegetation regeneration patterns and environmental variables.

Soil stabilization and rehabilitation treatments are often used to minimize the impact of fire on ecosystems (MacDonald and Larsen, 2009; Napper, 2006; Robichaud, 2009; Robichaud, et al., 2000, 2006). Among these techniques, mulching and seeding are extensively used (Bautista et al., 2009; Beyers, 2009) and have recently been applied in shrubland areas affected by wildfires in NW Spain, where their effectiveness in controlling soil losses have been evaluated (Fernández et al., 2011a, 2011b). The main aim of these measures is to reduce soil erosion and soil degradation, and it is generally assumed that they may also contribute to ecosystem recovery. However, information concerning the effects of such post-fire practices on soil microbiota is particularly scarce (Díaz-Raviña and Martín, 2012); such knowledge is important in establishing if these treatments reduce soil losses and substantially contribute to recovery of the soil, as microbial parameters are sensitive indicators of soil quality. The above is relevant for post-fire management decisions, although we are not aware of any previous studies that have evaluated the effect of mulching and seeding stabilization treatments on soil microbial properties in an experimentally burned shrubland area.

Experimental and prescribed burning enable better quantification of the magnitude of the impact of fire on soil properties than provided by opportunistic studies conducted after a wildfire, as pre-fire values of the relevant parameters can be measured and fire characteristics adequately assessed. Most investigations on experimental or prescribed fire effects have focused on forest areas (e.g. Boerner et al., 2000; Choromanska and DeLuca, 2001; Fontúrbel et al., 1995; Fritze et al., 1993; Giai and Boerner, 2007; Pietikäinen and Fritze, 1995). However, less is known about shrubland ecosystems (Barreiro et al., 2010; D'Ascoli et al., 2005; De Marco et al., 2005; Fioretto et al., 2002; Guénon et al., in press; Palese et al., 2004; Saá et al., 1993; Úbeda et al., 2005). To address the lack of knowledge on the microbiological effects of the two most commonly used post-fire and stabilization and rehabilitation treatments, a study involving the use of an experimental fire in a shrubland ecosystem was carried out to characterize fire behavior and soil burn severity and to evaluate the effects of fire and post-fire treatments.

The aim of the present study was to examine the microbial response, in terms of biomass, activity and diversity, to an experimental fire combined with post-fire seeding and mulching treatments in a Galician shrubland.

2. Material and methods

2.1. Experimental design

The study was conducted in an enclosed experimental field located at an altitude of 660 a.s.l., in Cabalar (A Estrada, 42° 38′ 58″ N; 8° 29′ 31″ W; NW Spain), i.e. under a temperate rainy climate. The soil is developed over a parent material of granite and the slope of the plot is 38–54% oriented to the north. The vegetation is representative of many oceanic–climate shrubland ecosystems in Galicia, i.e. it is dominated by gorse *Ulex europaeus* L. and some *Pteridium aquilinum* (L.) Kuhn., *Ulex gallii* Planch., *Daboecia cantabrica* (Huds.) K. Koch and *Pseudoarrhenaterum longifolium* Rouy. The height of the vegetation was on average 123 cm and ground cover 100%. The area has a long history of repeated fires and low range count. The last fire occurred five years before the start of the study.

Sixteen experimental plots (30 × 10 m each) were established with the longest dimension parallel to the maximum slope. Twelve plots were burned and the remaining four plots were used as unburned controls. In June 2009, the shrubs were cut and laid directly on the ground to favor more homogeneous burning and higher fuel combustion, particularly of the litter and duff layers. Pre-fire fuel inventories were carried out and the mean shrub stratum loading was evaluated as 3.1 ± 0.19 kg m^{-2} (mean value ± SD); the litter layer loading was 1.7 ± 0.26 kg m^{-2}. In the month preceding the experimental fires, the mean daily precipitation was 3.9 l/m^2, and there was a period of 6 days without rain immediately before the fires. The monthly mean air temperature was 15.2 °C. The plots were burned on 14 and 15 October 2009 by use of the backfire technique to promote soil organic cover consumption and soil heating. Burning was conducted under a mean air temperature of 22 °C, relative humidity 55% and wind speed 2.2 (1.0–3.2) m s^{-1}. The moisture content of the organic layer and mineral soil was 61% (31–97%) and 58% (38–75%), respectively. The mean organic layer depth prior to burning was 3.7 (2.9–4.7) cm. The fires progressed slowly (0.30 to 0.33 m/min) and caused relatively high fuel consumption, whereas the duff was consumed unevenly. The mean fuel loading (from shrub stratum + litter layer) remaining after the fire was 0.3 ± 0.07 kg m^{-2}. Five temperature measurement points were selected at random in each experimental burn plot, and chromel alumel K type thermocouples (1 mm external sheath diameter) were inserted at two depths (mineral soil surface and 2 cm below mineral soil surface) at each point. The thermocouples were connected to data loggers to record the temperature regime during fire (Table 1). The data obtained indicated moderate soil warming at the soil surface but that the heat did not penetrate into the soil.

Four treatments were applied at random to the experimental plots in quadruplicate: a) unburned soil (US); b) burned soil (B); c) burned soil seeded at a rate of 45 g m^{-2} (*Lolium multiflorum*, 35%; *Trifolium repens*, 25%; *Dactylis glomerata*, 20%; *Festuca arundinacea*, 10%; *Festuca rubra*, 5%, *Agrotis tenuis*, 5%) (B + S); d) burned soil to which 230 g m^{-2} of straw mulch was applied (B + M). The soil stabilization treatments were applied manually to minimize soil perturbation, immediately after fire.

Soil mineral samples were taken from the A horizon (0–5 cm depth) after removing the litter layer, immediately before and 1, 90, 180 and 365 days after the fire and application of the treatments. Ten squares (10 × 10 cm), distributed uniformly around each plot were sampled. The samples were mixed and thoroughly

Table 1
Temperature regime during the experimental fire considering the twelve experimentally burned plots (n = 5 measurements per plot and soil depth).

	Mineral soil surface		2 cm below mineral soil surface	
	Mean	Range	Mean	Range
Mean maximum temperature (°C)	153	48–420	34	22–43
Duration of temperatures > 60 °C (min)	23.4	0.6–93.4	0	0–0
Duration of temperatures > 100 °C (min)	11.0	0–44.0	0	0–0
Duration of temperatures > 300 °C (min)	1.4	0–10.8	0	0–0

homogenized after sieving at 2 mm. All samples were stored at 4 °C for no longer than 2 weeks until analysis of biochemical and microbiological properties.

2.2. Physicochemical properties

Measurements of physicochemical characteristics were carried out on air-dried soil samples as described by Guitián-Ojea and Carballas (1976). Soil water-holding capacity was determined in a Richards' membrane-plate extractor at a pressure corresponding to a matrix potential of pF 2. The pH was measured in a 1:2.5 soil/water suspension and electrical conductivity (EC) in the extract soil/water 1:1. Total C was determined by dry combustion in a Carmhograph 12 (Wösthoff, Bochum, Germany).

2.3. Microbial biomass

The microbial biomass C was determined by both the fumigation-extraction (Cmic-FE) and substrate induced respiration (Cmic-SIR) methods. The fumigation-extraction technique was performed as described by Díaz-Raviña et al. (1992). After fumigation of the soil with $CHCl_3$ for 24 h, the organic C was extracted from the unfumigated and fumigated samples with 0.05 M K_2SO_4 at a soil–extract ratio of 1:4. The microbial biomass C values were calculated from the equation: biomass C = 2.64 EC, where EC is the extractable C flush (difference between the extractable organic C in the fumigated and unfumigated samples). The substrate induced respiration was measured according to the criteria of Anderson and Domsch (1978). Glucose was added to the soil as a solid after determination of the optimum rates (5 mg glucose g^{-1} dry soil), and the CO_2–C respiration was measured in an automated impedance-meter. The CO_2 output from the soil was determined by KOH absorption and subsequent changes in conductivity. The soil microbial biomass was calculated by the following equation: Cmic-SIR (mg kg^{-1} soil) = 40.04 X + 0.37, where X is the substrate induced respiration (ml CO_2 kg^{-1} h^{-1}).

2.4. Microbial activity

The soil respiration, an overall index of the activity of heterotrophic microorganisms, and the measurement of three specific enzyme activities related to the C (β-glucosidase), N (urease) and P (phosphatase) cycles were used as indicators of soil microbial activity. The soil respiration was determined by estimation of the amount of CO_2 emitted from fresh soil samples incubated for 24 h at 22 °C in an automated impedance-meter. The CO_2 output from the soil was determined by KOH absorption and subsequent changes in conductivity. The specific respiration rate or metabolic quotient (qCO_2) was calculated from the respiration rate and the microbial biomass C (Anderson and Domsch, 1990).

The β-glucosidase activity was measured following the procedure of Eivazi and Tabatabai (1988), which determines the *p*-nitrophenol released after incubation of the soil with a *p*-nitrophenyl glucosidase solution for 3 h at 37 °C. The urease activity was estimated by incubating the soil samples with an aqueous urea solution and extracting the NH_4^+ with 1 M KCl and 0.01 M HCl followed by the colorimetric NH_4^+ determination by a modified indophenol reaction (Kandeler and Gerber, 1988). The phosphatase activity was assayed following the method described by Trasar-Cepeda et al. (1985), which determines the amount of *p*-nitrophenol released after incubation of the soil with *p*-nitrophenyl phosphate for 30 min at 37 °C.

2.5. Microbial community diversity

Laboratory assays on the community level physiological profiles (CLPPs) of the soil microflora utilizing various carbon substrates were performed to determine the functional capabilities and diversity of the soil microbial community (Garland and Mills, 1991). Biolog Ecoplates® were used to assess bacterial diversity. Soil suspensions in Milli-Q water (1:10 w/v) were physically dispersed in a Waring blender (13,500 min^{-1}, 3 cycles × 1 min). After carefully pipetting 25 ml of the soil suspension and centrifugation at 10,000 g (20 min, 20 °C), 10 ml of the resulting supernatant, which included the cells, was transferred to 250-ml bottles containing 90 ml of Milli-Q water. Dilution factors were determined in accordance with the results of preliminary tests. The Ecoplates were inoculated with 130 μl suspension and incubated at 28 °C in the dark. The optical density (OD) was measured (at 590 nm) every 24 h for 7 days in an automated plate reader, and plate readings were recorded after 72 h of incubation. The normalized OD data [corrected with the average well color development (AWCD) value (Garland and Mills, 1991)] were used. The microbial richness (MR) was expressed as the number of oxidized C substrates in the microplates. The Shannon–Weaver diversity index (H′) was calculated as: $H' = -\sum p_i (\ln p_i)$, where p_i is the ratio of

Table 2
Physicochemical properties in the treated soils (mean value ± SD of four field replicates) at different sampling times (1, 90, 180 and 365 days) after the experimental fire. Treatments: U, unburned soil; B, burned soil; B + S, burned soil plus seeding; B + M, burned soil plus application of straw. For the same sampling time different letters indicate significant differences among treatments ($p<0.05$).

	Time (days)	U	B	B + S	B + M
Moisture (%)	1	37.8 ± 2.1a	36.2 ± 5.5a	34.2 ± 6.5a	36.0 ± 2.7a
	90	42.8 ± 2.5a	41.4 ± 1.7a	44.3 ± 2.8a	42.4 ± 2.6a
	180	38.5 ± 1.5a	35.0 ± 2.2a	34.7 ± 4.2a	37.8 ± 1.2a
	365	34.5 ± 3.1a	35.9 ± 1.0b	37.6 ± 2.2a	38.2 ± 1.8a
pH water	1	3.85 ± 0.08a	4.17 ± 0.04b	4.18 ± 0.06b	4.10 ± 0.15b
	90	3.74 ± 0.09a	4.21 ± 0.09b	4.37 ± 0.16b	4.34 ± 0.12b
	180	3.79 ± 0.05a	3.99 ± 0.10b	3.98 ± 0.06b	4.01 ± 0.06b
	365	3.67 ± 0.06a	3.94 ± 0.13b	3.86 ± 0.10b	3.92 ± 0.07b
Electric conductivity (μS cm^{-1})	1	117 ± 30a	149 ± 10a	164 ± 32a	193 ± 45a
	90	52 ± 5a	49 ± 4a	41 ± 11a	42 ± 8a
	180	44 ± 8a	44 ± 5a	40 ± 3a	51 ± 8a
	365	45 ± 10a	43 ± 5a	40 ± 5a	50 ± 9a
Water retention at field capacity (g water kg^{-1})	1	853 ± 96a	780 ± 56a	836 ± 81a	800 ± 40a
	90	868 ± 57a	866 ± 59a	893 ± 100a	826 ± 65a
	180	820 ± 23a	806 ± 45a	856 ± 101a	844 ± 46a
	365	758 ± 53a	701 ± 53a	745 ± 70a	742 ± 56a
Total C (g kg^{-1})	1	180 ± 12a	165 ± 13a	168 ± 9a	175 ± 13a
	90	177 ± 11a	186 ± 19a	185 ± 15a	179 ± 16a
	180	182 ± 5a	166 ± 14a	187 ± 14a	182 ± 10a
	365	182 ± 16a	171 ± 4a	177 ± 15a	176 ± 10a

the activity on each substrate (OD_i) to the sum of the activities on all substrates ($\sum OD_i$).

2.6. Statistical analysis

In order to evaluate the effect of the different treatments on the biochemical and microbiological properties, two way analysis of variance (ANOVA2) was applied to determine the percentage of variation attributable to the treatment (F) and time (T) factors. For the same sampling time, the data were analyzed by standard analysis of variance (ANOVA1) and, in the cases of significant F statistics, the Tukey's minimum significant difference test was used to compare the mean values. The homogeneity of variances and normality of the data were previously checked by the Levene and Shapiro–Wilk tests, respectively.

The simple linear regression technique was used to examine the possible influence of a set of variables related to soil physicochemical properties on microbial parameters. In addition, a principal component analysis (PCA) was also carried out on physicochemical and microbial data. In these cases, the mean values for treatments at each sampling time were used (16 variables, 16 soil samples). The SPSS (2004) statistical package was used to carry out the respective analyses.

3. Results

3.1. Soil physicochemical properties

The main soil characteristics after the different treatments and at different sampling times are summarized in Table 2. All the physicochemical characteristics of the study site, such as low pH and relatively high content of organic matter, are representative of soils developed over igneous rocks and under shrubland vegetation in NW Spain (Martín et al., 2010). Briefly, the experimental fire led to a slight increase in the soil pH and did not modify the other physicochemical properties analyzed. In comparison with burning, the seeding and mulching treatments did not induce changes in the soil physicochemical properties analyzed. Soil cover was slightly increased by the seeding treatment, whereas this effect was not apparent in the mulched plots.

3.2. Microbial biomass

The values for the total microbial C determined by both fumigation-extraction (Cmic-FE) and substrate induced respiration (Cmic-SIR) methods are shown in Fig. 1. In the unburned soil, the Cmic-FE values varied from 1577 to 2251 mg kg^{-1} (mean ± SE, 1883 ± 140 mg C kg^{-1}), and represented between 0.9 and 1.2% of total soil C (1.04 ± 0.06%), whereas in the burned soils the values ranged from 1076 to 1906 mg kg^{-1} (1524 ± 85 mg kg^{-1}), representing between 0.6 and 1.1% of total soil C (0.87 ± 0.05%). The microbial C values estimated by the SIR method were lower than those obtained by FE, and varied from 963 to 1454 mg kg^{-1} (average 1153 ± 106 mg C kg^{-1}), representing between 0.5 and 0.8% of total organic C (mean 0.64 ± 0.06%) in the unburned soil, and from 909 to 1545 mg C kg^{-1} (average 1212 ± 57 mg C kg^{-1}), representing 0.5–0.9% of total soil C (average 0.69 ± 0.04%), in the burned soils. The results of the ANOVA2 indicated that Cmic-FE values were significantly affected by the experimental fire and the sampling time; the latter explained most of the variance (45%), and the fire treatment accounted for a further 16% of the variance. The experimental fire caused an initial significant reduction in Cmic-FE values; this effect persisted over time, but differences between the unburned and burned soil samples were not significant after 365 days. For Cmic-SIR values, the effect of the experimental fire was not significant, whereas the sampling time accounted for a similar percentage of variability (46%) as that observed for Cmic-FE. No differences between soil stabilization treatments were detected.

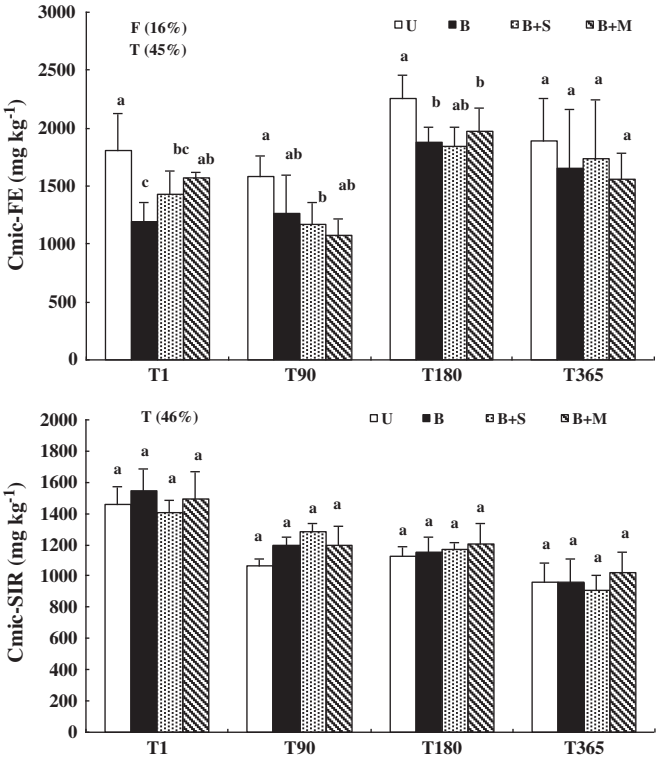

Fig. 1. Microbial C biomass determined by fumigation-extraction (Cmic-FE) and substrate induced respiration (Cmic-SIR) methods in different soil treatments (mean value ± SE of four field replicates) at different sampling times (1, 90, 180 and 365 days) after the experimental fire. For each analyzed parameter ANOVA 2 (F, experimental fire; T, sampling time; F×T, experimental fire × sampling time interaction) were performed, but only proportion of variance explained by significant factors ($P<0.05$ level) are indicated. Treatments: U, unburned soil; B, burned soil; B + S, burned soil plus seeding; B + M, burned soil plus application of straw. For same sampling time different letters indicate significant differences among treatments ($p<0.05$).

3.3. Microbial activity

The CO_2 evolved after 2 days of incubation of the unburned and burned soil samples varied from 12.8 to 14.0 μg CO_2 g^{-1} h^{-1} (mean 13.3 ± 0.3 μg CO_2 g^{-1} h^{-1}) and from 11.4 to 13.1 μg CO_2 g^{-1} h^{-1} (average 12.4 ± 0.2 μg CO_2 g^{-1} h^{-1}), respectively (Fig. 2). The analysis of variance revealed that soil respiration was significantly affected by the experimental fire, which explained 17% of the variance. Burning caused a slight decrease in soil respiration immediately after the fire and in the short-term, although the reduction was only significant for up to 180 days. The specific respiration rate or metabolic quotient (qCO_2) calculated from Cmic-FE data ranged from 6.0 to 8.2 μg CO_2 mg $Cmic^{-1}$ h^{-1} (mean 7.2 ± 0.5 μg CO_2 mg $Cmic^{-1}$ h^{-1}) for the unburned soil samples, and from 6.2 to 11.5 μg CO_2 mg $Cmic^{-1}$ h^{-1} (8.5 ± 0.5 μg CO_2 mg $Cmic^{-1}$ h^{-1}) for the burned soil samples, whereas that obtained from the Cmic-SIR data ranged from 9.6 to 13.3 μg CO_2 mg $Cmic^{-1}$ h^{-1} (mean 11.7 ± 0.8 μg CO_2 mg $Cmic^{-1}$ h^{-1}) and from 8.0 to 13.2 μg CO_2 mg $Cmic^{-1}$ h^{-1} (10.4 ± 0.4 μg CO_2 mg $Cmic^{-1}$ h^{-1}) for the unburned and burned soil samples, respectively. No significant effect of the fire was detected.

The glucosidase activity ranged from 67 to 150 μg *p*-nitrophenol g^{-1} h^{-1} (average 106 ± 17 μg *p*-nitrophenol g^{-1} h^{-1}) for the unburned soil samples and from 30 to 110 μg *p*-nitrophenol g^{-1} h^{-1} (79 ± 8 μg *p*-nitrophenol g^{-1} h^{-1}) for the burned soil samples (Fig. 2). The two-way analysis of variance revealed a significant effect of the two factors considered, experimental fire and sampling

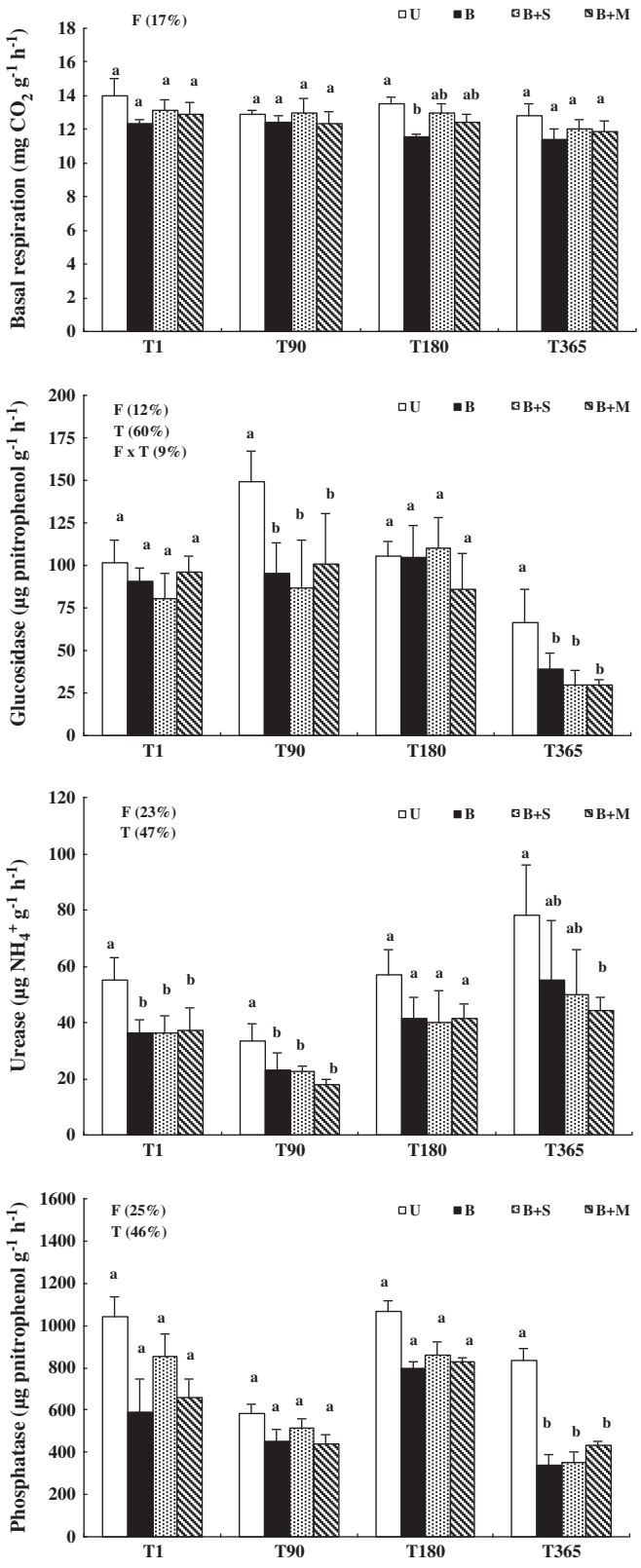

Fig. 2. Microbial activity (basal respiration and enzyme activities) in different soil treatments (mean value ± SE of four field replicates) at different sampling times (1, 90, 180 and 365 days) after the experimental fire. For each analyzed parameter ANOVA 2 (F, experimental fire; T, sampling time; F×T, experimental fire × sampling time interaction) were performed, but only proportion of variance explained by significant factors ($P<0.05$ level) are indicated. Treatments: U, unburned soil; B, burned soil; B + S, burned soil plus seeding; B + M, burned soil plus application of straw. For same sampling time different letters indicate significant differences among treatments ($p<0.05$).

time. These factors were not independent, as indicated by the significant effect of the interaction between them. The sampling time explained most of the variance (60%), the experimental fire accounted for only 12% of the variation, and the interaction between both factors explained a further 9%. The experimental fire caused a slight initial decrease in the glucosidase activity, and the effect became more marked over time, particularly after 90 and 365 days.

The urease activity varied from 34 to 78 µg $NH_4^+ g^{-1} h^{-1}$ (mean 56 ± 9 µg $NH_4^+ g^{-1} h^{-1}$) for the unburned soil samples and from 18 to 55 µg $NH_4^+ g^{-1} h^{-1}$ (37 ± 3 µg $NH_4^+ g^{-1} h^{-1}$) for the burned soil samples. These values were significantly affected by the experimental burning and sampling time, and the interaction was not significant. The sampling time explained 47% of the variation in urease activity, and the experimental burning explained 23% of the variance. Immediately after fire, the urease activity was significantly lower in the burned soil samples than in the unburned soil; this effect persisted for more than 365 days.

The phosphatase activity varied from 583 to 1068 µg *p*-nitrophenol $g^{-1} h^{-1}$ (mean 894 ± 111 µg *p*-nitrophenol $g^{-1} h^{-1}$) for the unburned soil samples and from 338 to 857 µg *p*-nitrophenol $g^{-1} h^{-1}$ (592 ± 58 µg *p*-nitrophenol $g^{-1} h^{-1}$) for the burned soil samples (Fig. 2). The two-way analysis of variance revealed that both the sampling time, which accounted for most of the variance (46%), and experimental fire (25% variation) had a significant effect on this enzyme activity. The phosphatase activity decreased after the experimental burning, although the effect was not significant because of the high variation among field replicates. However, after 365 days the phosphatase activity was lower in the burned soil than in the unburned soil.

There were no significant differences in microbial activities between any of the treated soils and the burned soil.

3.4. Microbial diversity

The mean well-color development (AWCD) values varied from 0.70 to 0.83 (mean 0.77 ± 0.04) for the unburned soil samples and from 0.90 to 1.23 (0.99 ± 0.03) for the burned soil samples (Fig. 3). The results of the analysis of variance revealed that these values were significantly affected by both factors considered (experimental burning and sampling time), and the interaction between them was not significant. The experimental burning had a more pronounced effect (30% of variance) than the sampling time (12%). Initially the fire caused a significant increase in the AWCD values; this effect tended to persist over time although after 180 days the values in the burned soil were not significantly different from those in the unburned soil.

Microbial richness (MR) ranged from 21.8 to 23.3 (mean 22.6 ± 0.3) for the unburned soil samples and from 24.3 to 27.8 (25.7 ± 0.3) for the burned soil samples. In this case, experimental burning explained 31% of the variance and sampling time a further 9%. The MR values tended to increase as a consequence of burning throughout the entire study period, although the effect was only significant after 90 days.

The values of the Shannon–Weaver diversity index (H′) varied from 2.91 to 2.99 (mean 2.96 ± 0.02) for the unburned soil samples and from 3.02 to 3.15 (3.08 ± 0.01) for the burned soil samples. The variation in these values can be explained by experimental burning and sampling time, which explained 31% and 11% of the variance respectively. An increase in the H′ values in the burned soil with respect to the corresponding unburned control soil was observed throughout the study period (1, 90, 180 and 365 days), although the differences were only significant after 90 days.

3.5. Combined interpretation of all physicochemical and microbial parameters

Overall, the results showed that microbial biomass and activity were generally reduced by the fire, and microbial diversity was

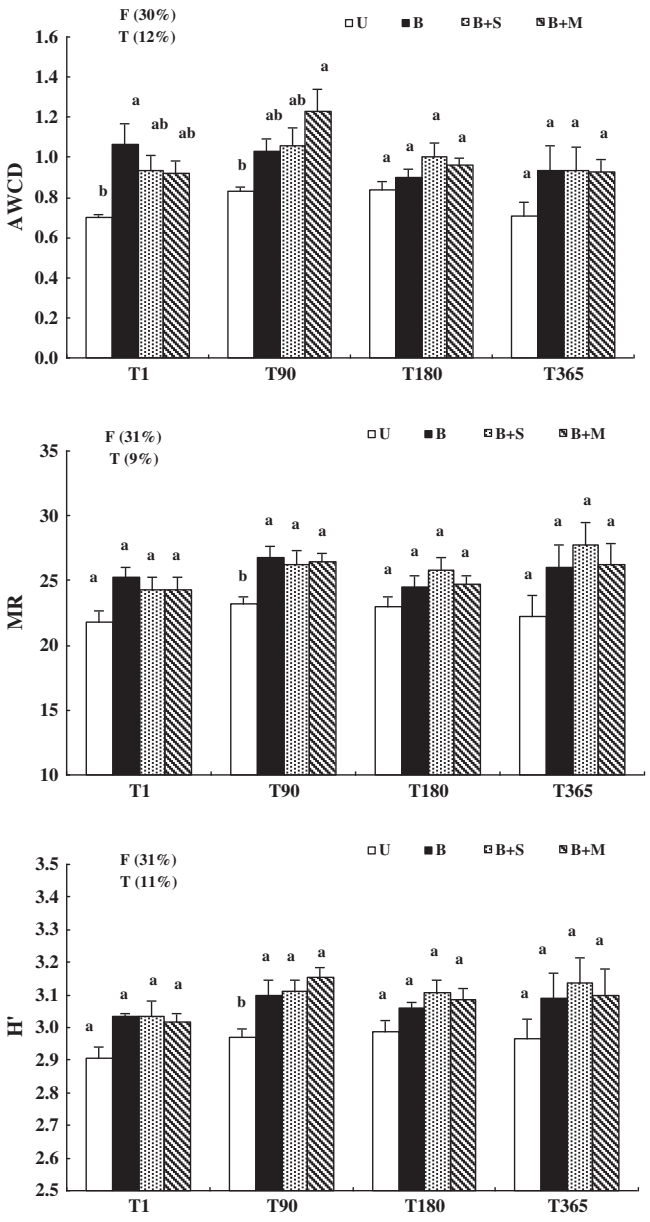

Fig. 3. Microbial diversity mean values. AWCD (average well color development), MR (microbial richness, expressed as the number of oxidized C substrates in the microplates) and H′ (the Shannon–Weaver diversity index) in different soil treatments (mean value ± SE of four field replicates) at different sampling times (1, 90, 180 and 365 days) after the experimental fire. For each analyzed parameter ANOVA 2 (F, experimental fire; T, sampling time; F×T, experimental fire × sampling time interaction) were performed, but only proportion of variance explained by significant factors ($P<0.05$ level) are indicated. Treatments: U, unburned soil; B, burned soil; B + S, burned soil plus seeding; B + M, burned soil plus application of straw. For same sampling time different letters indicate significant differences among treatments ($p<0.05$).

increased by the fire. In general, the measured parameters changed with sampling time, and the variations in biomass and activity values were of higher magnitude than fire-induced changes. Furthermore, the implementation of mulching and seeding after the experimental fire did not modify the effects of the fire during the study period.

The correlation coefficients for the variables analyzed are shown in Table 3. Cmic-FE was positively related to the enzyme activity of urease and phosphatase and negatively related to soil pH. Basal respiration was significantly and positively correlated with water retention at field capacity and with the enzymatic activities of phosphatase and glucosidase. The response of the microbial community to different substrates, measured by the mean values of the community level physiological profiles (AWCD, MR and H′), was positively and significantly correlated with pH and negatively correlated with Cmic-FE, basal respiration and phosphatase and urease activities.

Principal component analysis (PCA) of the physicochemical and microbial data, revealed that PC1 accounted for 41% of the total variation, and PC2 for 29% of the total variation. Therefore, 70% of total variance was explained by these two components (Fig. 4). The PC1 tended to separate the burned treatments from the unburned treatments. The burned soils (having positive values along PC1) were associated with high levels of pH and microbial diversity (AWCD, MR and H′) and the unburned soils (with negative values in PC1) with high levels of Cmic-FE and urease and phosphatase activities. The PC2 tended to separate samples of same treatment collected at different sampling times. The samples collected 365 days after the fire (having negative values along PC2) were associated with low values of electric conductivity, water retention, Cmic-SIR, respiration and glucosidase and phosphatase activities. The distribution of samples in PCA clearly indicated that the main differences in the soil quality were due to experimental burning and to a lesser extent to sampling time.

4. Discussion

4.1. Microbial biomass estimates

Immediately after burning, the microbial biomass C, estimated by FE and SIR methods, was scarcely affected (21% of reduction with respect to the unburned soil) or not affected, respectively, by the experimental fire. Significant decreases in soil or humus Cmic have been reported after experimental or prescribed fire (Andersson et al., 2004; Fritze et al., 1993; Palese et al., 2004; Pietikäinen and Fritze, 1993, 1995) and attributed to high fire severity. On the contrary, no effects or only slight increases in Cmic following experimental fires of short duration or low severity have been found (Andersson et al., 2004; Basanta et al., 2004; D'Ascoli et al., 2005; De Marco et al., 2005). In the present study, the low response can be explained by the low values and short duration of the maximum temperatures reached during the fire at the soil surface.

For most soil samples, Cmic-FE estimates were 1.1–2.0 times higher than those obtained by the SIR method. Furthermore, differences were more marked for the unburned soil samples (1.67 ± 0.18 times) than for the burned samples (1.30 ± 0.11 times). The results are in accordance with findings reported by Andersson et al. (2004), who observed a different microbial response on the basis of Cmic determined by FE and SIR methods. In contrast, a strong correlation between Cmic-FE and Cmic-SIR estimates was observed by Dumontet et al. (1996), in a study of a chronosequence of forest fires in Mediterranean pine ecosystems, and by Pietikäinen and Fritze (1995), in a study of the effect of prescribed burning in coniferous forests. The discrepancies between SIR and FE estimates are probably related to the fact that the methods measure different components of the total soil microbial biomass. The FE technique provides a relative measure of the (chloroform sensitive) total microbial biomass, and the SIR may be more sensitive to the active (glucose-responsive) microbial biomass (Wardle and Parkinson, 1990). In fact, the increased availability of C after soil heating (Díaz-Raviña et al., 1992) may modify the response of this fraction of glucose-responsive biomass, which may be a limitation of the SIR method in burned soils. Moreover, Cmic-FE estimates showed a higher influence of treatment (Fig. 1) and stronger correlations with other physico-chemical and microbial variables than Cmic-SIR estimates (Table 3). Therefore, the Cmic-FE method appeared to be more useful than Cmic-SIR for detecting the impact of fire on the soils analyzed in the present study.

The negative impact of the experimental fire persisted for at least six months, which is consistent with the results of a previous study

Table 3
Pearson's correlation coefficients (r) for some physicochemical and microbiological variables (n = 16).

	pH	WR	Cmic-FE	Cmic-SIR	BR	Glu	Ure	Pho	AWCD	MR
WR	ns									
Cmic-FE	−0.77**	ns								
Cmic-SIR	ns	ns	ns							
BR	ns	0.61**	ns	0.50*						
Glu	ns	0.77**	ns	ns	0.52**					
Ure	−0.83**	−0.57*	0.74**	ns	ns	ns				
Pho	ns	ns	0.63**	ns	0.69**	ns	ns			
AWCD	0.87**	ns	−0.69**	ns	−0.50*	ns	−0.80**	−0.55*		
MR	0.59*	ns	−0.50**	ns	−0.63**	ns	−0.53*	−0.80**	0.77**	
H′	0.62**	ns	ns	ns	−0.62**	ns	−0.53*	−0.67**	0.82**	0.94**

ns, not significant; WR, Water retention at field capacity; Cmic-FE, microbial C biomass determined by fumigation-extraction; Cmic-SIR, microbial C biomass determined by substrate induced respiration; BR, basal respiration; Glu, glucosidase; Ure, urease; Pho, phosphatase; AWCD, average color development in Biolog microplates; MR, number of C substrates oxidized in the microplates; H′, the Shannon–Weaver diversity index of the microbial communities growing in the microplates.
** Significant at p = 0.01.
* Significant at p = 0.05.

performed in the same area (Barreiro et al., 2010). The reductions in Cmic-FE may be attributed to indirect fire effects such as changes in nutrient supply due to destruction of the plant cover (Smith et al., 2008). Marked variations in Cmic-FE were found over time, which is consistent with the findings of previous studies on soils in the same area, in which seasonal changes were observed (Díaz-Raviña et al., 1995; 2005), and can be explained by fluctuations in different factors such as climatic conditions (temperature, moisture) and the availability of substrate derived from roots or from material incorporated into the soil.

4.2. Microbial activity

Immediately after the experimental fire there was no change in soil respiration which remained over the first study period. Six months after the fire, there was a significant decrease in soil respiration in the burned soil samples. Consequently, the specific respiration rate or metabolic quotient values, calculated from the soil respiration and Cmic-FE (μg CO_2–C mg $Cmic^{-1}$ day^{-1}), were slightly higher in the burned than in the unburned soil samples. The latter result is consistent with the reduced basal respiration and increased specific metabolic rate observed after prescribed fires in different studies, in pine forest (Fritze et al., 1993; Pietikäinen and Fritze, 1993) and in Mediterranean maquis (D'Ascoli et al., 2005). In contrast, Andersson et al. (2004) and De Marco et al. (2005) observed a short-term increase in both basal soil respiration and metabolic quotient after experimental fires, attributed to changes in soil water content, soluble C and nutrient availability. In the present study, no significant differences were found in soil moisture content between treatments for the sampling dates considered.

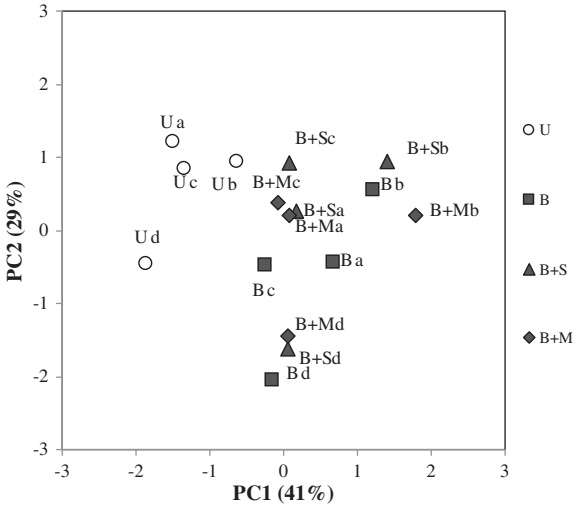

Fig. 4. PCA results of the physicochemical and microbiological properties classified by treatments and sampling time. Treatments: U, unburned soil; B, burned soil; B + S, burned soil plus seeding; B + M, burned soil plus application of straw. Lowercase letters indicate sampling time: a, 1 day; b, 90 days; c, 180 days; d, 365 days after the experimental fire.

The observed decreases in β-glucosidase and urease in the burned soil samples confirmed those observed in previous field studies performed in shrubland areas located in the same temperate humid zone (Galicia, NW Spain), indicating the sensitivity of both of these enzymes for detecting the immediate and medium-term impact of fire (Barreiro et al., 2010; Basanta et al., 2004). The reduction in β-glucosidase is also consistent with other results observed in different ecosystems (Ajwa et al., 1999; Boerner and Brinkman, 2003; Boerner et al., 2000; Eivazi and Bayan, 1996), although the contrary has been also reported (Guénon et al., in press). Fire severity, nutrient depletion, changes in the quality of soil organic matter (SOM) and reduction in microbial biomass are often suggested as factors explaining such results. In relation to β-glucosidase, a drastic change in both the quantity (C content did not change) and quality of SOM (low heating temperatures in mineral soil surface) does not seem likely. Another possible explanation for the decrease in β-glucosidase activity may be a correlative reduction in plant density and cover in the burned plots and a corresponding decrease in the source of cellulose. However, this is not consistent with the greater decrease observed 365 days after the fire, when vegetation regrowth was high. Thus, the acute drought in the previous summer (precipitation less than half of the mean values for the last thirty years) may explain the pronounced decrease in this enzyme activity, which was particularly apparent in the burned plots. The lack of differences between the mulched and seeded plots and the untreated burned plots may be due to the small amount of mulch used and the similar plant cover in all plots.

The phosphatase activity also tended to decrease after the experimental fire, although significant differences were only observed 12 months after the fire. However, Saá et al. (1993) reported large reductions of 80–90% in acid phosphatase activity only after a wildfire of high intensity in pine stands in NW Spain, whereas no change or only a slight increase was observed following a low intensity controlled fire in gorse shrubland. The differences between both soils were attributed to the large differences in the soil heating level. However, in the present study, the relatively low temperature of the fire would not explain the slight decrease. The greater reduction in the activities in the burned soils than in the unburned ones was detected 365 days after the fire (following a severe summer drought), suggesting that the seasonal effect may determine the response. Fire-related decreases in phosphatase activity have also been observed in forests soils (Boerner and Brinkman, 2003; Boerner et al., 2000; Eivazi and

Bayan, 1996) and attributed to changes in organic C and decreased SOM quality, factors that do not appear likely in our case. DeBano and Klopatek (1988) found no changes in phosphatase activity between one and 90 days after prescribed burning in pinyon pine and juniper soils, in wet soil subjected to a low rate of heating. Likewise, Boerner et al. (2006) and Giai and Boerner (2007) did not detect any changes in phosphatase activity after fire.

There was considerable temporal variation in soil enzyme activities in both the unburned and burned soil (CV = 25–32% and 32–58% for unburned and burned soil samples, respectively). This was confirmed by the ANOVA 2 analysis, which showed that for the whole set of specific enzyme activities, the variation attributed to sampling time was greater than that attributed to the experimental fire. The marked variations in microbial activity over time are consistent with previous findings for soils located in the same area (Barreiro et al., 2010; Díaz-Raviña et al., 1993, 2005). In a study in a tropical savannah woodland, Andersson et al. (2004) also found a more marked influence of seasonal variation on microbial activity (determined on the basis of specific soil enzymes) than that induced by a low severity experimental fire. Likewise, similar behavior was observed by Gutknecht et al. (2010) in a recent study on the effect of a wildfire on extracellular enzyme activity in a grassland soil.

4.3. Microbial diversity

The values of the three diversity indices (AWCD, MR and H′) varied depending on the experimental fire and to a lesser degree on sampling time. This contrasted with what was observed for microbial activity and biomass, for which sampling time explained a larger part of the variation than fire. The three diversity indices tended to be higher in the burned soil samples than in the respective unburned soil samples, although the differences were only significant in 25% of cases. The lack of response is consistent with previous findings indicating scarce and temporary changes in functional diversity after fires (Staddon et al., 1997). D'Ascoli et al. (2005) observed a slight and transient change in C-substrate utilization profiles after fire in the Mediterranean region, only in the first week after burning, and attributed this to changes in SOM quality, indicating that the microbial community quickly recovered its functional diversity. In the present study, the higher values of the diversity indices and pH detected immediately after the fire suggested that more favorable soil conditions for bacterial populations may stimulate the bacteria to use C-substrates. The positive and significant correlations between soil pH and three microbial diversity indices appear to confirm this hypothesis. This finding is also supported by recent studies showing that soil pH is the most important factor determining microbial composition (community structure assessed by PLFA pattern), either directly affecting the activity of main functional microbial groups such as fungi and bacteria or indirectly by altering abiotic factors such as carbon and nutrient availability, and metal solubility (Bååth, 1998; Bååth et al., 1995; Fernández-Calviño et al., 2010; Kermit et al., 2006; Rousk et al., 2010). Some authors have reported a discrepancy between the changes in microbial diversity and in microbial activity (Giai and Boerner, 2007; Guénon et al., in press), suggesting that each may reflect different soil processes. Whereas BIOLOG is assumed to mirror only the potential of microbial community to respond to various C-substrates, enzyme activities reflect the potential microbial metabolism in situ (Guénon et al., in press). Again, there was no evidence of any mulching effect on microbial diversity. This result is in contrast with the increased soil microbial diversity reported by Huang et al. (2008) in response to application of a mulch consisting of chipped vegetation, which may be explained by differences in type and doses of mulch applied (lower rate of mulch with a higher C/N ratio in the present study).

4.4. Combined interpretation of all physicochemical and microbial parameters analyzed

To compare the soil quality in the unburned and burned treated soil samples, all the physicochemical and microbiological parameters should be considered simultaneously. The PCA is an adequate statistic technique for examining combinations of variables or parameters as well as to determine if there is differentiation between treatments based on these variables. Thus, the PCA was used to analyze 16 variables monitored for 1 year from the four soil treatments studied. The distribution of soil samples in plane defined by PC1 and PC2, which explained 70% of the total variance, somehow makes it possible to differentiate the unburned samples from the burned ones and the soil samples collected 1 year after fire from the resting samples. The data clearly showed the usefulness of PCA analysis in order to quantify the importance of both experimental burning and sampling time as sources of variation in soil environment.

To analyze the effect of the treatments on soil microbial population (mass, activity and diversity), separate analysis of variance (ANOVA 2, ANOVA1) were made for each microbial parameter. The data indicated that although the experimental fire alone or in combination with some restoration technique (straw mulching and seeding) affected soil microorganisms, the effect on biomass and activity values was lower than that exerted by the sampling time, which appears to indicate that the fire does not greatly disturb microorganisms in these temperate humid ecosystems. This appears to be consistent with the relatively low degree of soil heating reached during the fire, the fast regrowth of vegetation and the mild climate conditions. In fact, the low temperature reached in the soil during the fire and the reduced effects on most of the physicochemical soil properties analyzed indicated that the fire can be considered as a low severity fire. Moreover, only a small amount of mulch was applied.

The data confirmed different patterns for microbial biomass and activity (negative fire effect) than observed for microbial diversity indices (positive fire effect). The negative relationships between some microbial diversity indices and parameters based on microbial mass and activity appear to support this different response. The significant positive relationships between MR and H′ with soil heating temperature suggest that ash and residues from the combustion of vegetation may provide a source of nutrients.

At each sampling time, the response of the soil to the post-fire stabilization treatments (mulching and seeding) was similar to that of the burned soil, apparently indicating that these treatments did not modify the soil quality during the study period. There are several possible explanations for this result. In relation to mulching, the total weight of mulch used in the plots was fairly low (0.2 kg m^{-2}). The role of mulch in modifying the abiotic environment was therefore presumably limited. Moreover, the meteorological conditions during the study were predominantly temperate and moist, especially during the first months of the experiment, when the plant cover was low. Erosion was also low in the untreated burned soil (Vega et al., 2010), indicating that the capacity for soil surface degradation was scarce. Finally, the C/N ratio in the straw mulch was presumably high, so that its capacity to supply available sources of carbon was low. The scarce response to seeding appears to be a consequence of the low rate of germination and its scarce contribution to the soil cover by plants. In fact, most of the cover was obtained by regrowth of native shrub. Information concerning the effect of mulching and seeding treatments on microbial communities of burned soils is very scarce. The absence of any response of the microbial community to the mulching and seeding treatments is consistent with recent findings that the application of these soil stabilization treatments in a forest area affected by a high severity wildfire had no effects on microbial biomass and activity estimates (Díaz-Raviña and Martín, 2012).

5. Conclusions and management implications

The results suggest that the application of low severity experimental fire under the specific conditions of these shrublands of NW Spain (fuel load, moisture in vegetation, soil properties, and climatic conditions) did not have any major effects on soil microbial characteristics, irrespective of whether experimental fire was combined with seeding and mulching treatments or not. The results also showed that fire-induced changes in soil microorganisms (mass and activity) appear to be of minor importance in comparison with microbial seasonal changes. The experimental fire failed to reproduce the conditions of a very severe summer fire and the question arises as to whether the soil stabilization treatments used would produce a marked effect on soil microbial parameters when applied under other conditions (dose rates, mixture of seeds) following severe fire. In fact, the characteristics of the experimental fire were similar to those of a prescribed fire. From this point of view, and taking into account the long history of frequent fires in the region and the pronounced fire-adaptive features exhibited by these shrubland systems, the weak impact of the fire is perhaps not surprising. The rapid native plant regrowth and cover in the area are further examples of this response.

Depending on the management objectives and the time span considered, the perspective of fire, its use and effects on this type of ecosystem, may differ. Whether the goal is basically to ensure the sustainability and biodiversity of ecosystems, the priority should be to avoid severe fires, which trigger intense post-fire erosive events and contribute to generation of CO_2. Under this scenario, periodic low intensity prescribed burning may be a suitable method of maintaining fuel accumulation in a lower level, without any loss of microbial diversity. A slight reduction in overall microbial activity may even have positive effects, in terms of delaying the release of carbon. An additional positive effect would be to reduce the recalcitrant SOM produced in a severe wildfire, although this SOM fraction may also contribute to C sequestration.

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