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ORIGINAL PAPER

Long-term response of soil microbial communities to fire and fire-fighting chemicals

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Abstract Wildfires are a global problem that can require the application of fire-fighting chemicals for combating them. These compounds can have negative effects on forest ecosystems; however, there are few studies about their influence on the soil microbial community. The aim of this work is to analyse under field conditions the impact of a prescribed fire and the addition at normal field doses of three different fire-fighting chemicals (foaming agent, ammonium polyphosphate, Firesorb) on different soil properties, 10 years after the fire and retardant addition. The study was performed in a Humic Cambisol under scrubland located in Galicia (NW Spain). Several physical, physicochemical, chemical (water holding capacity, pH, electric conductivity, total C and N, hydrosoluble C and carbohydrates), biochemical and microbiological [biomass C, enzyme activities, respiration, bacterial growth, fungal biomass and growth, biomass and community composition by the phospholipid fatty acid (PLFA) pattern] properties were analysed in the 0–2- and 2–5-cm soil layers. A marked effect of soil depth and no effects of prescribed fire on most analysed properties were observed, suggesting that soil chemical quality was recovered after 10 years, although changes in microbial community composition were still detected 10 years after the prescribed fire and the retardant addition. The PLFA pattern combined with principal component analysis allows us to differentiate the microbial communities according to both soil depth and soil treatment. The ammonium polyphosphate was the fire-fighting chemical with the strongest effects on the composition of soil microbial communities, which is in accordance with marked changes observed in vegetation.

Keywords Prescribed fires · Fire retardants · Biochemical properties · Bacterial and fungal growth · PLFA pattern

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Introduction

Fire has become one of the major disturbance agents of forest ecosystems causing the destruction of the vegetation and the degradation of the physicochemical, chemical and biological soil properties (Neary et al. 1999; Mataix-Solera et al. 2009; Certini 2005; Cerdá and Robichaud 2009; Carballas et al. 2015). Nowadays, the use of fire-fighting chemicals is a widespread practice due to the huge increase of both the number and the size of the wildfires all over the world. In some fire-prone regions, fire retardants have been used massively; for instance, in the Mediterranean area, more than 2000 t of these compounds are used each year to combat fires (Luna et al. 2007).

The retardants are basically mixtures of water and inorganic salts usually present in agricultural fertilizers (i.e. ammonium, sulphates and phosphates) with a few additives as thickening agents or a mixture of surfactants, foam stabilizers, wetting agents and solvents; the additives make the water denser and turn fuels less flammable. The retardants are normally applied at the front of an advancing fire or along its flanks to reduce fire intensity and spreading ability. The main types of fire-fighting chemicals include short-term retardants (those that do not reduce the combustion after evaporation of the water from the fuel) and long-term fire retardants (those that form a long-term barrier after evaporation of the water). Despite the common use of fire retardants, relatively little information is available in

relation with their potential environmental impacts. It is well known that these compounds can cause adverse environmental effects when the rate or intensity of use is sufficiently great, as chemicals can enter plants, soil and ground and underground water (Bradstock, Sanders, and Tegart 1987; Kalabokidis 2000; Giménez et al. 2004; Cruz et al. 2005; Pappa, Tzamtzis, and Koufopoulou 2006; Michalopoulos et al. 2016). The release of fire-fighting chemicals into the environment could have short-term toxic effects on organisms, due to the amount of available ammonia that they may be able to deliver (Giménez et al. 2004), and because some of them are recalcitrant (Sutherland, Haselbach, and Aust 1997). These chemicals are often applied in natural areas or environmentally sensitive zones, most times from aerial delivery systems (Blakely 1990), being impossible to avoid that unburnt areas receive part of these compounds; for this reason, it is necessary to determine their potential effects on the terrestrial ecosystems (Couto-Vázquez and González-Prieto 2006; García-Marco and González-Prieto 2008; Couto-Vázquez, García-Marco, and González-Prieto 2011; Fernández-Fernández, Gómez-Rey, and González-Prieto 2015; Michalopoulos et al. 2016).

Despite the widespread use of fire-fighting chemicals, there are few studies, all of them from the last decade, about short- or medium-term effects of the fire retardants on the soil microbial community. Laboratory studies (Basanta et al. 2002, 2003, Basanta, Díaz-Raviña, and Carballas 2003; Díaz-Raviña et al. 2006) showed positive effects on the enzyme activities and microbial biomass, with minor effects on the microbial community composition and negative effects on N mineralization, after the addition of Firesorb (Fi) (a short-term retardant). The few field studies that analysed the effect of the addition of different retardants showed the following results: (a) using short-term retardants, Basanta et al. (2004) did not find any effect on the enzyme activities and microbial biomass; (b) with long-term retardants, García-Villaraco Velasco et al. (2009) found that the microbial community composition changed slightly; (c) using both types of retardants plus a foam agent, Barreiro et al. (2010) found effects on the carbohydrate concentration, enzyme activities, microbial biomass and community composition immediately after the fire and the retardant addition, but 5 years later, they only found changes in the community composition of the samples treated with Fi. All these studies reported on the impact of retardant addition at short and/or medium term; however, no long-term field studies (more than 5 years) have been made. Therefore, we decided to prolong our preceding work (Barreiro et al. 2010) for 5 years more, taking advantage of the already established experimental design, although studying the impact of fire and fire-fighting chemicals not only on the topsoil (0–2 cm) but also on the soil layer 2–5-cm depth.

The objective of this work was to study, under field conditions, the long-term effects (10 years) of a prescribed fire and the addition of three commercial fire retardants, commonly used in the Mediterranean area, foaming agent (Fo), ammonium polyphosphate (Ap) and Firesorb (Fi), on the soil properties, especially on the activity, biomass and composition of the microbial community, of a Humic Cambisol located in Galicia (NW Spain). This soil type is representative of the main forest ecosystems affected by wildfires and experimental fires in the temperate humid area of the NW of the Iberian Peninsula. The work hypothesis is that prescribed fires and fire-fighting chemicals can have a significant effect on soil properties after such a long time.

Material and methods

Experimental design

This work was performed in an experimental field located in the Alto da Pedrada (Tomiño, Galicia, NW Spain) at an altitude of 455 m a.s.l. and with 29 $T^{05}182^{46}509$ UTM coordinates. The monthly mean temperature ranges from a minimum of 5 °C to a maximum of 25 °C, and the average annual air temperature and precipitation are 13 °C and 1.997 mm, respectively. The unburnt soil, a Humic Cambisol developed over paragneisses and under a heath dominated by *Ulex* sp., *Chamaespartium* and *Erica* 50–60 cm in height, had sandy texture, acidic pH, relatively high soil organic matter (SOM) content and high C/N ratio values. The prescribed fire was conducted with the backfire technique; according to the thermocouples used for monitoring the burning, the average temperatures of the 16 burnt plots measured during the fire were 428 °C in the soil surface and 55 °C at the 2-cm depth, producing charred material but also an ash layer (Fontúrbel, personal communication). As previously described (Couto-Vázquez and González-Prieto 2006; Barreiro et al. 2010), after a prescribed fire (with the fire extinguished but the soil still warm), the experimental design was established by considering the following five different treatments: (a) unburnt soil (U) as a reference; (b) burnt soil with 2 L m^{-2} of water (B); (c) burnt soil with 2 L m^{-2} of water plus foaming agent at 1 % (B + Fo); (d) burnt soil with 2 L m^{-2} of water plus ammonium polyphosphate at 20 % (B + Ap); and (e) burnt soil with 2 L m^{-2} of water plus Firesorb at 1.5 % (B + Fi). The burnt soil treatments were arranged in a fully randomized design with four replications and 1 m of separation between each plot (4 × 4 m), whereas the four unburnt soil replicates were established along the slope (18–19 %) and adjacent to the burnt ones. The physicochemical and chemical characteristics of the fire-fighting chemicals were determined by Couto-Vázquez and González-Prieto (2006). The Fo (RFC-88, a foaming agent from Auxquimia S.A) is mainly composed of surfactants and acts by increasing the water efficiency; the long-term retardant Ap is a fertilizer, composed of ammonium phosphate salts (Chemische Fabrik Budenheim KG), which

acts by forming a combustion barrier between the fire and the fuel; and the Fi is a synthetic acrylic acid-acrylamide copolymer and acts as superabsorbent (Stockhausen GmbH). In the unburnt and burnt soils, 7 months after the prescribed fire and the addition of retardants, four 1-year old pine seedlings (supplied by a forest nursery from a genetically similar stock) were planted in each plot to assess how the fire and flame retardants affect post-fire reforestation.

Ten years after the prescribed fire and the addition of the retardants, soil samples were taken, after removing the litter, from the A horizon at two different depths (0–2, 2–5 cm). Five 15 × 15-cm squares, uniformly distributed around each plot, were sampled, and the soil subsamples were mixed to form a composite sample and thoroughly homogenized after sieving at 2 mm. All samples were stored at 4 °C until analyses of the biochemical and microbiological properties. A subsample of each composite sample was frozen immediately after homogenizing and then lyophilized for the phospholipid fatty acid (PLFA) analysis. The soil-plant system at the study site was also characterized by Fernández-Fernández et al. (2015).

Soil analysis

Physical, physicochemical and chemical properties

The pH in H_2O was measured in a soil/water suspension ratio of 1:2.5; the electric conductivity (EC) was determined in a soil/water extract ratio of 1:5; and the water holding capacity (WHC) was estimated using a Richards pressure plate apparatus (pF = 2). The contents of total C and N were measured on finely ground samples (<100 μm) with an elemental analyser (EA) coupled on-line with an isotopic ratio spectrometer (Finnigan MAT Delta, Bremen, Germany). The hydro-soluble C and carbohydrates (CH) were extracted from the soil by successive extractions with deionized water at 22 °C for 2 h (soluble C, C22; soluble carbohydrates, CH22) and at 80 °C for 16 h (extractable C, C80; extractable carbohydrates, CH80), in a soil/water relation of 1:5 (*w*/*v*) for both phases; the extracted C was measured by digestion with $K_2Cr_2O_7$ and H_2SO_4 followed by colorimetric determination, and the carbohydrates were estimated in the same water-soil extracts by the anthrone method, with glucose as standard (Doutre et al. 1978).

Biochemical and microbiological properties

The microbial biomass C was determined as described by Vance et al. (1987). Briefly, after soil fumigation with $CHCl_3$ for 24 h, the organic C was extracted from the fumigated and non-fumigated samples with 0.05 M K_2SO_4 using a 1:4 soil-extract ratio and the organic C of the extracts was measured by digestion with $K_2Cr_2O_7$ and H_2SO_4 followed by colorimetric determination. The microbial biomass C values were calculated from the equation

$$\text{Biomass C} = 2.64\ E_C$$

where E_C is the extractable C flush (difference between the extractable organic C from the fumigated and non-fumigated samples).

The ß-glucosidase activity was measured following the procedure of Eivazi and Tabatabai (1988), which determines the released *p*-nitrophenol after incubation of the soil with *p*-nitrophenyl β-D-glucopiranoside for 2 h at 37 °C. The urease activity was estimated by incubating the soil samples with an aqueous urea solution, and the NH_4^+ liberated by the soil was extracted with 1 M KCl and 0.01 M HCl and measured by a modified indophenol colorimetric reaction (Kandeler and Gerber 1988).

The respiration was measured by transferring 1 g of soil to a 20-ml glass vial and purging with pressurized air. The vial was sealed and incubated for 24 h at 22 °C and without light, after which the concentration of the released CO_2 was estimated using gas chromatography.

The bacterial growth was estimated using the leucine incorporation technique (Bååth et al. 2001). Briefly, 1 g of soil was mixed with 20 ml of water, shaken in a vortex for 3 min, and centrifuged at low speed of 1000×*g* for 10 min to create a bacterial suspension in the supernatant. Aliquots of 1.5 ml of this bacterial suspension were transferred to 2-ml micro-centrifugation tubes, where 2 μl of labelled leucine (^{3}H Leu) and 2 μl of unlabelled leucine were added. After 2 h of incubation at 22 °C, the bacterial growth was stopped using trichloroacetic acid. The amount of the incorporated radioactivity was determined using a scintillator, and the amount of leucine incorporated per hour and gram of soil was used as a measure of the bacterial growth.

The fungal growth was estimated using the ^{14}C-acetate incorporation into ergosterol method (Bååth 2001). Briefly, 1 g of soil was incubated at room temperature with 20 μl of labelled ^{14}C-sodium acetate and 30 μl of no labelled sodium acetate for 4 h at 22 °C, after which 1 ml of 5 % formalin was added to stop the fungal growth. The samples were centrifuged at 1000×*g* for 5 min, and the supernatant containing non-incorporated acetate was aspirated and discarded. The ergosterol in the soil was then extracted with 5 ml of 10 % KOH in methanol, sonicated for 15 min, heated at 70 °C for 60 min, and partitioned twice with 2 ml of cyclohexane. The combined cyclohexane phases were evaporated to dryness under N_2, dissolved in 200 μl methanol, heated at 40 °C for 15 min and filtered through a 0.45-μm filter, and the total amount of ergosterol was analysed by an HPLC with a UV detector (282 nm) according to Rousk and Bååth (2007). The ergosterol peak was collected, and the amount of ^{14}C-acetate incorporated into it was determined using a scintillator and the

amount of the incorporated radioactivity was used as a measure of the fungal growth while the total amount of ergosterol was used as an indicator for fungal biomass.

The microbial community composition was determined by PLFA using the procedure and nomenclature described by Frostegård et al. (1993). The lipids were extracted from the soil with a chloroform/methanol/citrate buffer mixture (1:2:0.8 *v*/*v*/*v*) and separated into neutral lipids, glycolipids and phospholipids using a pre-packed silica column. The phospholipids were subjected to a mild alkaline methanolysis, and the fatty acid methyl esters were identified by gas chromatography (flame ionization detector, Thermo Scientific) by their relative retention times, using methyl nonadecanoate (19:0) as an internal standard. The total microbial biomass was estimated as the sum of all the extracted PLFAs (totPLFAs). The sum of the PLFAs considered to be predominantly of bacterial origin was used as an index of the bacterial biomass (bactPLFAs), and 18:2ω6 was used as an indicator of the fungal biomass (fungPLFAs) (Frostegård and Bååth 1996). The i14:0, a15:0, i16:0 and 10Me18:0 PLFAs are predominantly found in Gram-positive (G^+) bacteria, and the cy17:0, cy19:0, 16:1ω7c and 18:1ω7 PLFAs characterize Gram-negative (G^-) bacteria (Zelles 1999).

Statistical analysis

All results were expressed on the basis of oven-dry (105 °C) weight of soil. Mean values ± SE of four replicates were used to compare the different soil treatments. The data were analysed by a two-way analysis of variance (ANOVA 2) to determine the percentage of variation attributable to the factors depth and treatment. For each depth, the data were statistically analysed by a standard analysis of variance (ANOVA 1), and in the cases of significant *F* statistics, Tukey's minimum significant different test was used to separate the means. The values corresponding to the concentrations of all the individual PLFAs, expressed in mole per cent and logarithmically transformed, were subjected to a principal component analysis (PCA) to elucidate the main differences in the PLFA patterns. All the statistical analyses were done using the SPSS 23.0 statistical package.

Results

Physical, physicochemical and chemical properties

Soil pH values varied from 4.04 in the top layer of the U treatment to 4.68 in the deeper layer of the B + Fi treatment (Table 1) with a significant effect of depth and treatment (explaining 69 and 19 %, respectively, of data variation). The pH was significantly higher (8–11 %) in the deeper layer (2–5 cm) than in the top layer (0–2 cm) for all the treatments. The prescribed fire caused a significant increase (6 %) of the pH in the burnt soil compared with that of the unburnt soil, in the top layer. No significant effects of the addition of the retardants were found in both depths, except for the addition of Ap, which caused a slight but significant decrease (3 %) in the 0–2-cm layer.

The EC was similar in both layers for each treatment and ranged from 14 μg S cm^{-1} in the 2–5-cm layer of the B + Fi treatment to 30 μg S cm^{-1} in the 0–2-cm layer of the B treatment (Table 1), the treatment being responsible for 72 % of the variance while depth only explained 5 % of the variance.

The WHC varied from 513 to 661 g H_2O kg^{-1} and was significantly higher in the top than in the deeper layer (6–16 %) (Table 1); treatments did not affect WHC, and depth is the unique responsible for 33 % data variation of the samples.

The contents of total C and N ranged from 100 to 150 g kg^{-1} and from 5.8 to 8.8 g kg^{-1}, respectively, and were significantly higher in the top than in the deeper layer for all treatments (22–31 and 24–29 % for C and N, respectively) (Table 1). There were no significant differences between the unburnt and the different burnt samples. Only the depth showed a significant effect on these properties, explaining 61 % of the variance for C and 95 % for N.

The mean values of the hydro-soluble C and carbohydrates (CH) extracted with water at 22 and 80 °C for all studied soils were 378 ± 61 and 4316 ± 1289 μg C g^{-1} soil and 46 ± 3 and 2457 ± 52 μg C-Glu g^{-1} soil, respectively, in the 0–2-cm layer. Similarly, in the 2–5-cm layer, the mean values were 223 ± 31 and 2195 ± 153 μg C g^{-1} and 23 ± 2 and 796 ± 81 μg C-Glu g^{-1}, respectively (Fig. 1). Both hydro-soluble C and CH significantly decreased with depth (38–47 and 40–55 % for C22 and CH22, respectively; 42–61 and 62–71 %, for C80 and CH80, respectively). The highest amounts of hydro-soluble C and CH fractions in both layers were those extracted at 80 °C (91 and 98 % of hydro-soluble C and CH, respectively). All hydro-soluble C and CH fractions represented a low percentage of the total C, 3 and 2 %, respectively, in the top layer and 2 and 1 %, respectively, in the deeper layer. ANOVA 2 showed a significant effect of depth on all labile C fractions (49–95 % of data variance) and a smaller effect of the treatments (14–17 % of data variance) on the hydro-soluble C. Except for C80 at the 0–2-cm depth, no effect of the soil treatments was observed for all labile C fractions.

Biochemical and microbiological properties

The microbial C ranged from 1102 to 1385 μg g^{-1} and from 854 to 996 μg g^{-1} soil in the 0–2- and 2–5-cm layers, respectively, and represent around 1 % of the soil organic C (Fig. 2a). The values in the top layers were significantly higher than those in the deeper layers (21–30 %); however, for each depth no significant differences among treatments were observed.

Table 1 Main characteristics of the soil studied (mean values ± SE of four field replicates) 10 years after the prescribed fire and the addition of the retardants at two depths (0–2 cm, 2–5 cm)

	ANOVA 2	Depth	Treatments				
	(T, D)	(cm)	U	B	B + Fo	B + Ap	B + Fi
pH H_2O	D (69 %)	0–2	4.04 ± 0.03a	4.30 ± 0.04c	4.28 ± 0.03c	4.16 ± 0.01b	4.33 ± 0.03c
	T (19 %)	2–5	4.41 ± 0.03a	4.67 ± 0.05ab	4.73 ± 0.03b	4.55 ± 0.01ab	4.68 ± 0.10ab
Electric conductivity (μS cm^{-1})	D (5 %)	0–2	18 ± 2a	30 ± 2b	19 ± 1a	25 ± 1b	16 ± 1a
	T (72 %)	2–5	16 ± 2a	26 ± 2b	17 ± 2a	23 ± 1b	14 ± 1a
Water holding capacity (g H_2O kg^{-1})	D (33 %)	0–2	661 ± 24a	621 ± 36a	624 ± 35a	611 ± 23a	611 ± 36a
		2–5	555 ± 10a	567 ± 26a	563 ± 30a	513 ± 20a	572 ± 15a
Total C (g kg^{-1})	D (61 %)	0–2	150 ± 11a	130 ± 7a	144 ± 4a	144 ± 5a	132 ± 10a
		2–5	114 ± 2ab	117 ± 4b	112 ± 2ab	100 ± 2a	102 ± 3a
Total N (g kg^{-1})	D (95 %)	0–2	8.8 ± 0.6a	7.9 ± 0.5a	8.2 ± 0.3a	8.5 ± 0.3a	7.7 ± 0.5a
		2–5	6.3 ± 0.1a	6.0 ± 0.2a	6.1 ± 0.2a	6.0 ± 0.2a	5.8 ± 0.2a
C/N	D (99 %)	0–2	17.0 ± 0.4a	16.4 ± 1.0a	17.5 ± 0.3a	16.9 ± 0.4a	17.1 ± 0.3a
		2–5	18.1 ± 0.2a	19.4 ± 0.4a	18.4 ± 0.3a	16.7 ± 0.7a	17.6 ± 0.4a
TotalPLFAs (nmol g^{-1})	DxT (23 %)	0–2	265 ± 61a	263 ± 20a	214 ± 28a	232 ± 47a	200 ± 32a
		2–5	215 ± 23ab	215 ± 61ab	399 ± 82b	125 ± 28a	320 ± 92ab
Bacteria (nmol g^{-1})	DxT (24 %)	0–2	111 ± 28a	97 ± 7a	80 ± 12a	94 ± 20a	75 ± 12a
		2–5	92 ± 8ab	83 ± 23ab	154 ± 32b	52 ± 12a	122 ± 34ab
$Gram^+$ Bacteria (nmol g^{-1})	DxT (24 %)	0–2	30 ± 8a	25 ± 2a	21 ± 3a	26 ± 6a	20 ± 3a
		2–5	22 ± 2a	22 ± 6a	41 ± 10a	14 ± 3a	33 ± 9a
$Gram^-$ Bacteria (nmol g^{-1})	DxT (22 %)	0–2	62 ± 15a	56 ± 4a	46 ± 7a	50 ± 11a	42 ± 7a
		2–5	56 ± 5ab	48 ± 13ab	90 ± 18b	30 ± 7a	70 ± 19ab
Fungi (nmol g^{-1})	T (21 %)	0–2	9 ± 1a	14 ± 2a	8 ± 2a	10 ± 2a	9 ± 1a
		2–5	7 ± 2ab	7 ± 2ab	3 ± 1b	14 ± 4a	11 ± 4ab

For the same depth, different lowercase letters denote significant differences ($P < 0.05$) among treatments. ANOVA 2 analysis (D, depth; T, treatment) was performed for each parameter, but the only percentages of variance explained by the significant factors ($P < 0.05$ level) are indicated

U unburnt soil, *B* burnt soil + water, *B + Fo* burnt soil + foam agent, *B + Ap* burnt soil + ammonium polyphosphate, *B + Fi* burnt soil + Firesorb.

ANOVA 2 showed a significant effect of depth (39 % of variance) and treatments (18 % of variance) on the microbial C.

Soil respiration was significantly higher (33–46 %) in the topsoil (between 8 and 12 μg CO_2 g^{-1} h^{-1}) than in the deeper layer (between 6 and 8 μg CO_2 h^{-1} g^{-1}) for all the treatments except for B + Fi (Fig. 2b). ANOVA 2 showed a significant effect of depth and treatment on this property, explaining 47 and 15 % of the variance, respectively; nevertheless, as occurred for microbial C, no significant differences among treatments were observed for each depth.

The β-glucosidase and urease activities ranged from 69 to 117 μg p-nitrophenol g^{-1} h^{-1} and from 36 to 92 μg NH_4^+ g^{-1} h^{-1}, respectively (Fig. 2c, d). The β-glucosidase activity was significantly higher (32 %) in the topsoil than in the 2–5-cm layer only in the B soil, whereas for the urease activity the values were significantly higher in the 0–2-cm layer (41–48 %) in most cases (U, B and B + Fo). For both enzyme activities, there were no differences between the U and B soils indicating that the effect of the prescribed fire had disappeared. Nevertheless, the addition of Ap to the burnt soil (B + Ap) significantly decreased (37 %) the β-glucosidase activity in the topsoil compared with that of B, whereas the urease activity was not affected by the addition of the retardant. ANOVA 2 showed that for the β-glucosidase activity, treatment and depth were responsible for 38 and 24 %, respectively, of data variation while for urease activity the depth explained 52 % of data variation.

Bacterial growth ranged from 10 to 87 pmol leucine g^{-1} h^{-1} and was similar in both layers for all the soils, except in the B soil in which this property was significantly higher (about two times) in the 2–5-cm layer than in the top soil (Fig. 3a). Similarly, the prescribed fire did not affect the bacterial growth in the top layer whereas the 2–5-cm layer produced a significant stimulation (eight times) in the B soil compared with the U one. The addition of the retardant did not provoke a significant effect in the top-layer values whereas in the deeper layer the addition of Fo or Ap significantly decreased (55 and 70 %, respectively) the bacterial growth values in B + Fo and B + Ap soils. The data showed that 10 years after the fire, the bacterial growth was stimulated in the B soil and decreased by the

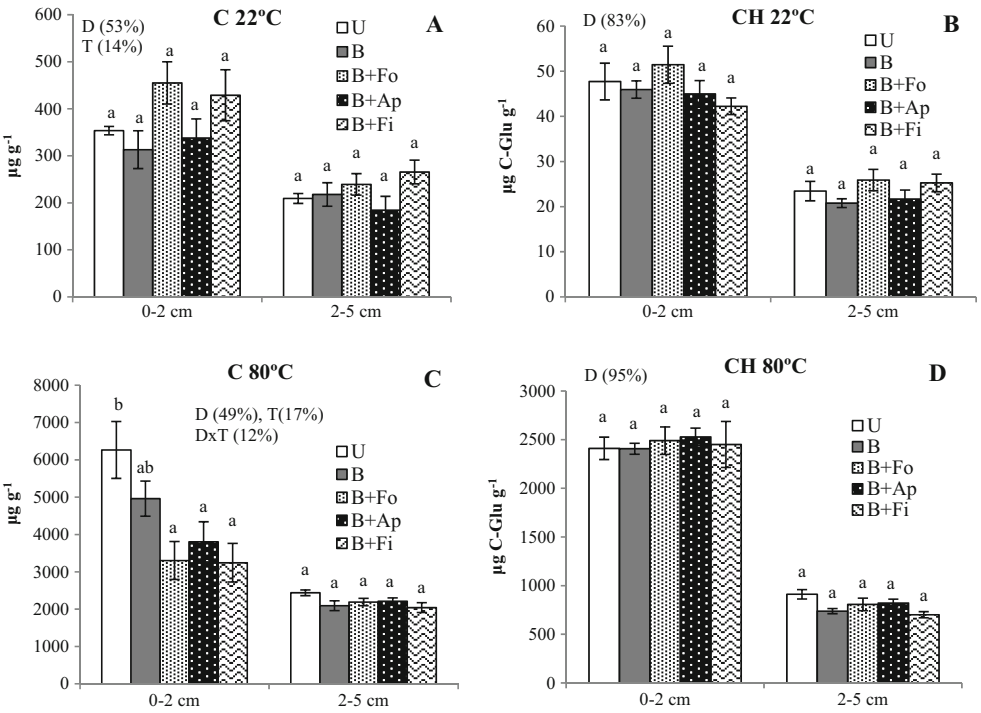

Fig. 1 Hydro-soluble C and carbohydrates (CH) (mean values ± SE of four field replicates) extracted at 22 and 80 °C, 10 years after the prescribed fire and the addition of the retardants, at two depths (0–2 cm, 2–5 cm) of the studied soils. Treatments: *U*, unburnt soil; *B*, burnt soil + water; *B* + *Fo*, burnt soil + foam agent; *B* + *Ap*, burnt soil + ammonium polyphosphate; and *B* + *Fi*, burnt soil + Firesorb. For the same depth, different letters denote significant differences ($P < 0.05$) among treatments. ANOVA 2 analysis (*D*, depth; *T*, treatment) was performed for each parameter, but the only percentages of variance explained by the significant factors ($P < 0.05$ level) are indicated

addition of some retardants. ANOVA 2 showed that the treatment was responsible for 31 % of the variance of samples.

The fungal growth ranged from 100 to 248 pmol acetate g^{-1} h^{-1} (Fig. 3b) and fungal biomass from 6 to 12 µg ergosterol g^{-1} (Fig. 3c); the fungal biomass of B, B + Fo and B + Ap treatments was significantly higher in the top layer than in the deeper one (46–48 %), and the same occurred for the fungal growth only in the B + Fo plots (36 %). Both the fungal biomass and the fungal growth were significantly affected neither by the prescribed fire nor by the addition of fire-fighting chemicals after 10 years. ANOVA 2 showed that the depth was responsible for 13 and 59 % of the variance in the

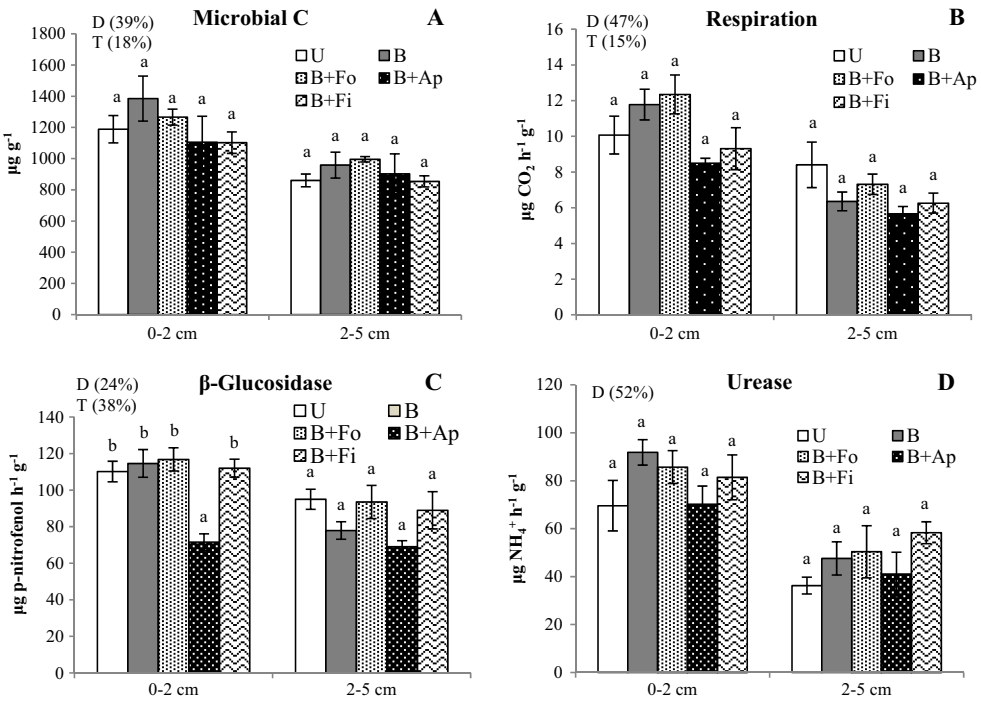

Fig. 2 Biochemical properties (mean values ± SE of four field replicates), 10 years after the prescribed fire and the addition of the retardants, at two depths (0–2 cm, 2–5 cm) of the studied soils. Treatments: *U*, unburnt soil; *B*, burnt soil + water; *B* + *Fo*, burnt soil + foam agent; *B* + *Ap*, burnt soil + ammonium polyphosphate; and *B* + *Fi*, burnt soil + Firesorb. For the same depth, different letters denote significant differences ($P < 0.05$) among treatments. ANOVA 2 analysis (*D*, depth; *T*, treatment) was performed for each parameter, but the only percentages of variance explained by the significant factors ($P < 0.05$ level) are indicated

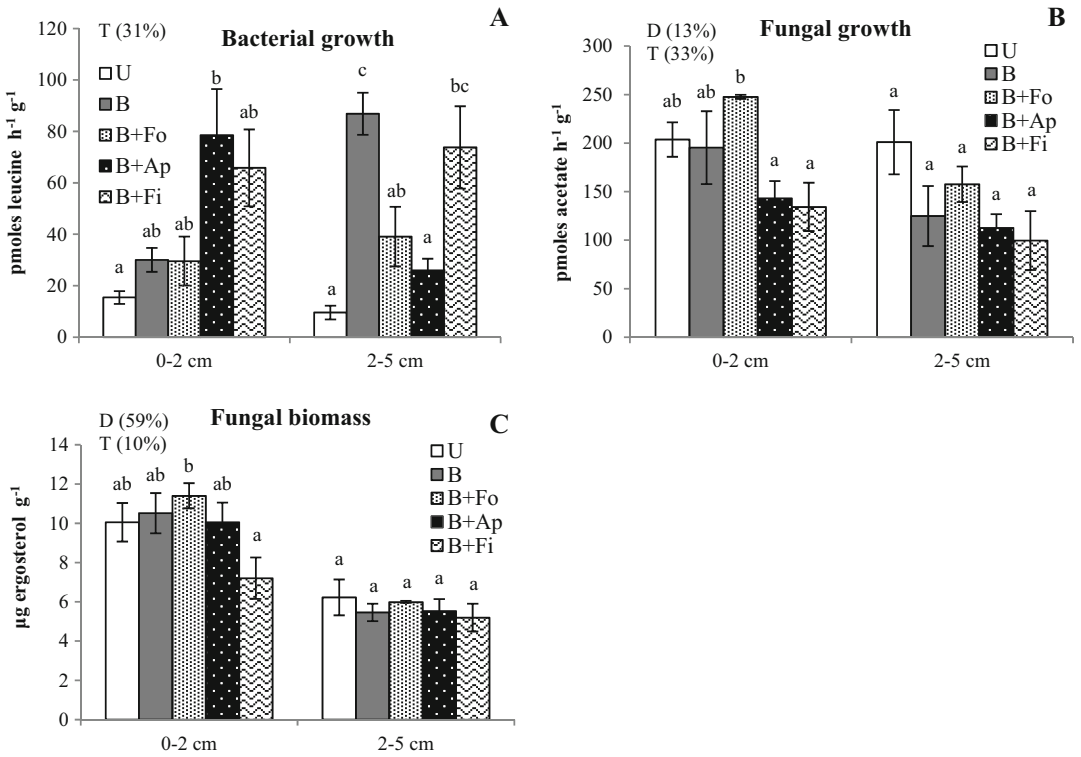

Fig. 3 Bacterial growth and fungal biomass and growth (mean values ± SE of four field replicates), 10 years after the prescribed fire and the addition of the retardants, at two depths (0–2 cm, 2–5 cm) of the studied soils. Treatments: *U*, unburnt soil; *B*, burnt soil + water; *B* + *Fo*, burnt soil + foam agent; *B* + *Ap*, burnt soil + ammonium polyphosphate; and *B* + *Fi*, burnt soil + Firesorb. For the same depth, *different letters* denote significant differences ($P < 0.05$) among treatments. ANOVA 2 analysis (*D*, depth; *T*, treatment) was performed for each parameter, but the only percentages of variance explained by the significant factors ($P < 0.05$ level) are indicated

fungal biomass and the fungal growth, respectively, and the treatment was responsible for 10 and 33 %, respectively, of the variance.

Microbial biomass, estimated as totPLFAs, ranged from 200 ± 32 to 265 ± 20 nmol g^{-1} and from 125 ± 28 to 399 ± 82 nmol g^{-1} in the 0–2- and 2–5-cm layers, respectively (Table 1); in general, there were no significant differences between the values of the totPLFAs in the top layer and those of the deeper one, although in the 2–5-cm layer the lowest value was exhibited by the B + Ap treatment and the highest by the B + Fo treatment. The quantities of PLFAs that were chosen to represent the bacterial (bacPLFAs) and fungal (funPLFAs) biomass varied between 52 and 154 and from 3 to 14 nmol g^{-1}, respectively, and comprised 37–43 and 2–5 %, respectively, of the total amount of PLFAs. The amounts of the PLFAs representative of the groups $Gram^+$ and $Gram^-$ bacteria ranged from 14 to 41 nmol g^{-1} and from 30 to 90 nmol g^{-1}, respectively, and comprised 10–11 and 21–26 %, respectively, of the total amount of PLFAs. The totPLFAs were positively and significantly correlated with the biomass of the specific microbial groups (bactPLFA, fungPLFA, $Gram^+$PLFA, $Gram^-$PLFA, $r = 0.999$, $P < 0.001$); therefore, a similar behaviour was observed for the totPLFAs and for the biomass of specific groups. ANOVA 2 showed a significant effect of the interaction of depth and treatment, explaining 22–24 % of data variation.

The PCA performed with the whole PLFA data (Fig. 4) showed that the main differences in the PLFA pattern were due to the soil depth. The first component, which explained 27 % of the variance, mainly distinguished between samples from the 0–2-cm layer and those of the 2–5-cm layer, but consistently differentiated the unburnt treatment from the burnt treatments also along this component (along PC1, $P < 0.001$ from ANOVA) (Fig. 4). The second component, which explained 25 % of the variance, separated the soil samples that were treated with Ap from the other samples (along PC2, $P < 0.001$ from ANOVA). The samples from the 0–2-cm layer (differing along PC1) were characterized by high concentrations of the PLFAs indicative of bacteria. The samples treated with Ap (having positive values in PC2) were mainly characterized by a low concentration of the 18:1ω9 and 18:2ω6 PLFAs, both indicative of fungi (Fig. 4).

Discussion

As was mentioned above, the selected unburnt soil is representative of the soils developed on acid rocks and under scrublands of the temperate humid zone of the NW of the Iberian Peninsula, which have acid pH; high buffer power that avoid great variations of pH; high content in organic matter; a

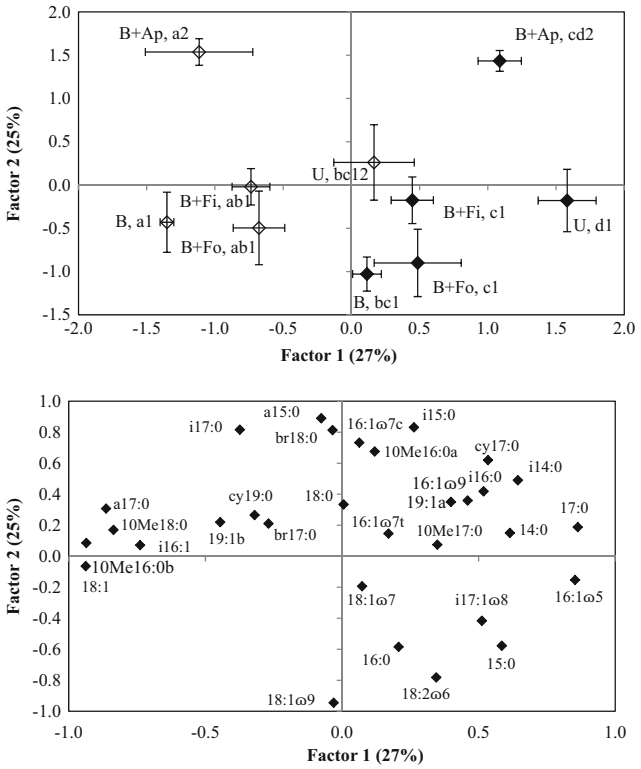

Fig. 4 Score (mean values ± SE of four field replicates) and loading plots from principal component analysis performed on the PLFAs of the soil samples collected 10 years after the prescribed fire and the addition of the retardants at two depths (0–2 cm, filled symbols; 2–5 cm, no filled symbols). Treatments: *U*, unburnt soil; *B*, burnt soil + water; *B + Fo*, burnt soil + foam agent; *B + Ap*, burnt soil + ammonium polyphosphate; and *B + Fi*, burnt soil + Firesorb. Different letters denote significant differences along PC1 from ANOVA ($P < 0.001$), and different numbers denote significant differences along PC2 from ANOVA ($P < 0.001$)

desaturated cation exchange complex with abundant H^+ ions due to the abundance of organic acids; low content of exchangeable Ca and Mg; predominated exchangeable K and Na, which can be easily lost by water infiltration due to sandy textures; high content of exchangeable Al^{3+}; low content of available P and in general high content of cryptocrystalline Fe and Al oxides, which form very stable complexes, particularly those with Al, with some components of the SOM pool and with P, immobilizing this important nutrient. They have abundant organic N that has to be mineralized to have available forms of N, predominating in these soils' ammonification on nitrification in the process of soil mineralization; as for its texture in general, the fine and gross sand predominate and the clay is scarce, and therefore, the organic matter plays a fundamental role not only as cement of the aggregates and then on the soil structure but also in the exchangeable cation complex (Carballas et al. 2015). These particular characteristics could explain what happens in this experience, in line with previous studies performed in the same region (Carballas et al. 1993; Carballas 1997; Prieto-Fernández et al. 2004; Martín et al. 2012; Lombao et al. 2015) or even in the same experimental design (Couto-Vázquez and González-Prieto 2006; García-Marco and González-Prieto 2008; Barreiro et al. 2010; Couto-Vázquez et al. 2011; Fernández-Fernández et al. 2015).

A marked effect of the depth was observed for all the analysed soil properties of the samples collected 10 years after the fire, this factor explaining around 13–99 % of data variation, while the treatments (fire combined or not with retardants) usually showed a minor influence, explaining only 14–19 % of data variance of the physicochemical and chemical properties and between 10 and 38 % of data variation of the properties related with the mass and activity of the soil microorganisms (Table 1; Figs. 1, 2, and 3).

As expected, the pH increased with depth while the SOM content and the physicochemical and chemical related properties (water holding capacity, electrical conductivity, hydro-soluble C and CH) decreased, which confirms what was reported for forest soils by Prieto-Fernández et al. (1998) and Lombao et al. (2015) or for non-tillage soils by Mahía et al. (2009) and González-Prieto et al. (2013). Likewise, biochemical and microbiological properties (enzyme activities, microbial biomass and activity) also decreased with depth, which is related with the decrease of the SOM content that is determinant for most biochemical and microbiological soil properties analysed (Burns 1978; Fritze et al. 2000; Matinizadeh et al. 2008), particularly on these soils of the temperate humid zone, and in lesser extent, with variations between different soil niches (aeration, moisture, temperature, pH, available C, available nutrients, etc.) (Nannipieri et al. 2003; Certini 2005), which, in turn, are associated with different depth (García-Villaraco Velasco et al. 2009). This is supported by the significantly positive coefficients of correlations among SOM and most biochemical and microbiological parameters analysed (data not showed). The principal component analysis performed with the PLFA data confirmed the importance of soil depth as one of the main factors responsible for the microbial community composition (Fig. 4).

Initially, as usual in the soils of the zone, the prescribed fire produced an increase of soil pH and EC likely due to the abundant basic ions supplied by the ashes from the combustion of the vegetation, deposited in the top layer, and the reduction of organic acids of SOM (Carballas et al. 1993; Certini 2005; Couto-Vázquez and González-Prieto 2006), as well as in burned soils treated with retardant, to the cations supplied by the fire-fighting chemicals and the neutral pH of its water solutions (Couto-Vázquez and González-Prieto 2006). Usually, these changes were more or less rapidly attenuated with time due to the loss of the ashes by post-fire wind or pluvial erosion and the leaching of basic ions (Certini 2005; Úbeda and Outeiro 2009), so that 5 or 10 years after the fire usually there were no differences between the burnt and the unburnt soils (Carballas et al. 1993). However, in our experiment we have still detected a slight increase in the pH on B, B + Fo and B +

Fi soils and a higher and significant increase of the EC on B and B + Ap soils, compared with the unburnt soil, 10 years after the fire (Table 1). The higher values of available P and Al and NO^{3-}-N in the B + Ap soil, 10 years after the fire (Fernández-Fernández et al. 2015), could have some influence.

With respect to the organic matter content (total C and total N), the fire initially did not cause significant changes (Couto-Vázquez and González-Prieto 2006). This can be explained by a partial combustion of the SOM due to the temperatures reached in the top layer during the prescribed fire, which is compensated by the incorporation of organic matter from the burnt vegetation (Johnson and Curtis 2001; González-Pérez et al. 2004; Certini 2005). The lack of effect of the prescribed fire and the retardants on SOM was also found after 10 years (Table 1), as was also reported by Fernández-Fernández et al. (2015) for the N content; similarly, no effects on SOM 5 and 10 years after the fire were reported by Carballas et al. (1993) and Prieto-Fernández et al. (2004) in soils of the same zone. On the contrary, a significant effect in both soil C and N was found by Johnson and Curtis (2001) more than 10 years after the fire, and the response was dependent on the type and severity of the fire. A behaviour parallel to that observed for SOM was shown by WHC (Table 1), two highly correlated soil properties; the same behaviour was found by Carballas et al. (1993) in soils affected by wildfires of the same zone.

The most labile fractions of organic matter (microbial-C, hydro-soluble C and CH) and the soil enzyme activities were drastically reduced immediately after the prescribed fire and the retardant addition, but most of them recovered at medium term (Barreiro et al. 2010) or long term, 10 years after the fire, as revealed in our study (Figs. 1 and 2). In line with this, Prieto-Fernández et al. (1998) reported that the microbial C and N and the extractable C of burnt soils of the temperate humid zone were still at a lower level between 4 and 13 years after the fire than that in the unburnt soils. The wide range of results that can be found in the literature for the reestablishment of microbial C under field conditions depends on diverse factors such as fire severity, changes in soil, post-fire climate and degree of vegetation recovery that supply labile OM (Mataix-Solera et al. 2009). The lack of differences that we found between the burnt and unburnt soils after 10 years for the hydro-soluble C and CH contents (Fig. 1) was expectable because, although the cellulose and hemicellulose fractions are the most altered by the burning followed by the water-soluble fraction (Fernández et al. 1997), between 2 and 5 years after fire the burning appeared to have no consistent effect on the hydro-soluble C and CH fractions (Carballas et al. 1993; Martín et al. 2009).

The lack of effects of the burning on soil enzyme activities (β-glucosidase and urease), observed by Barreiro et al. (2010) on the same plots 5 years before, were confirmed by our results 5 years later (Fig. 2c, d). Nevertheless, Carballas et al. (1993) reported effects of burning on diverse enzyme activities of burnt soils of the humid temperate zone up to 10 years after the fire; this is because the enzyme activities can be affected by burn severity but also for changes directly or not related to the heating (pH, changes in the organic matter, moisture, vegetation, geographic location, etc.) (Nannipieri et al. 2012), which may explain the wide range of time of disturbance of the soil enzyme activity by the fire as shown in the literature (1–40 years) (Mataix-Solera et al. 2009). At long term, effects of the addition of the retardant on most physicochemical, chemical and biochemical soil properties were not observed; only the hydro-soluble C levels at 80 °C of the burnt soils treated with retardants remained significantly lower than those of the U soil, and the same trend was observed for the β-glucosidase activity of the B + Ap soil (Figs. 1 and 2). Both effects may be related because β-glucosidase activity is regulated by the substrate availability (Boerner et al. 2005), and the decrease of the labile C fraction may affect this enzyme activity; moreover, as reported by Kuderyarova et al. (1991), the application of phosphorus fertilizers, such as Ap, increases the solubility of SOM and promotes the migration of C producing loss of OM four times higher on the treated soil than that on the control soil, 2 years after its application to soil. On the other hand, the positive effect of Fi on the β-glucosidase activity, reported at short and medium term in laboratory and field experiences (Basanta et al. 2002; Barreiro et al. 2010), is not noticeable at long-term field experiences (Fig. 2).

It should be noticed, however, that most properties analysed (physicochemical, chemical and biochemical) showed similar values to those observed in the corresponding unburnt control soil. Hence, if present, the long-lasting changes induced by the prescribed fire were small. In addition, taking into account the magnitude of changes and the spatial and temporal variations of most analysed chemical and biochemical properties (total C, total N, labile organic matter fractions, respiration, soil enzymes) in soils of the same temperate humid zone (Díaz-Raviña et al. 1993, 1995; Martín et al. 2009, 2011), the combined interpretation is that soil conditions had recovered 10 years after the prescribed fire. This is consistent with data reported by Fritze et al. (1993) who found that recovery of prescribed burning occurred within 12 years.

Ten years after the fire, no significant differences were found between the burnt soil and the corresponding unburnt control for most biological parameters (Figs. 2 and 3), except for bacterial growth determined by the leucine incorporation technique and fungal biomass and growth estimated by ^{14}C-acetate incorporation to ergosterol. In general, as compared with the unburnt soil, bacterial growth was higher in the soils affected by the prescribed fire while fungal biomass and growth decreased (Fig. 3). This is consistent with results obtained by other authors concerning the higher sensibility of fungi to temperatures reached during experimental fires and the predominance of bacteria favoured by post-fire conditions such as an increase of pH (Vázquez et al. 1993; Acea and Carballas 1996; Certini

2005; Bárcenas-Moreno et al. 2011). Likewise, the dominance of bacteria growth over fungi growth in the burned soils after such a long time can be explained by the negative interaction or antagonist effect between these two microbial decomposer groups likely due to exploitation competition as occurred during colonization of new substrate or natural soils (Rousk et al. 2008).

The use of phospholipid analysis in combination with principal component analysis allows to study the changes in the composition of the main microbial groups in soils under different management (Frostegård et al. 1993; Díaz-Raviña et al. 2006; Fernández-Calviño et al. 2009; Mahía et al. 2011), on different soil niches (Steer and Harris 2000) and along the soil profile (Fritze et al. 2000; Fierer et al. 2003; Lombao et al. 2015). Our PLFA data indicated that the first differentiating factor of the soil samples was the depth followed by the fire (separation of burnt samples from the corresponding unburnt control samples) and even to the different retardant treatments (B + Fo and B + Fi that were grouped more closely to the corresponding burnt control and clearly separated from the B + Ap treatment) (see Fig. 4). This is in agreement with data of García-Villaraco Velasco et al. (2009) who found that factors associated to the soil depth were more important than fire retardants for functional diversity of soil microbial communities measured using the Biolog EcoPlate technique 1 year after the fire. Somehow, these data of PLFA pattern are also consistent with our results of soil enzyme activities, bacterial and fungal growth and fungal biomass, estimated by ergosterol, and the biomass of specific groups determined from PLFA data, indicating that both B + Fi and B + Ap, particularly the latter, were the treatments showing a stronger effect on all these parameters. As a consequence of B + Ap treatment in the surface layer, the β-glucosidase activity significantly decreased, and slight but not significant decreases were also observed for respiration and urease activity values (Fig. 2); in addition, as a consequence of the B + Ap and B + Fi treatments, fungal growth significantly decreased and bacterial activity increased (Fig. 3). Likewise, the PLFA pattern obtained 5 years after the prescribed fire and application of treatments also allows us to differentiate the communities of burnt and unburnt soils as well as the different retardant effects, although in this case at shorter term the strongest effect was observed for the B + Fi treatment (Barreiro et al. 2010). A greater effect of the treatments B + Fi and B + Ap on the availability of macronutrients and micronutrients was revealed by studies performed in the same soil plots, 5 and 10 years after the prescribed fire (Couto-Vázquez et al. 2011; Fernández-Fernández et al. 2015). The effect of these two treatments, B + Fi and B + Ap, on soil microbiological properties can be explained by their chemical nature, a synthetic polymer very difficult to biodegrade (Fi), which sometimes had a stimulation effect while in others it had an inhibitory effect on soil microorganisms (Basanta et al. 2002, 2003; Díaz-Raviña et al. 2006), and a fertiliser salt (Ap), which can affect positively or negatively to soil microorganisms due to the direct supply of N and P or indirectly through its effects on some soil properties such as pH, soil aggregation, moisture, oxygen, etc. (García-Villaraco Velasco et al. 2009). This is supported by previous studies showing that the Ap, due to its composition (high content of N and P), acts as a fertilizer both at short (Couto-Vázquez and González Prieto 2006) and long terms on the soil-plant system (Couto-Vázquez et al. 2011; Fernández-Fernández et al. 2015). Likewise, a possible effect of different additives of commercial fire-fighting chemicals on biochemical and biological properties cannot be discarded (Bashan et al. 2016). Other factors that may explain the contrasting results is that N and P addition often leads to changes in plant species composition and diversity (Fernández-Fernández et al. 2015), which in turn may affect activity, biomass and composition of soil microbial communities with inputs of different quality and quantity of plant debris, including rhizodeposition (Geisseler and Scow 2014).

It should be noticed that the soil treatments studied (fire, retardant addition) differed notably with respect to vegetation growth and cover and that both fire and ammonium phosphate addition had significant effects on the soil-plant system after 10 years, since the pines in the unburnt plots were higher and wider than in the burnt treatments except for B + Ap, where the tallest and widest trees were found, although half of them were either dead (the second highest mortality after B + Fi) or had a distorted trunk (Fernández-Fernández et al. 2015). In this study, B + Ap was the treatment with the strongest effects on the plants showing *E. umbellate* as having the lowest coverage and height, *P. tridentatum* as having the highest coverage and *U. micranthus* as having one with the lowest coverage and being the only treatment where *Genista triacanthos*, a N fixer species, was absent. The microbial communities were grouped according to the PLFA pattern in a similar way to that observed for the vegetation data suggesting that the vegetation was the main driving force determining the microbial community composition of these burnt soils. The data are consistent with earlier investigations showing that the microbial component can vary greatly with vegetation (Grayston et al. 1998; Priha et al. 2001; Mahía et al. 2006; Álvarez et al. 2009; Lombao et al. 2015).

Conclusions

In the present study, the long-term impact of the prescribed fire was small and similar to that observed in other experimental fires in shrublands located in the same area and very different to medium- and high-intensity wildfires affecting shrublands or forest ecosystems causing negative or even irreversible soil quality modification (physical, chemical and biological degradation). Overall, the addition of retardants did not lead to any

major direct adverse effect on activity, biomass and composition of soil microbial communities as compared with the untreated soils. Nevertheless, the PLFA technique used was able to detect changes caused by the fire combined or not with fire-fighting chemicals at long term as occurred in previous studies performed at short and medium terms. B + Ap was the treatment with the strongest effects on activity, biomass and composition of soil microbial communities 10 years after the fire, which is associated with indirect changes of this retardant on the vegetation growth and cover after such a long time. It should be noticed, however, that although the values of most physicochemical, chemical and biochemical properties analysed suggested that the soil chemical quality is not affected, the PLFA and vegetation data clearly showed that the composition of the below-ground and above-ground organisms at the community level was affected by both the prescribed fire and the retardant addition. PLFA analysis provides us with information on the composition of the major microbial taxonomic groups of microorganisms, which can be considered as a broad approximation of biodiversity measure of soil at the community level, although it does not reveal any information at the species level. To this respect, the data suggest that, in order to preserve the above-ground and below-ground biodiversity, prescribed fires combined or not with fire-fighting chemicals as a tool to reduce the impact of wildfires should be applied with precaution, particularly in ecosystems of special interest such as natural protected or sensible areas.

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