SJSS
SPANISH JOURNAL OF SOIL SCIENCE

Medium-term impact of post-fire emergency rehabilitation techniques on a shrubland ecosystem in Galicia (NW Spain)

Impacto a medio plazo de las técnicas de rehabilitación de emergencia post-incendio sobre un ecosistema de matorral en Galicia (NO de España)
Impacto a médio prazo das técnicas de reabilitação de emergência pós-fogo em um ecossistema de matagal na Galiza (NW Espanha)

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Received: 13.12.2017 | **Revised:** 16.03.2018 | **Accepted:** 21.03.2018

ABSTRACT

The aim of this work was to study the effectiveness of two post-fire emergence rehabilitation techniques (seeding and mulching) for reducing soil erosion as well as their effects on the soil quality; therefore in the field, experimental plots of unburnt soil, burnt soil, burnt soil plus seeding and burnt soil plus mulching were established. Soil samples were collected from the A horizon and a wide range of physical, chemical and biological soil properties were analyzed to evaluate soil quality. The effect of fire on the vegetation cover was observed after one year and changes in soil properties persisted even after four years. The phospholipid fatty acids pattern showed that in the medium-term (8-48 months after the fire), the fire may modify the soil microbial communities by altering the plant community via plant-induced changes in the soil environment. No effect of seeding or mulching on the vegetation cover was observed. The mean efficiency in preventing soil erosion between 8 and 12 months after the fire and the application of the treatments was 11% for seeding and 65% for mulching. These stabilization treatments had a minor influence on the post-fire soil quality in the medium term (48 months); therefore, taking into account its effectiveness for reducing soil erosion, the mulching treatment is recommended as the best post-fire stabilization technique.

RESUMEN

El objetivo de este trabajo fue estudiar la eficacia de dos técnicas emergentes de rehabilitación post-incendio (siembra y acolchado) para reducir la erosión del suelo así como sus efectos sobre la calidad del suelo. Para ello, en la experiencia de campo se implementaron las siguientes parcelas: suelo no quemado, suelo quemado, suelo quemado más siembra y suelo quemado más acolchado. Se tomaron muestras de suelo del horizonte A y un amplio rango de propiedades físicas, químicas y biológicas fueron analizadas para evaluar la calidad del suelo. El efecto del fuego sobre la cubierta vegetal fue detectado un año después del incendio y los cambios sobre las propiedades del suelo persistieron incluso 4 años después del mismo. El patrón de los ácidos grasos de los fosfolípidos demostró que a medio plazo (8-48 meses después del incendio) el fuego puede modificar las comunidades microbianas del suelo por alteración de las comunidades vegetales a través de los cambios inducidos en el suelo. No se observó ningún efecto de la siembra ni del acolchado sobre la cubierta vegetal. La eficiencia media para la prevención de la erosión del suelo durante el período de 8 y 12 meses después del incendio y la aplicación de los tratamientos fue del 11% para la siembra y del 65% para

DOI: 10.3232/SJSS.2018.V8.N3.03

el acolchado. Estos tratamientos de estabilización tuvieron una menor influencia sobre la calidad del suelo post-incendio, a medio plazo; por lo tanto, teniendo en cuenta su eficacia, se recomienda el acolchado como la mejor técnica de estabilización post-incendio.

RESUMO

O objetivo deste artigo foi estudar a eficácia para reduzir a erosão do solo de duas técnicas emergentes de reabilitação pós-fogo (sementeira e mulching), bem como seus efeitos sobre a qualidade do solo. Assim, na experiência de campo, foram implementadas as seguintes parcelas: solo não queimado, solo queimado, solo queimado mais sementeira e solo queimado mais mulch. As amostras de solo foram retiradas do horizonte A e uma ampla gama de propriedades físicas, químicas e biológicas foram analisadas para avaliar a qualidade do solo. O efeito do fogo sobre a cobertura da vegetação foi observado um ano após o incêndio e as alterações nas propriedades do solo persistiram até quatro anos depois. O padrão dos ácidos gordos dos fosfolípidos mostrou que, a médio prazo (8 a 48 meses após o incêndio), o fogo pode modificar as comunidades microbianas do solo por alteração das comunidades vegetais através de mudanças induzidas no solo. Não foi observado efeito da sementeira ou da aplicação do mulch na cobertura vegetal. A eficiência média para a prevenção da erosão do solo durante o período de 8 e 12 meses após o incêndio e a aplicação dos tratamentos, foi de 11% para a sementeira e de 65% para o mulching. Estes tratamentos de estabilização tiveram, no médio prazo, uma menor influência na qualidade do solo pós-fogo; assim, tendo em consideração a sua eficácia na redução da erosão do solo, o mulching é recomendado como a melhor técnica de estabilização pós-fogo.

KEY WORDS
Wildfires, seeding and mulching, soil quality, vegetation recovery, soil erosion, PLFA pattern.

PALABRAS CLAVE
Incendios no controlados, siembra y acolchado, calidad del suelo, recuperación de la vegetación, erosión del suelo, patrón de los PLFA.

PALAVRAS-CHAVE
Fogos incontrolados, sementeira e mulching, qualidade do solo, recuperação da vegetação, erosão do solo, padrão PLFA.

1. Introduction

In the last decades, fire has become a major disturbance of forest ecosystems by destroying the vegetation cover, causing soil degradation and increasing soil losses by erosion (Chandler et al. 1983). Fires can affect physical, chemical and biological soil properties to a varying extent; usually they can produce loss of nutrients by volatilization and leaching, partial or total combustion of soil organic matter (SOM), increase of hydrophobicity, alteration of aggregate stability, deterioration of soil structure and marked alterations in the number, activity and composition of the soil microbial communities (Neary et al. 1999; Certini 2005). Consequently, fires can produce a reduction in the soil quality and cause huge economic and ecological damage that will probably be worse in the foreseen scenario of climate change (Shakesby 2011).

Galicia (NW Spain) and the north of Portugal are the most affected areas by forest wildfires worldwide, with the greatest number of fires per hectare or inhabitant (Carballas et al. 2009). Throughout recent decades, this area has been affected by a temporal and spatial change in fire regime, resulting in a dramatic annual increase in the surface burnt by wildfires due to human action, changes in land use, policies and climatic fire risk. Most of these wildfires occur in forest and shrubland areas, which are often located on sloping terrain, in soils with moderate erodibility mainly due to high capacity of soil infiltration, which is related to sandy texture, high SOM content and high stability of soil aggregates (Benito et al. 2010; Varela et al. 2010). However, these factors, together with post-fire meteorological conditions (abundant high-intensity rainfall events in the autumn period, immediately after summer wildfires), tend to increase runoff and erosion processes of different orders of magnitude in the soil surface horizon (Díaz-Fierros et al. 1990; Vega et al. 2005; Fernández et al. 2008). In Galicia, in the last 42 years 250,000 forest fires have been registered affecting 1,711,000 ha (86% of its forest area) and causing dramatic effects (Carballas et al. 2009). In 2006, two thousand fires occurred in 12 days, affecting 75,000 ha approaching urban centers, although most of them were concentrated in small villages and affected 50% of their forest surface (Carballas et al. 2016); and in October 2017, 1,300 ha were affected in only 1 day (MAPAMA 2018). They caused serious off-site damages by runoff and deposition of sediments in downstream terrestrial and aquatic ecosystems as a consequence of torrential rainfall occurring at the end of the summer (Carballas et al. 2016).

When the vegetation cover is partial- or completely destroyed by the fire, the interception of rainfall decreases, causing destruction of aggregates and dispersion of the fine particles that clog the pores; this decreases the infiltration capacity of water in the soil and increases the runoff and soil loss due to water erosion (Carballas et al. 2016). Therefore, in some burnt areas very susceptible to erosion, emergency stabilization treatments should be applied as early as possible to accelerate re-vegetation or provide a soil cover with plant material, as naturally occurs with needles or leaves of the unconsumed canopy, to reduce post-fire soil erosion (Cerdà and Doerr 2008). Straw mulching and herbaceous seeding are often the techniques recommended for this purpose; however, rigorous quantitative studies concerning their effects on the yield of sediments coming from the burnt hillslopes are scarce and have been made mostly in the USA (Robichaud et al. 2000, 2006; Wagenbrenner et al. 2006; Groen and Woods 2008), in the north and central areas of Portugal (Prats et al. 2012, 2016), in the semiarid region of Spain (Bautista et al. 1996; Badía and Martí 2000; Kribeche et al. 2013), and in some other countries (Prosdocimi et al. 2016). Recently, the Galician Forest Service has initiated a post-fire stabilization program to reduce the potential increase in runoff and erosion. Although the research on the effectiveness of different post-fire erosion mitigation techniques has increased (Vega et al. 2013), results on their effects on soil quality are still necessary. The first results of a recent study performed under field conditions following a high severity wildfire in a shrubland ecosystem in the NW of Spain showed that both rye seeding and straw mulching did not modify the quality of the burnt soil and significantly reduced the post-fire hillslope erosion rates over the first four months after their application (Díaz-Raviña et al. 2012), which can be interpreted as a control of soil quality at short-term. Although these results are promising, it is necessary to study the effects of these treatments on the vegetation recovery, soil quality and erosion control at the medium-term, particularly during the first year, when the risk of erosion is greater.

The effects induced by wildfires on soil quality are highly variable and depend on fire severity, soil type, topography and post-fire conditions (climatology and recovery of the soil-plant system), among other factors, and so in some cases the results obtained in one area cannot be compared to those produced in another. Soil quality has been defined as the capacity of a specific kind of soil to function, with naturally or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and soil quality, and support human health and habitation (Karlen et al. 1997). Several parameters have been proposed for the assessment of quality of burnt soils but most investigations analyze soil parameters separately (Cerdà and Jordán 2010).

On the other hand, most studies or even reviews concerning this topic, performed by diverse researchers, are focused on the short-term effects on diverse soil properties (Neary et al. 1999; Certini 2005; Mataix-Solera et al. 2009). However, to date, studies performed on the same soil combining the information of fire effects on physical, chemical and biological properties and therefore on the overall soil quality of burnt soils are scarce (those performed mainly by our research group). In addition, despite the fact that microorganisms are known to be the main responsible agent for soil functioning, their detailed study, including aspects related with their biomass, activity and diversity, were not taken into account in most of these investigations (Mataix-Solera et al. 2009). Hence, for the same soil we have to attempt to combine the results from different soil properties, including the biological ones, for a comprehensive understanding of the wildfire effects as well as the effects of the soil rehabilitation treatments on soil quality.

The aim of the present study, therefore, was to evaluate the medium-term (8-48 months) impact of a wildfire (comparing the burnt soil versus an unburnt soil; i.e. control) and two different post-fire stabilization treatments (comparing the burnt soil with seeding and mulching versus the burnt soil) on the vegetation and the soil quality of a shrubland located in the NW of Spain as well as to determine the efficacy of the treatments to control post-fire erosion (8-12 months). This is one of the few studies that combine: (a) the recovery of the vegetation cover; (b) the evaluation of the overall soil quality (physical, chemical and biological properties) after the fire and application of the soil rehabilitation treatments; and (c) the effectiveness of these techniques for post-fire erosion mitigation.

2. Materials and Methods

2.1. Experimental Design

The study was performed in an area of special interest due to its proximity to the Serra do Invernadoiro Natural Park (Laza, Galicia, NW Spain). The climate is temperate (mean annual temperature 6-8 °C) and humid (1600-1800 mm•year^{-1} of precipitation) as registered at the nearest meteorological station (Cabeza de Manzaneda) during the studied period (first year after the fire). The soil was an Entisol (Udorthent) (Soil Survey Staff 2010) developed over phyllites under a shrubland (*Erica* spp., *Vaccinium myrtillus* L., *Pterospartum tridentatum* Willk., *Cistus* spp.) reforested with *Pinus sylvestris* (planted in rows and separated 5 m; mean height of 1-1.60 m), which was the dominant vegetation prior to the wildfire. The soil is representative of the soils developed over metamorphic rocks and under shrubland vegetation in the temperate humid zone of NW Spain, which have acid pH, high SOM content and a desaturated cation exchange complex (Carballas et al. 2016). This ecosystem, highly susceptible to soil erosion due to the steep relief and high rainfall erosivity, suffered a wildfire in September 2010, affecting 1,700 ha. A hillslope of the burnt and unburt areas, separated by a firewall, was selected for the study because of its homogeneity in terms of fire severity, vegetation, slope, and the presence of a comparable unburnt control soil near the burnt area (U.T.M. 29T PG34168-71422, 1566 m above sea level and 30% slope, with orientation 245-250° WSW). After the fire the pines died and the prevalence of white ashes over black ones as well as the total consumption of the shrubland (vegetation and soil litter layers) suggested that the fire severity had been from moderate to high (Vega et al. 2013). One week after the wildfire, the field experimental burnt and unburnt areas were delimited by metal fences to avoid soil perturbation by other factors than the rehabilitation treatments (i.e. presence of animals). Nine experimental plots (4 × 20 m each) were established between the pine planting rows across the hillslope in a randomized block design in the burnt area; in the same way, three control plots with the same dimensions and slope position were established in the unburnt area. To study the effect of the wildfire and two selected post-fire stabilization techniques, four treatments were considered in triplicate: unburnt control soil (U); burnt soil (B); burnt soil plus native *Secale cereale* L. seeds at a rate of 10 g•m^{-2} (B+S); and burnt soil plus 250 g•m^{-2} of native wheat straw mulch (B+M); seeds and straw being spread manually immediatelly after the wildfire at recommended field dose. The straw mulching and the seeding were selected because they are considered the most effective treatments to reduce surface runoff and keep soil in place (Robichaud 2009). Metallic sediment collectors, Gerlach troughs, were located on the downhill portion of the burnt plots to measure soil erosion.

2.2. Soil Sampling

Soil sampling was carried out 8, 12 and 48 months after the wildfire and several physical, chemical and biological properties were analyzed. At each sampling time, from each plot, after removing the litter (4-8 cm thick) of the unburnt plots, the ash layer of the burnt plots and also the straw of the mulched plots, 10 soil subsamples from squares (15 cm × 15 cm) uniformly distributed in zig-zag along the plot were taken from the top layer (0-2 cm). They were mixed to form one representative composite soil sample and refrigerated (4 °C) until processing in the laboratory. Additionally, soil samples at 2-5 cm depth were collected 48 months after the wildfire in all treatments and in the same soil surface than those collected at 0-2 cm. This allowed us to determine if the effect of the treatments could be detected in the deeper layer (if this occurs it means that rhizosphere influence and straw incorporation to soil took place). The soil samples were passed through a sieve with a 2 mm diameter mesh; fractions bigger than 2 mm soil were discarded and the soil fractions < 2 mm were thoroughly homogenized and used in all subsequent analyses. They were divided into: (a) fresh subsamples maintained at -4 °C for the measurement of moisture content, water retention capacity (WRC), hydrosoluble carbon and carbohydrates and biochemical properties; (b) fresh subsamples lyophilized and frozen to -15 °C for the assessment of Phospholipid Fatty Acids (PLFAs) analysis; and (c) air-dried subsamples for analysis of the rest of the physical and chemical soil properties.

2.3. Vegetation Monitoring

The percentage of soil covered by vegetation was estimated in 10 subplots, each one covering an area of 1 m^2, uniformly distributed and systematically selected along each burnt plot, that were photographed at each sampling time, immediately and 4, 8 and 12 months after the fire. The images were analyzed in the laboratory, using a Photoshop 5.0 program. On each image a mesh (26 cm x 18 cm) with 10 x 14 squares was placed and the square numbers fully covered by straw mulching and plants were registered. Then, the total average percentage of ground cover by the dominant herbaceous and shrub species was calculated for each plot.

2.4. Erosion Measurements

Soil erosion was assessed by the sediments arrived to the burnt plots by runoff, collected at two sampling times, 8 and 12 months after the fire, in metallic collectors, Gerlach throughs, with a modification of the method as previously indicated by Díaz-Raviña et al. (2012).

2.5. Physical, Physicochemical and Chemical Soil Properties

The soil properties monitored were the following: (a) physical: granulometric composition, aggregate stability, water repellency, moisture content and WRC; (b) physicochemical: pH (in water and KCl) and electrical conductivity (EC); and (c) chemical: total carbon (C) and nitrogen (N) contents and hydrosoluble C and carbohydrates. The methods described by Guitián-Ojea and Carballas (1976) were utilized to determine: granulometric composition, moisture, WRC, pH and EC. The aggregate stability and the water repellency were measured following the procedure described by Díaz-Raviña et al. (2012). The total C and N contents, and the different labile fractions of the SOM, hydrosoluble C and carbohydrates [soluble carbon (WSC) and carbohydrates (WSCH), hot water extractable carbon (HWC) and carbohydrates (HWCH)] were analyzed following the techniques previously described by Lombao et al. (2015b).

2.6. Biological Soil Properties

The biochemical properties [β-glucosidase and urease activities, microbial biomass C (Cmic), soil respiration, specific respiration rate or metabolic quotient (qCO_2, ratio between soil respiration and Cmic) and bacterial activity] were determined as described by Lombao et al. (2015b).

The microbial community structure and the total and relative bacterial and fungal biomass were estimated by PLFA analysis using the procedure and nomenclature described by Frostegård et al. (1993). The total amount of 10Me18:0, 10Me17:0 and 10Me16:0 PLFAs were used as an indicator of the actinobacteria biomass (ActPLFA). The sum of the PLFAs considered to be predominantly of bacterial origin (i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:1ω8, i17:0, a17:0, 17:0, cy17:0, 18:1ω7 and cy19:0) was used as an index of the bacterial biomass

(BactPLFAs), and the quantity of the 18:2ω6 PLFA was used as an indicator of the fungal biomass (FungPLFAs). The i14:0, i15:0, 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.

2.7. Statistical Analyses

All results were obtained from triplicate determinations and were expressed on the basis of oven-dry (105 °C) weight of soil. Mean values of three field replicates (values ± SE, standard error) were used to compare the different soil treatments. The values of the variables determined on samples collected at 0-2 cm depth at different times (8, 12, 48 months) after the fire were analyzed by Two-way Analysis of Variance (ANOVA 2) to determine the percentage of variation attributable to the soil treatment (U, B, B+S, B+M) and to the sampling time. Likewise, the data of samples collected 48 months after the fire at two depths, 0-2 cm and 2-5 cm, were analyzed by ANOVA 2 to determine the percentages of variance attributable to the soil treatment and to the depth. The significant differences among the different treatments at each sampling time and those for the same treatment at different depths were determined with the Bonferroni's test for the multiple comparisons at $p < 0.05$. The comparison of the B soil versus the U soil give us the wildfire effect and the comparison of B+S and B+M versus B give us the rehabilitation treatments effect; after 48 months, the comparison of the same treatment at 0-2 cm layer versus at 2-5 cm layer give us the depth effect. The vegetation data were statistically analyzed by ANOVA 2 following the same procedure indicated above. The One-way Analysis of Variance (ANOVA 1) was used to analyze the accumulated sediment yield during the first year after the fire and in the cases of significant F statistics, the Tukey's minimum significant difference test was used to separate the means and to determine the effect of the soil rehabilitation treatments (B versus B+S, B+M). The values corresponding to the concentrations of all the individual PLFAs, expressed in mole percent and logarithmically transformed, were subjected to a principal component analysis (PCA) to elucidate the main differences in the PLFA patterns of the microbial communities of the study soils. All statistical analyses were made using the SPSS 15.0 statistical package.

3. Results

3.1. Vegetation Monitoring

The results of ANOVA 2 showed a significant effect of the time elapsed from the fire on the total vegetation cover, explaining 89% of variance, and on the percentage of the ground cover for most plant species analyzed, explaining 44-59% of variance for herbaceous species and 67-88% for shrub species. The effect of the treatment was only observed on *Luzula lactea* L. and *Agrostris* spp. species (15-17% of variance) and on *Vaccinium myrtillus* L. (40% of variance). Similar results were obtained for data of the plant cover dynamics, the effect of the time elapsed from the fire explaining 20-90% of variance and the treatment a further 2-25% of variance only on *Luzula lactea* L., *Agrostis* spp. and *Pterospartum tridentatum* Willk.

The recovery of the vegetation cover did not happen during the first four months after the fire (percentage of the ground cover lower than 12%), slightly increased between the 4th and the 8th months (values lower than 18%), and reached values of 36-40% between the 8th and the 12th months (**Table 1**). The *Secale cereale* L. sown covered 5.4%, 7.1% and 1.9% of the soil surface 4, 8 and 12 months after the fire, respectively. The soil surface covered by straw mulching was reduced with time, showing values of 90%, 81%, 76% and 53% immediately and 4, 8 and 12 months after the fire, respectively. During the first eight months after the fire, the significantly ($p < 0.05$) higher (12-18%) and lower (5-10%) percentages of the ground cover by plants corresponded to B+S and B+M treatments, respectively; nevertheless, the significant ($p < 0.05$) highest values of the total ground cover corresponded to B+M because the wheat straw mulching covered 76-90% of the soil surface in the same period.

Table 1. Percentages of the ground cover by plants and plant cover dynamics for the principal plant species in the burnt soils and 4, 8, and 12 months after the fire and application of the post-fire stabilization techniques (Mean values of three field replicates ± SE). Treatments: B, burnt soil; B+S, burnt soil plus seeding; B+M. burnt soil plus straw mulching. For the same sampling time different letters denote significant differences ($p < 0.05$) among treatments.

Plant Species	**Time (months)**	**Treatment**			**Treatment**		
		B	**B+S**	**B+M**	**B**	**B+S**	**B+M**
		Ground cover (%)			Species (%)		
Total vegetation	4	7,4 ± 0.3 b	12,0 ± 0.3 c	4,5 ± 1.1 a			
Luzula lactea L.		4,7 ± 0.4 a	4,4 ± 0.4 a	2,6 ± 0.7 a	66,5 ± 8.1 b	32,8 ± 2.6 a	56,5 ± 4.8 b
Festuca spp.		1,6 ± 0.7 a	1,5 ± 0.3 a	1,2 ± 0.1 a	19,2 ± 7.9 a	12,7 ± 2.0 a	30,0 ± 3.9 a
Agrostis spp.		1,1 ± 0.2 a	0,7 ± 0.1 a	0,7 ± 0.2 a	14,3 ± 2.8 b	5,1 ± 0.7 a	13,5 ± 0.1 b
Total vegetation	8	12,4 ± 1.8 ab	18,3 ± 0.7 b	9,7 ± 0.0 a			
Luzula lactea L.		8,7 ± 1.1 a	7,4 ± 1.1 a	6,4 ± 1.6 a	72,8 ± 5.2 b	38,7 ± 5.6 a	65,1 ± 3.7 b
Festuca spp.		2,1 ± 0.5 a	2,4 ± 0.3 a	1,9 ± 0.1 a	17,2 ± 3.8 a	13,7 ± 1.8 a	19,2 ± 2.8 a
Agrostis spp.		0,9 ± 0.1 b		0,7 ± 0.3 b	5,7 ± 0.9 b		8,6 ± 3.0 b
Pterospartum tridentatum Willk.		0,1 ± 0.1 a	0,5 ± 0.2 a	0,1 ± 0.1 a	0,4 ± 0.4 a	2,7 ± 0.9 a	0,6 ± 0.6 a
Cistus spp.		0,5 ± 0.2 a	0,7 ± 0.3 a	0,5 ± 0.0 a	3,5 ± 1.1 a	3,5 ± 1.3 a	5,0 ± 0.7 a
Erica spp.		0,1 ± 0.1 a	0,1 ± 0.0 a	0,1 ± 0.1 a	0,4 ± 0.4 a	0,1 ± 0.1 a	1,5 ± 0.5 a
Vaccinium myrtillus L.			0,1 ± 0.1 a			0,1 ± 0.0 a	
Total vegetation	12	35,6 ± 3.7 a	36,5 ± 5.0 a	39,9 ± 2.7 a			
Luzula lactea L.		10,0 ±1.1 a	9,3 ± 1.9 a	6,5 ± 1.7 a	31,1 ± 2.1 a	27,2 ± 8.8 a	20,7 ± 1.6 a
Festuca spp.		3,6 ± 0.7 a	4,1 ± 1.4 a	4,3 ± 1.2 a	10,6 ± 1.6 a	11,5 ± 3.2 a	11,4 ± 3.6 a
Agrostis spp.		0,1 ± 0.1 a			0,2 ± 0.2 a		
Pterospartum tridentatum Willk.		10,0 ± 1.6 a	6,8 ± 2.6 a	11,3 ± 0.2 a	27,1 ± 1.6 b	18,1 ± 1.6 a	29,0 ± 1.7 b
Cistus spp.		4,2 ± 1.6 a	5,5 ± 2.0 a	6,5 ± 0.8 a	10,4 ± 3.3 a	16,0 ± 5.2 a	16,5 ± 0.7 a
Erica spp.		7,7 ± 0.5 a	8,9 ± 4.1 a	11,3 ± 3.3 a	20,6 ± 1.3 a	21,5 ± 7.1 a	22,4 ± 4.0 a

The total vegetation cover did not show significant differences among treatments 12 months after the fire. In the burnt soils (B, B+S, B+M), the most abundant plant species (21-73% of the total vegetation) was the pioneer specie *Luzula lactea* L. following by other herbaceous species, mainly *Festuca* spp. (11-30%) and *Agrostis* spp. (0.2-14%). The contribution of the shrub species *Pterospartum tridentamtum* Willk., *Cistus* spp. and *Erica* spp., which represent 0.1-5% of the total vegetation, to the regeneration of the vegetation of the burnt soils did not occur until 8 months after the fire and was very low. However, they were the most abundant plant species (58-68%) in the burnt soils one year after the fire.

3.2. Sediment Yield

The sediments collected from the B, B+S and B+M treatments in the periods of 4 to 8 months and 8 to 12 months after the fire were 36 $g{\bullet}m^{-2}$, 32 $g{\bullet}m^{-2}$ and 9 $g{\bullet}m^{-2}$, respectively, in the first period; and 9 $g{\bullet}m^{-2}$, 8 $g{\bullet}m^{-2}$ and 4 $g{\bullet}m^{-2}$, respectively, in the second period (**Figure 1**). Therefore, the efficiency of the seeding and mulching treatments in reducing the soil loss was 11% and 75%, and 11% and 56% respectively for the first and the second evaluation periods. For both periods (T8 and T12) the ANOVA 1 showed no significant effectiveness for B+S and significant effectiveness ($p < 0.05$) for B+M. One year after the fire and application of the treatments, the accumulated sediment yields were 249 $g{\bullet}m^{-2}$, 168 $g{\bullet}m^{-2}$, and 35 $g{\bullet}m^{-2}$ for B, B+S and B+M soils, respectively. In all the burnt soils B, B+S and B+M the biggest sediment production occurred during the first 4 months reaching 85%, 76% and 63%, respectively, of the total sediments (**Figure 1**).

3.3. Physical, Physicochemical and Chemical Soil Properties

The values of all these physical, physicochemical and chemical soil properties of the unburnt and burnt soils, 8, 12 and 48 months after the fire, are shown in **Figure 2** and **Table 2.** Except for the physical properties (granulometric fractions, aggregate stability) the results of ANOVA 2 for the 0-2 cm depth soil samples showed in all cases a significant effect of the soil treatments, explaining 38-83% of the variance, a slight effect of the sampling time that explained 4-36% of variance while the interaction of soil treatments x sampling time explained 6% and 36% of variance in the moisture content and the EC, respectively. During the study period, comparing the B soil

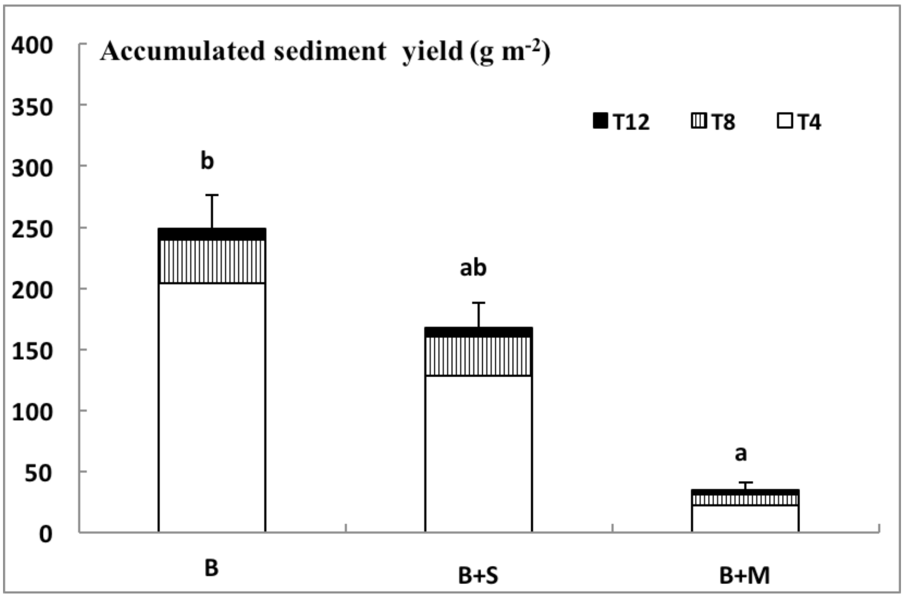

Figure 1. Accumulated sediment production for the burnt soils collected in the first year after the fire and application of treatments. T4, 0–4 months; T8, 4–8 months; T12, 8–12 months. T4 data taken from Díaz-Raviña et al. (2012). Mean values of three field replicates ± SE. Treatments: B, burnt soil; B+S, burnt soil plus seeding; B+M, burnt soil plus straw mulching. Different lowercase letters denote significant differences ($p < 0.05$) among treatments.

with the U soil, we observed: a) no appreciable changes in the granulometric fractions, aggregate stability and water repellency; b) an increase in soil pH (0.5 units); and c) a significant reduction (11-70%) in the moisture content, WRC, EC and total C and N contents (**Table 2**). Likewise, the labile fractions of the SOM (WSC, HWC, WSCH, HWCH) were drastically reduced (50%) by the wildfire and post-fire erosion processes (**Figure 2**). The data showed that the physical (moisture content and WRC), physicochemical and chemical properties, particularly the SOM labile fractions of the burnt soils, did not recover 4 years after the fire. Conversely, no effect of the B+S and B+M treatments was observed on the analyzed properties compared with the corresponding ones of the B soil (**Table 2**). The results of ANOVA 2 for the samples collected 48 months after the fire at 0-2 cm and 2-5 cm depth also showed a significant effect of the soil treatments on the analyzed properties (**Table 2**), which explained between 59% and 92% of variance, respectively, while the depth only explained 4-22% of variance. The results of the analyzed variables showed a clear effect of the burning, comparing B versus U, while no effect of the rehabilitation treatments was observed comparing B+S and B+M with B in both layers. An effect of the depth was observed on the pH of some burnt soils as well as on total C and N contents and all analyzed SOM labile fractions (WSC, HWC, WSCH, HWSCH) of the unburnt soils (**Table 2, Figure 2**).

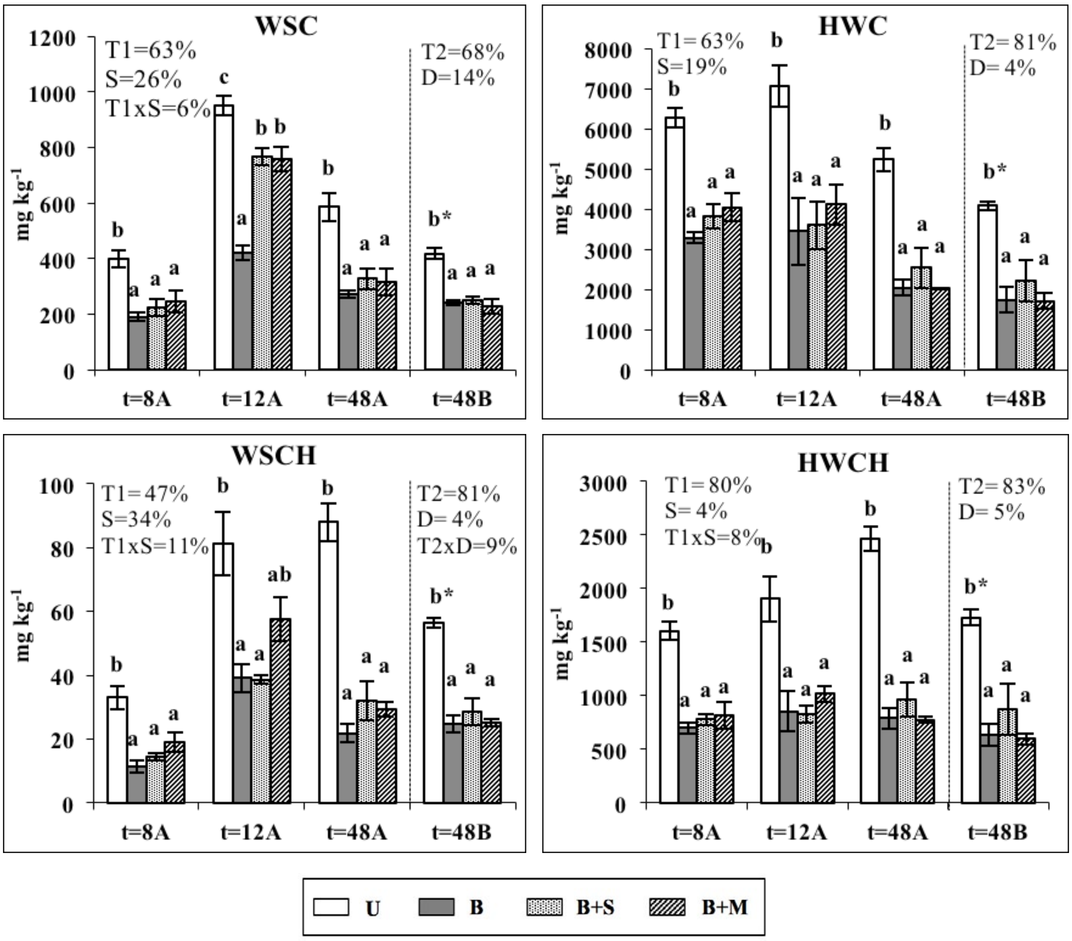

Figure 2. Organic matter labile fractions [(water soluble and extractable carbon (WSC and HWC) and carbohydrates (WSCH and HWCH)] in the different soil treatments 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques (mean values of three field replicates ± SE). Treatments: U, unburnt soil; B, burnt soil; B+S, burnt soil plus seeding; B+M, burnt soil plus straw mulching. Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected at 0-2 cm depth, according to an ANOVA 2 (T1, treatment; S, sampling time; T1xS, interaction treatment x sampling time). Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected 48 months after the fire at two depths, 0-2 cm (A) and 2-5 cm (B), according to an ANOVA 2 (T2, treatment; D, depth; T2xD, interaction treatment x depth). For the same sampling time different lowercase letters denote significant differences ($p < 0.05$) among treatments. For samples collected 48 months after the fire, * denotes significant differences ($p < 0.05$) between different depths.

Table 2. Soil physical, physicochemical and chemical properties analyzed in the different study soils, 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques (mean values of three field replicates ± SE). Treatment: U, unburnt soil; B, burnt soil; B+S, burnt soil plus seeding; B+M. burnt soil plus straw mulching. For the same sampling time different lowercase letters denote significant differences ($p < 0.05$) among treatments. For samples collected 48 months after the fire, * denote significant differences ($p < 0.05$) between different depths

Properties	Depth (cm)	Time (months)	Treatment			
			U	B	B+S	B+M
Sand (%)	0-2	8	25 ± 3 a	30 ± 2 a	29 ± 5 a	32 ± 1 a
		12	24 ± 5 a	30 ± 2 a	31 ± 3 a	31 ± 6 a
Silt (%)	0-2	8	56 ± 3 a	53 ± 3 a	54 ± 5 a	51 ± 3 a
		12	57 ± 1 a	54 ± 1 a	53 ± 3 a	52 ± 5 a
Clay (%)	0-2	8	20 ± 0 a	17 ± 1 a	17 ± 1 a	17 ± 2 a
		12	20 ± 2 b	17 ± 1 a	16 ± 1 a	17 ± 1 a
Aggregate stability (%)	0-2	8	96 ± 1 b	84 ± 8 a	87 ± 2 ab	92 ± 1 ab
		12	96 ± 2 a	90 ± 4 a	89 ± 3 a	90 ± 7 a
Water repellence	0-2	8	Very severe	Severe	Severe	Severe
		12	Very severe	Severe	Severe	Very severe
Moisture (%)	0-2	8	35 ± 2 a	29 ± 2 a	31 ± 6 a	31 ± 1 a
		12	26 ± 2 b	13 ± 3 a	14 ± 4 a	19 ± 3 ab
		48	40 ± 1 b	15 ± 1 a	19 ± 4 a	17 ± 3 a
	2-5	48	36 ± 2 b	19 ± 1 a	22 ± 3 a	20 ± 2 a
Water retention at field capacity (g kg^{-1})	0-2	8	872 ± 29 b	537 ± 28 a	565 ± 49 a	579 ± 65 a
		12	770 ± 74 b	501 ± 33 a	460 ± 83 a	488 ± 45 a
		48	1035 ± 13 c	530 ± 26 a	622 ± 65 ab	687 ± 60 b
	2-5	48	958 ± 39 b	636 ± 19 a	698 ± 34 a	699 ± 64 a
pH$_{water}$	0-2	8	3,4 ± 0.1 a	4,2 ± 0.2 b	4,2 ± 0.1 b	4,0 ± 0.1 b
		12	3,7 ± 0.0 a	4,1 ± 0.1 b	4 ± 0.1 b	4,1 ± 0.1 b
		48	3,6 ± 0.0 a	4,3 ± 0.0 bc	4,1 ± 0.0 b	4,4 ± 0.0 c
	2-5	48	3,5 ± 0.0 a	4,0 ± 0.0 b *	3,9 ± 0.0 b	4,0 ± 0.0 b *
pH$_{KCl}$	0-2	8	2,5 ± 0.0 a	2,9 ± 0.1 b	2,9 ± 0.1 b	2,9 ± 0.1 b
		12	2,6 ± 0.0 a	2,9 ± 0.0 b	2,9 ± 0.0 b	2,9 ± 0.0 b
		48	2,3 ± 0.0 a	2,8 ± 0.0 b	2,8 ± 0.0 b	2,9 ± 0.0 b
	2-5	48	2,4 ± 0.0 a	2,6 ± 0.0 a *	2,5 ± 0.0 a *	2,6 ± 0.0 a *
Electric conductivity (μS cm^{-1})	0-2	8	51 ± 5 b	27 ± 1 a	25 ± 4 a	26 ± 2 a
		12	23 ± 2 a	25 ± 3 a	30 ± 1 a	30 ± 5 a
		48	74 ± 2 b	22 ± 2 a	31 ± 8 a	26 ± 2 a
	2-5	48	82 ± 4 b	29 ± 2 a	35 ± 1 a	27 ± 5 a
Total C (g kg^{-1})	0-2	8	239 ± 11 b	143 ± 12 a	154 ± 13 a	157 ± 20 a
		12	216 ± 24 b	140 ± 12 a	132 ± 26 a	139 ± 20 a
		48	266 ± 4 b	121 ± 13 a	154 ± 2 a	171 ± 1 a
	2-5	48	195 ± 17 b *	116 ± 9 a	129 ± 2 a	136 ± 2 a
Total N (g kg^{-1})	0-2	8	12 ± 1 b	8 ± 1 a	9 ± 1 a	9 ± 1 a
		12	10 ± 1 b	8 ± 1 a	7 ± 1 a	8 ± 1 a
		48	11 ± 0 b	6 ± 0 a	7 ± 1 a	8 ± 0 a
	2-5	48	8 ± 0 b *	5 ± 0 a	7 ± 0 ab	6 ± 0 a

3.4. Biological Properties

The values of the biochemical properties of the unburnt and burnt soils, 8, 12 and 48 months after the fire are shown in **Figure 3**.

The results of ANOVA 2 performed for the biochemical properties of samples collected at 0-2 cm depth indicated that the soil treatments explained 8-72% of variance and the sampling time explained 18-70% of variance, whereas

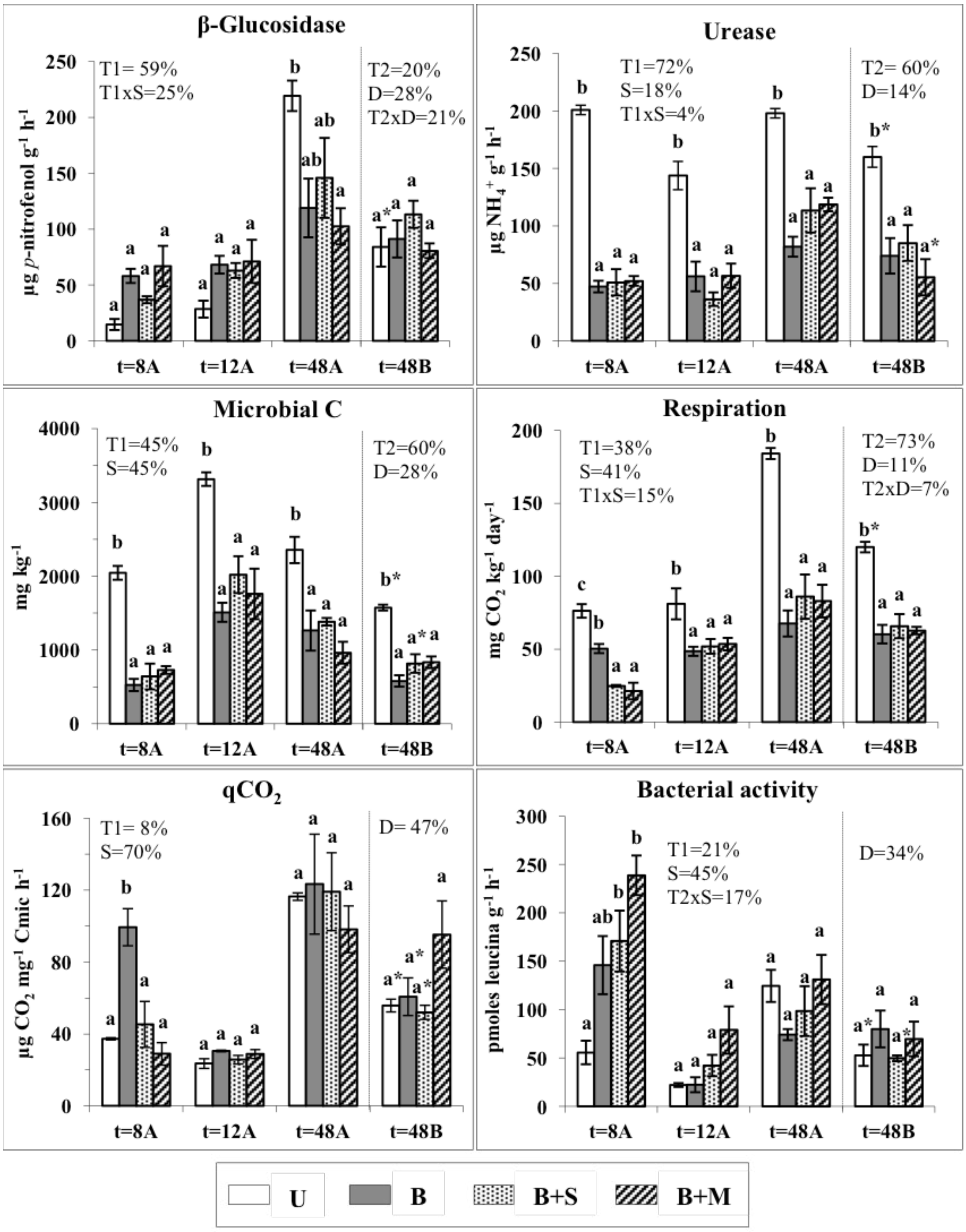

Figure 3. Soil biochemical properties in the different soil treatments 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques (mean values of three field replicates ± SE). Treatments: U, unburnt soil; B, burnt soil; B+S, burnt soil plus seeding; B+M, burnt soil plus straw mulching. Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected at 0-2 cm depth, according to an ANOVA 2 (T1, treatment; S, sampling time; T1xS, interaction treatment x sampling time). Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected 48 months after the fire, at two depths, 0-2 cm (A) and 2-5 cm (B) according to an ANOVA 2 (T2, treatment; D, depth; T2xD, interaction treatment x depth). For the same sampling time different lowercase letters denote significant differences ($p < 0.05$) among treatments. For samples collected 48 months after the fire, * denotes significant differences ($p < 0.05$) between different depths.

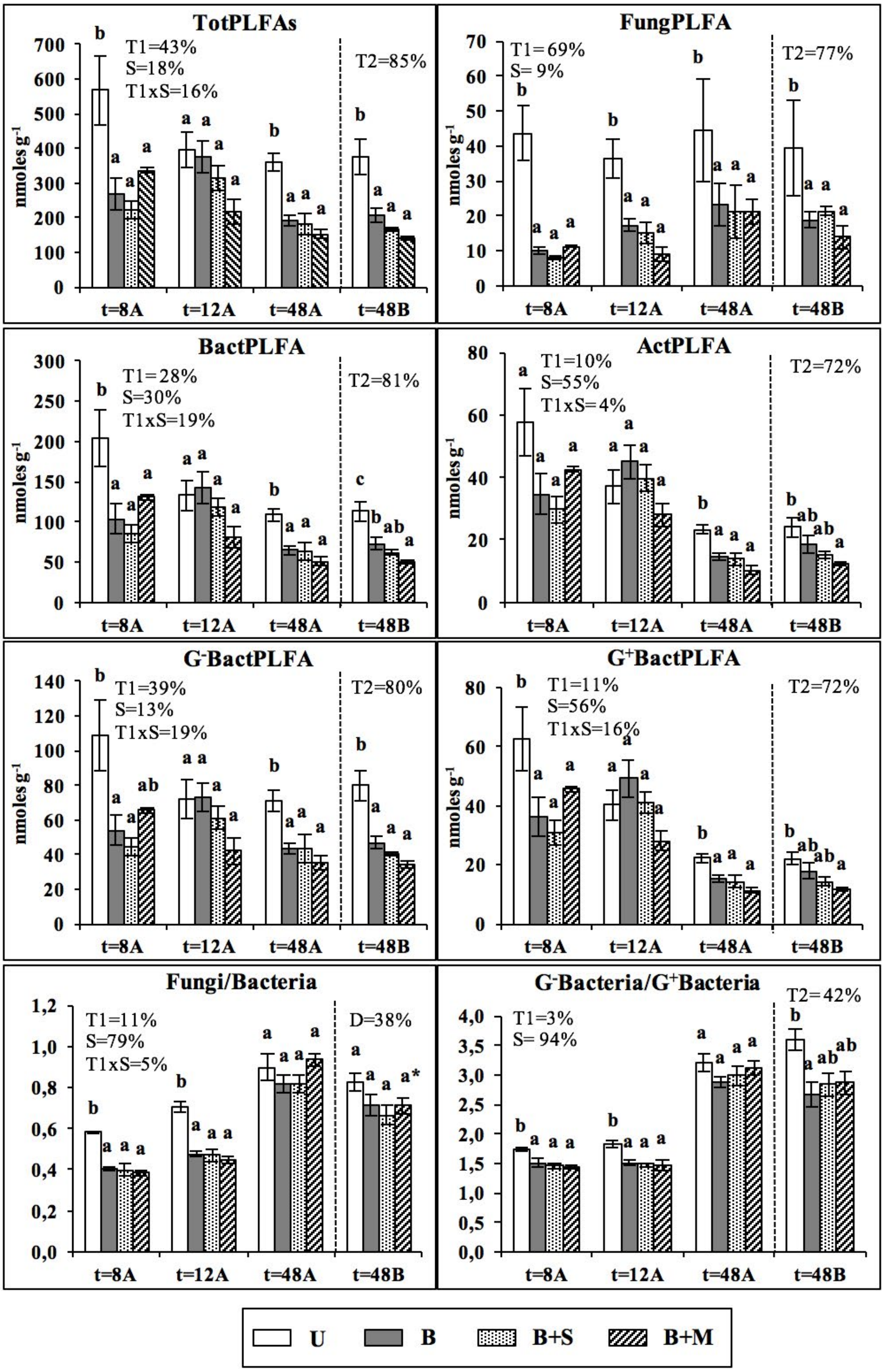

Figure 4. Total microbial biomass (TotPLFAs) and biomass of the specific groups: fungi (FungPLFA), bacteria (BactPLFA), actinobacteria (ActPLFA), G$^+$ bacteria (BactG$^+$PLFA), G$^-$ bacteria (BactG$^-$PLFA) and Fungi/Bacteria and G$^-$ bacteria/ G$^+$ bacteria ratios of the different study soils, 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques (mean values of three field replicates ± SE). Treatments: U, unburnt soil; B, burnt soil; B+S, burnt soil plus seeding; B+M, burnt soil plus straw mulching. Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected at 0-2 cm depth, according to an ANOVA 2 (T1, treatment; S, sampling time; T1xS, interaction treatment x sampling time). Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected 48 months after the fire at two depths, 0-2 cm (A) and 2-5 cm (B), according to an ANOVA 2 (T2, treatment; D, depth). For the same sampling time different lowercase letters denote significant differences ($p < 0.05$) among treatments. For samples collected 48 months after the fire, * denotes significant differences ($p > 0.05$) between different depths.

the interaction of treatments x sampling time explained only 4-25% of variance. During the study period, most biochemical properties were still disturbed 4 years after the fire but a different behaviour was observed depending on the property considered. For all sampling times a clear negative effect of the burning on urease activity, microbial C and soil respiration was observed. A similar trend was exhibited by these properties since in the B soil they did not recover and the magnitude of the reductions (40-63%) versus their values in the U soil still persisted 4 years after the fire. In general, an opposite behaviour of the fire was observed for bacterial activity and qCO_2, showing in the B soil values significantly higher than those in the U soil 8 months after the wildfire. This behaviour tended to disappear after one year and remained so for up to 48 months after the fire. For β-glucosidase activity a negative effect was found only 4 years after the fire. No significant effect of the mulching and seeding treatments versus the B soil was observed on the biochemical properties during the study period except on qCO_2, 8 months after the fire, showing values in the B soil significantly higher than those exhibited by B+S and B+M. It should be highlighted that this trend disappeared in the rest of the sampling times.

Likewise, the results of ANOVA 2 for samples collected 48 months after the fire at 0-2 and 2-5 cm depths also indicated an effect of the soil treatments, which explained 20-73% of variance, an effect of the depth that explained 11-47% of variance while the interaction of treatments x depth explained a further 7-21% of variance. In the 2-5 cm layer, a clear negative effect of the burning was observed on urease activity, microbial C and soil respiration 48 months after the fire, with the magnitude of the reductions being comparable to those observed in the surface layer. As in the surface layer, no significant effect of the stabilization treatments (seeding, mulching) versus the burnt control was observed in the 2-5 cm layer during the study period. Forty-eight months after the fire, a significant effect of the depth was only observed on all biochemical properties analyzed in the unburnt soil, on urease activity in the B+M treatment, on microbial C and bacterial activity in the B+S treatment and on qCO_2 in all treatments except the B+M treatment (**Figure 4**).

The total microbial biomass (TotPLFAs) data, as well as those of the different specific microbial groups (fungi, bacteria, actinobacteria, G^+ bacteria and G^- bacteria) of the unburnt and burnt soils, 8, 12 and 48 months after the fire, are shown in **Figure 4**. The results of ANOVA 2 for samples collected at 0-2 cm depth showed a significant influence of both the treatments and the sampling time on all variables analyzed, the treatments explaining 3-69% of variance and the sampling time 9-94% of variance, these factors not being independent since the interaction of treatment x sampling time explained a further 5-19% of variance. The TotPLFAs were positively and significantly correlated with the biomass of the specific microbial groups ($r = 0.999$, $p < 0.001$ for bacteria; $r = 0.787$, $p < 0.001$ for fungi; $r = 0.872$, $p < 0.001$ for actinobacteria; $r = 0.984$, $p < 0.001$ for G^- bacteria; $r = 0.855$, $p < 0.001$ for G^+ bacteria); therefore, a similar behavior was observed for these variables. The total microbial biomass, as well as that of the different specific microbial groups (fungi, bacteria, actinobacteria, G^+ bacteria and G^- bacteria) of the B soil versus the U soil were still diminished 8 months after the fire. In general, for the same sampling time the unburnt soil values were significantly higher than those of the burnt soil. These differences persisted after 48 months of the fire (**Figure 4**). The data also indicated that the fire tended to decrease the fungal/bacterial and the G^- bacteria/G^+ bacteria ratios; however, these decreases were attenuated 48 months after the fire. Comparison of the values of the studied variables in B+S and B+M treatments with those of the corresponding B soil showed that there was no effect of mulching and seeding on all these variables (**Figure 4**).

On the other hand, the results of ANOVA 2 for samples collected 48 months after the fire at 0-2 cm and 2-5 cm depths showed a significant effect of the soil treatments that explained 42-85% of variance and a depth effect for only the Fungi/Bacteria ratio that explained 38% of variance. An effect of the burning was observed in the 2-5 cm layer, whose magnitude was similar to that observed in the 0-2 cm layer. For all the studied variables, no effect of the B+S and B+M treatments compared with the B soil was observed at both depths. Similarly, no effect of the depth on these variables was detected, except for the fungi/bacteria ratio of the soil treated with mulching (B+M).

The PCA performed with the whole PLFAs data (**Figure 5**) showed that the main differences in the PLFA pattern were due to the sampling time. The first component that explained 45% of variance, mainly distinguished the samples collected at 48 months from the rest of the samples collected 8 and 12 months after the wildfire, although with the B+M treatment being consistently different from the B control also along this component (along PC1, $p < 0.001$ from ANOVA). The second component that explained 18% of variance was related to the wildfire effect, differentiating the unburnt samples (U8, U12 and U48) from the burnt ones. The samples collected 48 months after the fire with negative values along PC1, were mainly characterized by high concentrations of the 18:2ω6 and 18:1ω9 PLFAs, characteristic of fungi, and the monosaturated i17:1ω8, 18:1ω7 and 19:1a PLFAs. The unburnt samples, with negative values along PC2, were mainly characterized by high concentrations of the saturated fatty acids 16:0, 17:0 and 18:0. The data clearly indicated that fungi were the microbial group more affected by the wildfire, as indicated by the values of the fungal/bacteria ratio and the distribution of the variables along PC1 in the PCA (**Figures 4 and 5**).

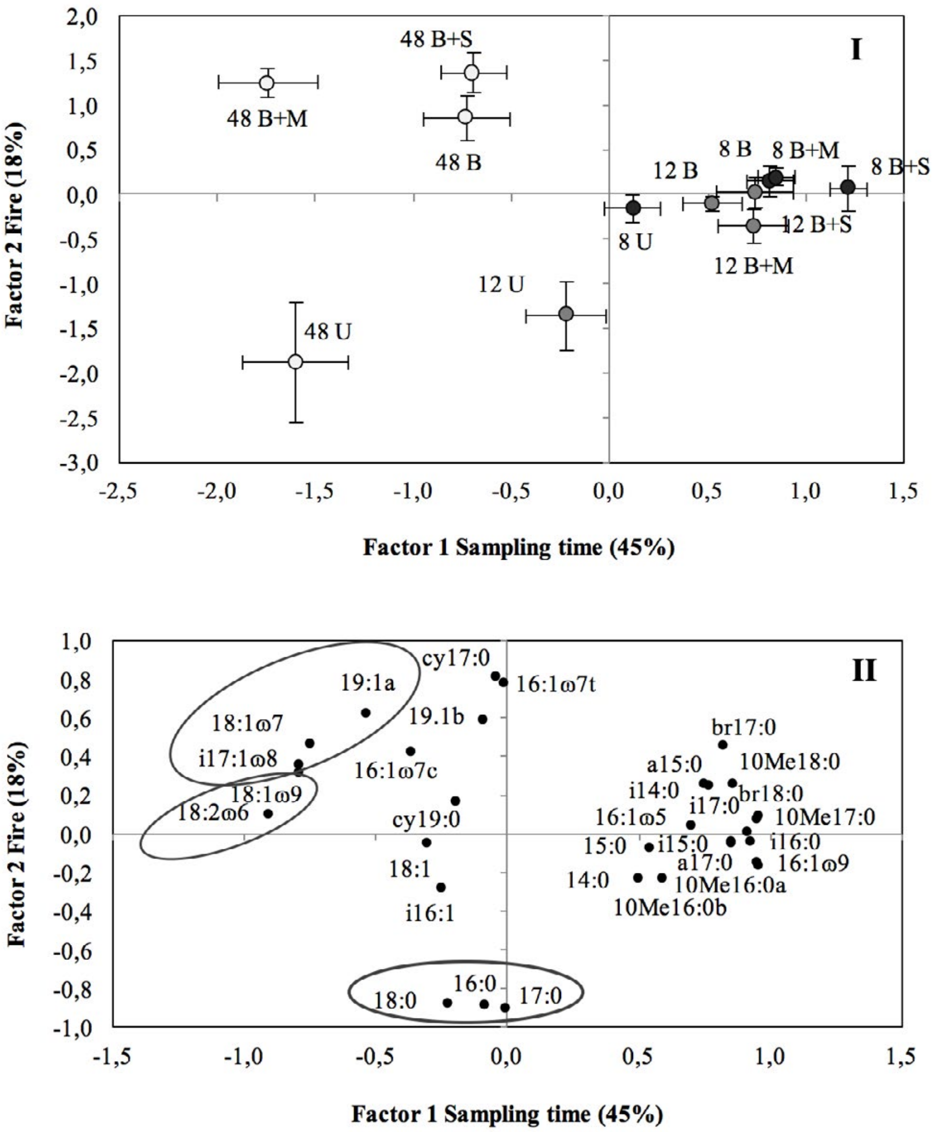

Figure 5. Results of the PCA performed for the whole PLFAs data set of the different soils collected 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques. Treatments: U, unburnt soil; B, burnt soil; B+S, burnt soil plus seeding; B+M, burnt soil plus straw mulching. Scores ± SE of three field replicates (I) and loading plots (II). To analyze in more detail the effect of the treatments at each sampling time, a PCA was also performed separately (**Figure 6**). Similar results were observed independently of the sampling time (8, 12 and 48 months) indicating that the main differences in the PLFA pattern were due to the soil burning.

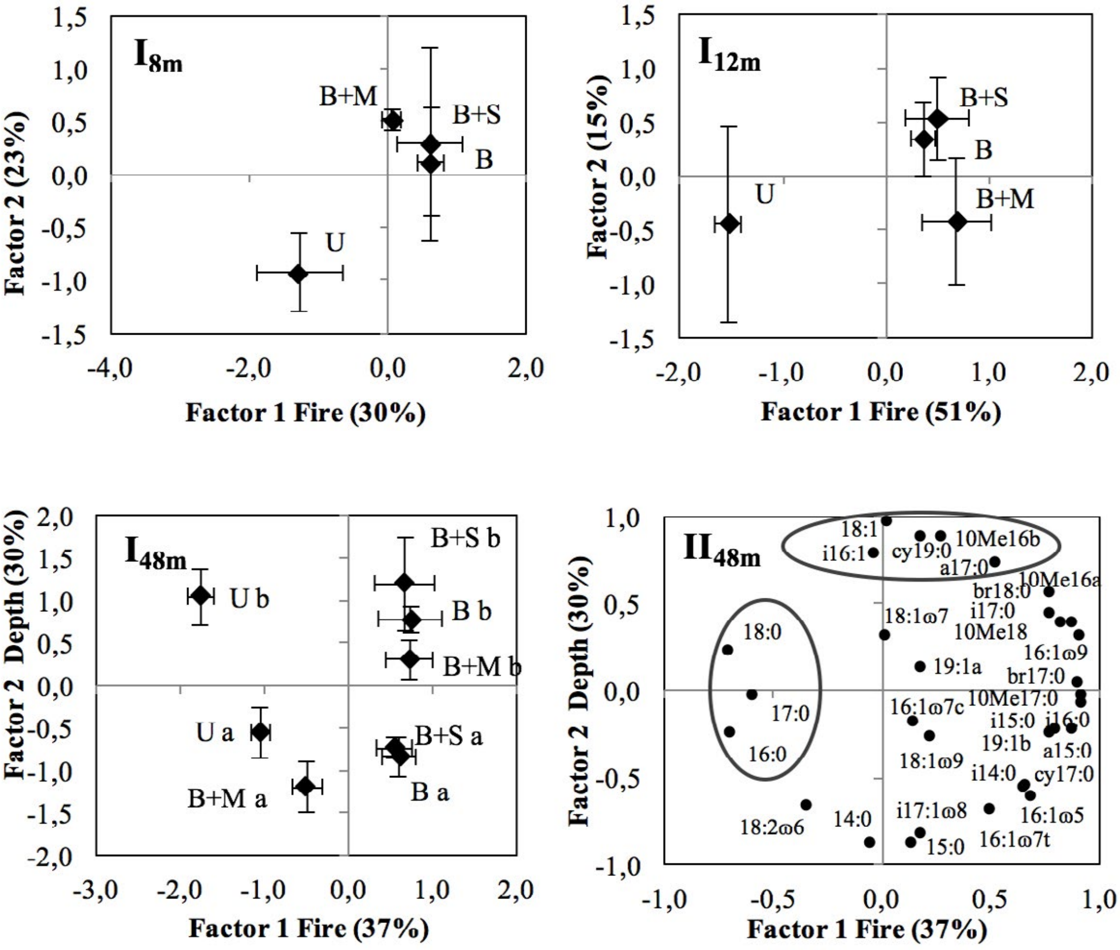

Figure 6. Results of the PCA performed for the PLFAs of the soils collected at different sampling times, 8, 12 and 48 months after the fire and application of the post-fire stabilization techniques. Treatments: U, unburnt soil; B, burnt soil; B+S, burnt soil plus seeding; B+M, burnt soil plus straw mulching. Scores ± SE of three field replicates (I) and loading plots (II). At 48 months, a and b correspond to 0-2 cm and 2-5 cm layers, respectively.

To analyze in more detail the effect of the treatments at each sampling time, a PCA was also performed separately (**Figure 6**). Similar results were observed independently of the sampling time (8, 12 or 48 months) indicating that the main differences in the PLFA pattern were due to the soil burning. For samples collected at 8 and 12 months after the fire, the first component, which explained 30-51% of variance, differentiated the unburnt samples from the burnt ones while the second component, explaining 15-23% of variance, differentiated the soil with mulching (B+M) from the other burnt soils (B, B+S). For samples collected 48 months after the fire, the first component, which explained 37% of variance, also separated the unburnt samples (U) from the burnt ones (B, B+S, B+M), whereas the second component, explaining 30% of variance, differentiated samples collected at 0-2 cm from samples collected at 2-5 cm depth. The unburnt samples, with negative values along PC1, were mainly characterized by high concentrations of the saturated 16:0, 17:0 and 18:0 PLFAs; and the samples collected at 2-5 cm depth, with positive values along PC2, were characterized by high concentration of the 18:1, cy19:0, i16:1, 10Me16:0b and a17:0 PLFAs. It should be highlighted that in the 0-2 cm layer the B+M treatment was different from the other burnt samples (B, B+S) along PC1 ($p < 0.01$ from ANOVA); however, this effect was not observed in the 2-5 cm layer (**Figure 6**).

4. Discussion

4.1. Effect of the Wildfire

The fire destroyed 100% of the vegetation cover in the studied soils. The recovery of the vegetation did not occur during the first 4 months (probably due to the unfavorable winter conditions) but took place mainly between the 8th and the 12th months after the fire, reaching a mean value of 38% of the vegetation cover one year after the fire, a value similar to that obtained by Barreiro et al. (2015) but lower than the 70% of vegetation cover registered by Vega et al. (2014) in experimental fires of the temperate humid zone. This behavior can be explained by the very low mean temperatures registered during the study period in the experimental plot area (-0.1 °C-1.3 °C between the 3th and 7th months after the fire and 6.7 °C–11.7 °C in the following months) as well as by the lower severity of the experimental fires. In our study the herbaceous cover represented 93-100% and 32-44% of the total vegetation cover the first 8 months and one year after the fire, respectively. *Luzula lactea* L. was the most abundant herbaceous species, particularly in the first 8 months after the fire, which could be attributed to the pioneer behavior of this type of plant whose rapid growth is favored by fire, the grasses *Festuca* spp. and *Agrostis* spp. following in importance. The shrub cover showed an opposite trend representing 4-7% and 56-68%, 8 months and one year after the fire, respectively, behavior that was observed by Vega et al. (2014) and Morgan et al. (2014) one and up to 6 years after the fire, respectively. *Pterospartum tridentatum* Willk., a strong re-sprouter, *Erica* spp., a germinator (Reyes et al. 2009) and *Cistus* spp. were dominant shrub species in all burnt soils one year after the fire, which was not surprising since they are the most common shrub species in the unburnt soil, although in this last one *Erica* spp. was the most abundant species (data not shown). This is line with data of Dubuy and Vallejo (2008), who consider that in a burnt Mediterranean area the vegetation formations are dominated by the sprouting species, which are more resilient to fire than those dominated by germinator plants.

At the end of the study period, 4 years after the fire, all burnt soils reached values of 60-80% of vegetation cover (data not shown). Earlier investigations (Fernández and Vega 2014b; Fernández et al. 2016a) showed that two years after wildfires, the vegetation had reached values equivalent to those reported for unburnt shrubland areas in the NW Spain, possibly reflecting both the re-sprouting capability of shrub species and favorable post-fire conditions. In our study, high temperatures were reached during the fire and therefore the pines died, the rest of the vegetation was destroyed and later on the shrubland exhibited lower growth and different species composition, which could also be associated with different soil characteristics. This indicates that although the vegetation cover recovers, it will need much more than 4 years to reach the same development as in the unburnt soil (Robichaud et al. 2013; Morgan et al. 2014).

A short-term study performed in the same experimental plots revealed that immediately after the wildfire, the combustion caused a marked decrease in the content of SOM and related properties (soil moisture, WRC), increases in soil pH, EC, and sand fraction, accompanied by decreases in the clay and silt fractions, and no changes in the aggregate stability and water repellency (Díaz-Raviña et al. 2012). The results obtained in our study 8, 12 and 48 months after the fire indicated that in most of the physical, physicochemical and chemical properties analyzed, the initial changes induced by the wildfire (Díaz-Raviña et al. 2012) were still maintained after 4 years, except in the soil texture, which tended to recover its initial granulometric composition (**Table 2**). The SOM labile fractions (WSC, HWC, WSCH and HWCH) were drastically reduced in the burnt soils during the study period, mainly due to observed changes in vegetation and microbial activity (Nannipieri et al. 2003; Certini 2005) and post-fire erosion processes (Gómez-Rey et al. 2013). These labile fractions did not reach the values of the unburnt soil 4 years after the fire, which confirms what has been reported for cellulosic and non-cellulosic carbohydrates of forest soils affected by wildfires of different severity, located in the same area, in the short-term (Martín et al. 2009), and for microbial and extractable C and N in the medium-term (Prieto-Fernández et al. 1998).

In the short-term study cited above, the microbial biomass, urease and bacterial activity values were markedly reduced immediately after the passage of the fire while the respiration and qCO_2 increased and the β-glucosidase activity was not affected. Our results obtained at 8, 12 and 48 months after the fire showed that most biochemical properties were still modified 4 years after the fire and depending on the soil property considered, a different behavior was observed. The microbial C, the soil respiration and the urease activity did not recover, the bacterial activity and qCO_2 did, while the β-glucosidase activity showed a variable and inconsistent trend (**Figure 3**). This behavior can be explained on the basis of the different information obtained from the diverse microbial indices (overall microbial biomass, overall metabolic activity, specific enzyme activities) and also to the different sensitivity of the analyzed parameters to detect the impact of fires (Certini 2005; Mataix-Solera et al. 2009; Díaz-Raviña et al. 2010; Lombao et al. 2015a,b). According Eivazi and Bayan (1996) lower temperatures than those reached in different soils after repeated prescribed burnings for several years have a long-term accumulative effect on the microbial C and the soil enzymes related to the cycles of C, N and P but did not affect the pH and organic C. The fact that the burning effects were observed on the labile fractions of SOM (WSC, HWC, WSCH, HWCH) and the biochemical properties, such as urease, Cmic and soil respiration, showed that these properties are the best indicators in the medium-term of the changes in the soil quality induced by fire.

The total microbial biomass, estimated by PLFA, and the biomass of specific microbial groups were reduced immediately after the fire (Díaz-Raviña et al. 2012) and as shown by our results this effect still persisted 4 years later (**Figure 4**). Immediately after the fire, the burnt soils exhibited lower Fungi/Bacteria and G^- bacteria/G^+ bacteria ratios than those in the corresponding unburnt soils, showing that the fungi and the G^- bacteria have a higher sensitivity to heating and/or they did not proliferate following post-fire conditions (Carballas et al. 2009; Bárcenas-Moreno et al. 2011; Holden et al. 2013; Barreiro et al. 2015). The trend of these ratios disappeared 4 years after the fire (**Figure 3**). Our results of the biomass of specific groups and the PLFA pattern showed that fungi play an important role in the recovery of these burnt soils since high concentrations were obtained in samples collected 48 months after the fire (**Figures 5 and 6**). The importance of fungi can be explained because they contribute much more to the microbial biomass than bacteria, increase soil C sequestration (Six et al. 2006) and soil aggregation (Helfrich et al. 2015), which will be especially important after a high severity forest fire when the C is drastically reduced and both soil structure and soil microbial biomass are strongly affected.

In general, a significant effect of the sampling time was also observed for the soil properties analyzed that was more accentuated for most labile fractions of the SOM (Cmic, WSC and WSCH) and the enzyme activities. These results are in accordance with marked seasonal fluctuations observed in properties of different forest soils located in the same area, which are associated with above ground vegetation effects and variation in climatic conditions (Díaz-Raviña et al. 1993, 1995; Martín et al. 2011). However, it should be highlighted that the changes induced by the sampling time were of a minor order of magnitude compared to the changes induced by the wildfire as is clearly indicated by the percentages of variance explained by ANOVA 2. Therefore, taking into account the magnitude of the changes and the spatial and temporal variation of most chemical and biochemical properties, the combined interpretation is that the soil conditions did not recover 4 years after the fire. This is consistent with data from other authors, who found that the recovery of soil microbiota affected by wildfires (Prieto-Fernández et al. 1998) and prescribed burnings (Fritze et al. 1993) occurs between 10 and 13 years after the fire.

In the unburnt samples, a depth effect was observed on total C, pH, labile fractions of

SOM (WSC, HWC, WSCH, HWCH) and all biochemical properties analyzed (β-glucosidase, urease, Cmic, respiration, qCO_2 and bacterial activity), the effect being more accentuated in the properties related to the activity of soil microorganisms (**Table 2, Figures 2 and 3**). For most soil properties analyzed (physical, chemical, biological) no effect of depth was generally observed for all burnt samples (B, B+S, B+M), likely due to the fact that the fire tends to homogenize the surface (0-5 cm) of the soil samples as well as to the removal of the burnt soil surface (wind, lixiviation, erosion). In contrast, a depth effect was observed on qCO_2 in B and B+S treatments, on Cmic and bacterial activity in B+S treatment and on urease and Fungi/Bacteria ratio in B+M treatment. The fire modified these properties in the 0-5 cm depth but was more noticeable in the first 2 cm, which support the data of other authors (Certini 2005; Zhan et al. 2005) showing that the burning could influence the enzyme activities and chemical properties of soil not only of the upper layers but also of the lower ones.

In summary, compared with the corresponding unburnt soil, significant reductions in the values of most physical, physicochemical, chemical and biochemical properties as well as changes in the microbial community structure were observed 4 years after the fire. This can be explained by the high temperatures reached during the fire as well as by unfavorable post-fire conditions (climate, vegetation, soil environment, erosion) and is consistent with studies performed in the same area by several authors (Prieto-Fernández et al. 1998; Carballas et al. 2009; Martín et al. 2009; Lombao et al. 2015a) showing a reduction of the soil quality and therefore a degradation of the soil following high severity wildfires. Therefore, effects on both soil and plants are concordant, indicating lasting changes induced by the wildfire on the soil-plant system 4 years after the fire event.

4.2. Effect of Post-Fire Stabilization Treatments

The vegetation recovery reached values between 36% and 40% one year after the fire in all burnt soils (B, B+S, B+M). However, during the first 8 months, as compared with the burnt treatment (B), the seeding had only a slight positive effect on the vegetation cover. This was probably because the germination percentage of the sown rye seeds was low due to the low temperatures registered during the period of germination and growth of the rye, or to the low dose of rye applied (10 $g \cdot m^{-2}$) in our case compared to the doses used by other authors (30-35 $g \cdot m^{-2}$) (Albadalejo-Montoro et al. 2000; Badía and Martí 2000) or to the competence with native plants (Beyers 2009). Our results are consistent with those obtained in the same area by Barreiro et al. (2015) in a shrubland affected by an experimental fire and application of the same treatments at similar doses as well as with other studies (Kim et al. 2008; Vega et al. 2014) showing neutral effects of seeding and mulching on the vegetation cover. In contrast, Fernández et al. (2016b) found that the straw mulching favored the recovery of the plant cover in the first year after the fire by conserving the soil moisture. Nevertheless, in our study, the mulching showed lower vegetation regeneration (< 10% of vegetation cover during the first 8 months) and the same occurred in B+S at the end of the study period. During the first year after the fire, the vegetation regeneration in the mulching treatment increased from 5% to 40% while the ground cover by the straw decreased from 90% to 53%, likely due to the natural regrowth of vegetation cover, the straw incorporation into soil and its removal by wind and overland flow (Wagenbrenner et al. 2006). A similar behavior was observed by Badía and Martí (2000) although two years after the fire and application of the treatments. However, the total ground cover in the mulching treatment remained 85-93% lower than those of Badía and Martí (2000) but it was effective to protect the soil in the B+M treatment (Robichaud et al. 2010).

In general, our results showed that the mulching and seeding did not seem to affect the plant diversity of the burnt soils in the first year after the fire although other researchers reported different effects of these treatments on the vegetation recovery and the plant community composition in the semiarid and temperate-humid zones of Spain (Badía and Martí 2000; Fernández et al. 2016b) and in soils of USA (Wagenbrenner et al. 2006; Morgan et al. 2014). A marked and significant effect of the time elapsed since the fire on the recovery of the vegetation cover and the plant community composition was observed independently of the treatment applied, showing a continuous increase of the total vegetation cover and the shrub cover, accompanied by a reduction of the herbaceous cover. This may be related to the soil availability of nutrients, especially N, after the fire, which can lead to the displacement of some plant species by other ones (Morgan et al. 2014).

Most of the soil surface was bare in the burnt soil during the first 4 months following the wildfire and therefore the vegetation could not mitigate the impact of the rainfall erosivity. The total precipitation during the first year after the fire was 1055 mm, distributed as follow: 468 mm in the first 4 months, 398 mm between the 4th and the 8th months, and 189 mm between 8th and 12th months. The total rainfall produced between the 4th and the 8th months was quite similar to that produced during the first 4 months after the fire; however, the sediment yields were very different (36 $g \cdot m^{-2}$ versus 204 $g \cdot m^{-2}$, respectively) (**Figure 1**). This seems to indicate that the sediment yield was not related to the total rainfall. After one year the accumulated soil loss from the burnt soil was 249 $g \cdot m^{-2}$ of sediment, a value that lies in the reported range for burnt soils of the temperate-humid zone using experimental plots of similar size (Díaz-Fierros et al. 1987; Vega et al. 2013). For all treatments, the accumulated sediment yield during the period between 0 and 12 months after the fire was similar to that obtained in the first 4 months (**Figure 1**). Cumulative sediment yield values corresponding to the burnt soils in the first year after the fire indicated that the mulching treatment was the most effective for controlling post-fire erosion since, compared to the burnt control, the soil losses were reduced by 86% whereas the seeding reduced the soil losses only by 33% (**Figure 1**).

Treatment effectiveness and especially soil loss diminished over time, particularly when the vegetation cover reached around 40%. In the medium term, between the 4th and 12th months after the fire, the mean efficiency for seeding and mulching was 11% and 71%, respectively, while in the short term, during the first 4 months, higher values were obtained (37% for seeding and 89% for mulching). Our efficiency values of the soil stabilization treatments fall within the reported values obtained in different contexts such as fire-affected areas, agricultural lands, rangeland and anthropic sites (Badía and Martí 2000; Fernández et al. 2012, 2016a; Prats et al. 2012; Fernández and Vega 2014a; Vega et al. 2014, 2015; Prosdocimi et al. 2016) and are, in part, concordant with the results for the vegetation cover obtained during the present study (18-40% from the 4th to the 12th months and lower than 12% during the first 4 months). The lower sediment yield in B+M relative to the B control plots was attributed to the immediate protection of soil, with around 90% ground cover in the mulch plots. Likewise, the lower sediment yield produced in B+S between 4 and 12 months after the fire can be explained by the protection of the vegetation cover and by the fact that, according to Díaz-Raviña et al. (2012), in the temperate humid zone (Galicia, NW Spain) the erosion is mainly produced by the saturation excess in the form of overland flow, and as the maximum WRC was not reached, the erosion was more reduced than should be expected according to precipitation values. We consider these results as an important contribution for land managers. For instance, when the natural ground cover with needles from the dead burnt pines is around 40% or higher, the implementation of seeding or mulching should not be done due to the high cost/benefit ratio, as was stated by Cerdá and Doerr (2008) and Prats et al. (2016). In addition, in our study seeding is not recommended due to its low efficiency as compared with that of mulching. Post-fire seeding ineffectiveness can be due to the steep slope (seeds can be washed away by water) and to the failure of germination and the establishment of seeded plants (unfavorable conditions such as temperature, humidity, etc.). This is also consistent with data of Beyers (2009), who point out that although non-native annual and perennial grasses commonly have been used to provide temporal ground cover until native plants are re-established, grass seeding compete with native vegetation and often do not effectively reduce the erosion.

The physical, physicochemical and chemical properties were not affected by seeding and mulching (**Table 2**), as happened in the same experimental area at the short term (4 months) (Díaz-Raviña et al. 2012). The same trend was observed by Gómez-Rey and González-Prieto

(2014) one year after the fire in the contents of macronutrients (N, P, Ca, Mg and K) and extractable trace elements (Al, Fe, Mn, Cu, Zn, Co and Bo). Likewise, in line with this, no effect of mulching regardless of its application form and dose were found for most physicochemical and chemical soil properties, including the labile C pools (WSH and WSCH) (Lombao et al. 2015b), which are considered to degrade rapidly and may be an immediate source of energy for microorganism (Nannipieri et al. 2003). However, in a study performed in a prescribed fire, Gómez-Rey et al. (2013) found that mulching and seeding had slight but significant positive effects on $\delta^{15}N$ and extractable K, Mg and Ca.

The analyzed biochemical properties of burnt soils were not affected in the medium-term by the stabilization treatments (**Figure 3**), as occurred at the short-term (Díaz-Raviña et al. 2012); however, an influence of the sampling time was observed on most of them. The results are also consistent with other studies in Galician soils developed under scrub, oak or pines that have suffered controlled fires and wildfires of different severity to which seeding, straw mulching (independently of the form of application), bark mulching and log erosion barriers have been applied (Fernández et al. 2011; Fontúrbel et al. 2012, 2015; Lombao et al. 2015a). These studies, after monitoring for 1-3 years, showed a higher effect of the fire than that of the post-fire rehabilitation techniques on the microbial biomass and activity as well as a great variability of these properties with the sampling time (Fontúrbel et al. 2015; Lombao et al. 2015b). This behavior is related, to a large extent, to the meteorological conditions (temperature, humidity), the availability of the substrate from the plant remains, and the vegetation. In contrast, in a study performed in the same experimental plots, Gómez-Rey and González-Prieto (2015) found a significant effect of both sampling time and mulching treatment on gross N mineralization and NH_4^+ immobilization rates of the soil.

The results of the PLFA pattern clearly showed that the main factor determining the composition of the microbial communities was the time elapsed after the wildfire, followed in order of importance by the fire and in a much lesser extent by the soil depth. In general, the results also showed the absence of any medium-term response of the microbial community to mulching and seeding. It should be highlighted, however, that the microbial community composition in the B+M soil at 0-2 cm depth was different than those exhibited by B and B+S soils four years after the fire. This is consistent with above results showing a significant effect of depth on urease and Fungi/Bacteria ratio in the B+M treatment likely due to the incorporation of straw in the first 0-2 cm depth, as well as with the decline of the straw mulching coverage found in the B+M treatment. In line with these results, several authors showed that mulching could affect the soil microclimate (soil moisture and temperature) (Bautista et al. 2009; Ferreira et al. 2015; Fernández et al. 2016b) and the C and N availability (Huang et al. 2008), which in turn can affect the microbial community structure. In fact, it has been showed that the incorporation of plant material with higher C/N ratio into the soil, such as wheat straw used as mulching, is more favorable to fungal than to bacterial growth (Rousk and Bååth 2007; Barreiro et al. 2016). Our PLFA data also seem to indicate that in the medium-term the vegetation rather than the fire was a key factor in determining the evolution of the soil microbial communities since initially the vegetation was totally destroyed and the natural regrowth increased progressively to reach a vegetation cover of 36-40% and 60-80% (data not shown), 12 and 48 months after the fire, respectively (the performed PCA clearly differentiated samples collected at 48 months from the rest of the samples; **Figure 5**).

The vegetation is crucial for soil formation and differences in plant residues quality and quantity (Hart et al. 2005), including root exudates (Grayston et al. 1998), can influence different soil properties such as C, N and P content and pH. Therefore, the role of the vegetation, litter and vegetation debris, as a source of C to the soil, can determine the quantity and quality of the SOM and can condition soil microbial biomass, activity and diversity (Wardle 1992; Grayston et al. 1998; Garveva et al. 2004). This is consistent with the post-fire vegetation dynamics as drivers of the soil microbial community structure and function (Hart et al. 2005). In other words, changes in the plant community structure in the years following the fire constitute a more dominant driver of soil microbiota than the wildfire itself. Bárcenas-Moreno et al. (2016) also found that the post-fire microbial recolonization process was different depending on the plant community studied. In the same line, Barreiro et al. (2016) in a recent laboratory study showed a clear effect on bacterial and fungal growth, particularly the latter, as well as on the PLFA pattern of a soil with different incorporated chopped materials (wheat straw, coconut fiber, *Eucalyptus* bark and wood chips). However, our PLFA pattern also differed due to the wildfire (the burnt soils were separated from the corresponding U soil) and even the stabilization treatments (the B+S soil was closely grouped with the corresponding B soil and was clearly separated from B+M) (see **Figures 5 and 6**). The clear differences observed between the unburnt and burnt soils in the PLFA pattern could be related to differences in the soil pH, the quantity and quality of the SOM, soil microbial properties and post-fire vegetation recovery. This is consistent with other studies performed in the same zone showing a clear effect of both prescribed fires and wildfires on the PLFA pattern (Díaz-Raviña et al. 2006, 2012; Barreiro et al. 2010, 2016; Lombao et al. 2015a). In general, in our study no effect of the depth was detected on the chemical and biochemical properties and PLFAs biomass of the burnt soils (B, B+S, B+M); nevertheless, marked and significant differences with soil depth were observed in the physicochemical, chemical and biochemical properties but not in the PLFAs biomass of the U soil. However, a depth effect was observed on the microbial structure (PLFA pattern), which is in agreement with other studies (Fritze et al. 2000; Fierer et al. 2003; Lombao et al. 2015a; Barreiro et al. 2016); this is related to the decrease of the SOM content that is determinant for soil microorganisms (Fritze et al. 2000; Martinizadeh et al. 2008) and, in a lesser extent, with variations in soil different niches (aeration, moisture, temperature, pH, available C and nutrients, etc.) (Nannipieri et al. 2003; Certini 2005). The data clearly showed the sensitivity of the PLFA analysis to detect the impact of different soil disturbances (Frostergåd et al. 2011) as well as its usefulness as an integrative tool to analyze the relative importance of different factors considered (fire, post-fire stabilization treatments, time, depth) on the microbial community composition and hence on the soil quality of these burnt soils. This lipid biomarkers technique is a rapid, inexpensive and both sensitive and reproducible analysis to explore environmental effects on soil microbiota. Therefore, we consider that PLFA analysis can be a good routine and complementary tool for helping land managers to take decisions concerning the implementation of determined forest practices on burnt soil ecosystems.

5. Conclusions

The vegetation cover was destroyed by the fire and the plant regeneration increased progressively until a 40% cover of the burnt soil surface was reached one year after the fire, the herbaceous and the woody species being dominant at short- and medium-term, respectively. Both seeding and mulching, especially the latter, were effective at reducing post-fire soil erosion at medium-term although their magnitude was lower than that observed during the first 4 months after their application. One year after the fire, the effectiveness of seeding and mulching to reduce post-fire erosion was 33% and 86%, respectively. In the medium term, 4 years after the fire, the initial soil quality had not recovered as indicated by the values of the whole set of physical, chemical and biological soil properties analyzed. The main factor determining the composition of the microbial communities was the time elapsed after the wildfire, followed in order of importance by the fire and to a much lesser extent by the soil depth. The PLFA pattern showed that the fire may modify the soil quality by altering the composition of the plant community via plant-induced changes in the soil environment due to the close relationships between the above (plant communities) and below ground communities (soil microbial communities) living in the burnt soil. In general, seeding and mulching did not modify the vegetation cover or the soil quality. However, the microbial community composition (PLFA pattern) of the soil treated with mulching at 0-2 cm depth was different from the burnt soil community and this effect was likely positive. Therefore, taking into account the absence of negative effects on the soil quality as well as its efficiency, mulching is the recommended rehabilitation technique.

6. Acknowledgements

This study was supported by the Consellería de Medio Rural de la Xunta de Galicia (08MRU-002400PR) and by the Ministerio de Ciencia e Innovación (AGL2008-02823), Spain. A. Barreiro and A. Lombao are recipients of FPU grants from Spanish Ministry of Education (AP2010-2284, AP2010-1900). The authors wish to acknowledge to Mª Cruz Mato for helping in identifying some plant species.

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