Effects of prescribed fire for pasture management on soil organic matter and biological properties: A 1-year study case in the Central Pyrenees

Antonio Girona-García[1*]; David Badía-Villas[1]; Clara Martí-Dalmau[1]; Oriol Ortiz-Perpiñá[1]; Juan Luis Mora[2]; Cecilia M. Armas-Herrera[1]

[1]Departamento de Ciencias Agrarias y del Medio Natural, Escuela Politécnica Superior de Huesca, Instituto de Investigación en Ciencias Ambientales (IUCA), Universidad de Zaragoza, Ctra. Cuarte s/n, 22071, Huesca, Spain

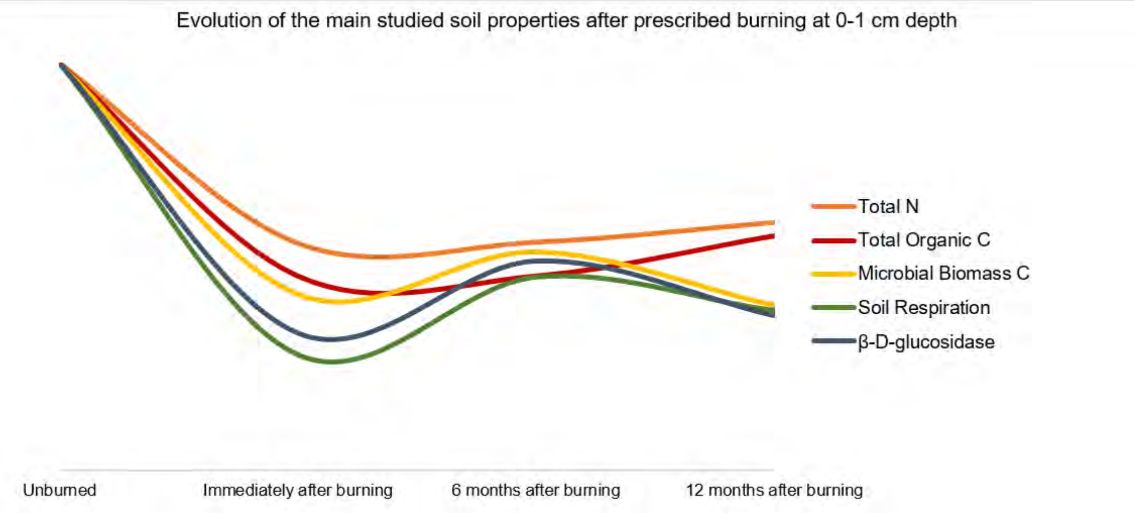
[2]Departamento de Ciencias Agrarias y del Medio Natural, Facultad de Veterinaria, Instituto de Investigación en Ciencias Ambientales (IUCA), Universidad de Zaragoza, C/ Miguel Servet 177, 50013, Zaragoza, Spain.

*Corresponding author: Antonio Girona-García. Departamento de Ciencias Agrarias y del Medio Natural, Escuela Politécnica Superior de Huesca, Instituto de Investigación en Ciencias Ambientales (IUCA), Universidad de Zaragoza, Ctra. Cuarte s/n, 22071, Huesca, Spain. E-mail: agirona@unizar.es . Telephone: +34 974292664

Highlights

- Prescribed burning is adopted as a tool to remove shrubs and recover pasturelands
- Burning effects were evaluated at 0-1, 1-2 and 2-3 cm mineral soil depth
- Fire severely affected the studied soil properties mainly at 0-1 cm depth
- No recovery on soil properties was observed 1 year after burning
- Further research is needed in order to assess the sustainability of this practice

Graphical Abstract



Abstract

Prescribed burning has been readopted in the last decade in the Central Pyrenees to stop the regression of subalpine grasslands in favour of shrublands, dominated among others by *Echinospartum horridum* (Vahl) Rothm. Nevertheless, the effect of this practice on soil properties is uncertain. The aim of this work was to analyse the effects of these burnings on topsoil organic matter and biological properties. Soil sampling was carried out in an autumnal prescribed fire in Buisán (NE-Spain, November 2015). Topsoil was sampled at 0-1 cm, 1-2 cm and 2-3 cm depth in triplicate just before (U), ~1 hour (B0), 6 months (B6) and 12 months (B12) after burning. We analysed soil total organic C (TOC), total nitrogen (TN), microbial biomass C (C_{mic}), soil respiration (SR) and β-D-glucosidase activity. A maximum temperature of 438°C was recorded at soil surface while at 1 cm depth only 31°C were reached. Burning significantly decreased TOC (-52 %), TN (-44 %), C_{mic} (-57 %), SR (-72%) and β-D-glucosidase (-66 %) at 0-1 cm depth while SR was also reduced (-45 %) at 1-2 cm depth. In B6 and B12, no significant changes in these properties were observed as compared to B0. It can be concluded that the impact of prescribed burning has been significant and sustained over time, although limited to the first two topsoil centimetres.

Key words: prescribed burning, soil organic matter, shrub encroachment, subalpine grassland, soil biological activity

1. Introduction

Livestock grazing has been a key factor in the traditional pasture management in the Central Pyrenees (NE-Spain) (San Emeterio et al., 2014). However, in the past decades, this activity has decreased due to changes in socio-economic conditions such as rural exodus and the reduction of stocking densities (Komac et al., 2013). In the present times, most of the European pasturelands are linked to mountain systems (Lasanta, 2010) and specifically in the Central Pyrenees, these lands occupy an approximate surface of 600,000 ha (Caballero et al., 2010). The mesophytic pastures that can be found in the Pyrenean mountains are composed by subclimax species that require grazing for its survival against shrubs (Halada et al., 2011). Therefore, the reduction in grazing can lead to a shrub encroachment that, in the Pyrenees, has remarkably increased by woody species such as the thorny cushion dwarf (*Echinospartum horridum* (Vahl) Rothm). This species can colonize several hectares forming large and dense monospecific patches that only let few other species survive in small gaps (Komac et al., 2011). Although encroachment is a natural stage in the grassland conversion to forest and plays an important role in pedogenesis, it entails a threat to biodiversity, pasture potential and flammability risk (Caballero et al., 2010).

In order to stop the regression of subalpine grasslands in favour of shrublands, prescribed burning has been readopted in the last decade in the Central Pyrenees. Prescribed burning can be defined as the planned use of fire to achieve precise and clearly defined objectives, which represents a more suitable and less risky practice than the non-regulated traditional agricultural burning (Fernandes et al., 2013). Furthermore, its use is less expensive and more practical in this type of landscape than the mechanical procedures (Goldammer & Montiel, 2010). Nevertheless, fire can affect most of soil physical, chemical and biological properties (Certini, 2005; Mataix-Solera et al., 2011), specially soil organic matter (SOM) and microorganisms (González-Pérez et al., 2004; Mataix-Solera et al., 2009). The extent and duration of burning effects depend mainly on fire severity, i.e. its intensity and duration which are highly influenced by the environmental parameters that determine the combustion process (Certini, 2005). For this reason, prescribed burning is carried out under favourable conditions of soil and fuel moisture, temperature and topography (Molina, 2009) in which the impact on soil is low (Vega et al., 2005). These factors can be very variable so a high heterogeneity is reported in the studies dealing with prescribed fire effects on soil properties. Prescribed burning can produce no effects (Alexis et al., 2007; Goberna et al., 2012; Fultz et al., 2016) or increase organic C and N content (Úbeda et al., 2005; Alcañiz et al., 2016) due to the incorporation of partly charred material or litter (González-Pérez et al., 2004). On the other hand, Armas-Herrera et al. (2016) observed a remarkable decrease in SOM after a *E. horridum* prescribed fire. Dooley & Treseder (2012), after a meta-analysis concluded that the impacts of prescribed fire on soil microbial biomass amount are negligible, although they can induce changes in fungal abundance and diversity. This is of vital importance since microbial biomass is the main factor driving SOM turnover rates and, therefore, regulates the C transfer between soil and the atmosphere (Knicker, 2007; Dooley & Treseder, 2012).

Grasslands, defined as ecosystems in which the dominant vegetation is composed by herbaceous species (Jones & Donelly, 2004), are of great ecological value since they provide food for livestock, habitat for wildlife and improve soil quality and productivity (Follett & Reed, 2010; Saha & Butler, 2017), storing 10-30 % of global SOM (Eswaran et al., 1993). Management practices such as burning can influence soil C sequestration since they can alter the rates of SOM inputs, its composition and how it is incorporated into the soil (Jones & Donelly, 2004; Follett & Reed, 2010). Therefore, knowing the role that these practices play in C cycle is of special interest in the context of climate change. Additionally, information regarding prescribed fire effects for pasture improvement in subalpine environments on soil properties is scarce (San Emeterio et al., 2014; Armas-Herrera et al., 2016)

We hypothesized that given the high soil water content and the low fire intensity that characterises prescribed burning, there would be a low affection on C-related soil properties and in a limited depth. We also argued that the probable low effect of burning would disappear in the short-term due to vegetation recovery. The general aim of this study was to analyse the effects of *E. horridum* prescribed burning for pasture reclamation on topsoil SOM and biological properties and their evolution in the short-term in the Central Pyrenees. Specifically, the effects on: soil total organic C and N, microbial biomass C, basal respiration and β-D-glucosidase activity; immediately, 6 months and one year after the prescribed burning.

2. Material and methods

2.1. Area of study

The area of study comprises 3.8 ha in Buisán, Central Pyrenees (NE-Spain; 42°36’04.4” N 0°00’43.3” E) at 1760 masl dominated by *E. horridum* where the mean annual temperature is 5.7 °C and the mean annual precipitation 1270 mm. The average slope is 12-30 % facing south (SE to W). Soils in the study area range from Eutric to Calcaric Cambisols (IUSS Working Group WRB, 2014) and the main properties of a representative soil profile are given in Table 1. The study area is located in a zone with great pastoral value in the limit of Ordesa and Monte Perdido National Park. In the past, more than 20,000 sheeps pastured these lands, while at the present times, this number has decreased below 10,000 animals. Until 1980, shepherds eliminated the incipient *E. horridum* by small burnings but due to the prohibition of fire use in that decade, the area has been invaded by this species. The study site is nowadays occupied during summer by shepherds that still practice transhumance to the flat lands of the Ebro Valley. This guarantees pastoral pressure and cattle trampling in the plot.

Table 1. Chemical and physical soil properties of the study area (Eutric Cambisol)

Horizon	Ah$_1$ (0-5 cm)	Ah$_2$ (5-15 cm)	Bw$_1$ (15-25 cm)	Bw$_2$ (25-40 cm)	C (40-65 cm)
pH (H_2O, 1:2.5)	6.7	6.4	6.7	6.6	6.5
pH (KCl, 1:2,5)	5.9	5.6	5.6	5.4	5.2
EC$_{1:5}$ (µS/cm)	115	80.5	50.5	36.4	32.3
CEC (cmol(+)/kg)	33.1	24.2	19.9	17.9	14.3
OM (g/kg)	173	89.3	53.2	39.1	27.7
C/N	12.9	10.1	9.1	8.1	7.6
Clay (g/kg)	228	318	310	370	370
Silt (g/kg)	661	602	612	550	554
Sand (g(kg)	111	79.9	77.9	80.1	76.1
Textural class (USDA)	Silty loam	Silty clay loam			
FC (g/kg)	546	409	337	325	302
PWP (g/kg)	394	252	202	189	174
AWC (g/kg)	152	157	135	136	128

EC: electrical conductivity; CEC: cation Exchange capacity; OM: organic matter; FC: water content at field capacity; PWP: water content at permanent wilting point; AWC: available water holding capacity

2.2. Prescribed fire specifications and soil temperature record

The prescribed burning was carried out within the prescription parameters established for *E. horridum* in November 2015 by qualified firefighters of the EPRIF (Wildfire Prevention Teams) of Huesca and BRIF (Reinforcement Brigades against Wildfires) of Daroca units. No rainfall events occurred during 10 days prior to the burning and air relative humidity was between 35-70 % while the maximum temperature was of 15 °C with a wind speed <8 km/h. The area had a 75 % surface cover of *E. horridum* and > 90 % of it was eliminated by fire. Burning was applied shrub-to-shrub and fire spread was of 0.64 ha/hour with a maximum flame length of 1.5 m and 1 m high. The lack of winds during most of the burning, given the safe conditions under which it was carried out, increased the required time to accomplish the desired burned surface. Soil temperatures were recorded during the burning via type-K thermocouples placed at mineral soil surface and at 1, 2 and 3 cm depth in one of the sampling points.

2.3. Soil sampling

Soil samples were collected by triplicate in areas with similar vegetation cover, slope and parent material; following a triangle shape with a separation of 5 meters between vertices (Figure 1). The organic horizons were removed prior to soil sampling. Then, a ruler was inserted into the soil to serve as a depth reference and mineral layers were carefully scrapped from the topsoil Ah horizon using a spatula at 0-1, 1-2 and 2-3 cm depth. These samples were taken as unburned controls (U) in the early morning. A couple of hours later prescribed fire was applied and as soon as it cooled down, contiguous plots to U were sampled following the sample procedure, removing ashes and remaining organic horizons, in order to assess the immediate effect of the burning (B0). The area was covered by snow one week after the burning and six months after the fire, just after snow melted in spring 2016, burned soils were sampled again (B6). To monitor the evolution of the selected soil properties further in time, soil was sampled one year after the fire in November 2016 (B12). The visual appearance of the study site over the sampling periods is represented in Figure 2. All samples were collected in plastic bags to avoid desiccation and stored as soon as possible at 4°C to maintain the fresh conditions.

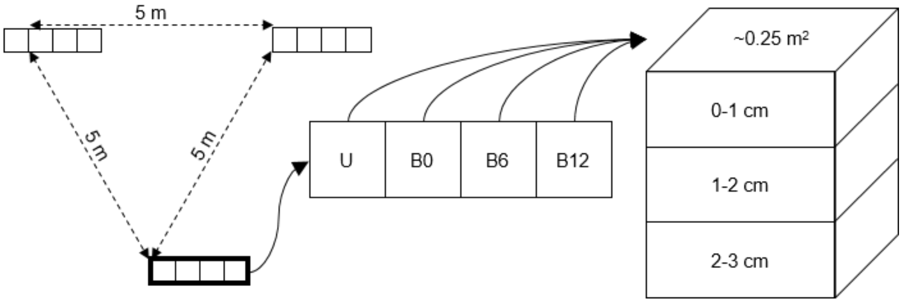


Figure 1. Design of the sampling plots. Unburned (U), immediately after (B0), 6 months (B6) and one year (B12) after burning sampling

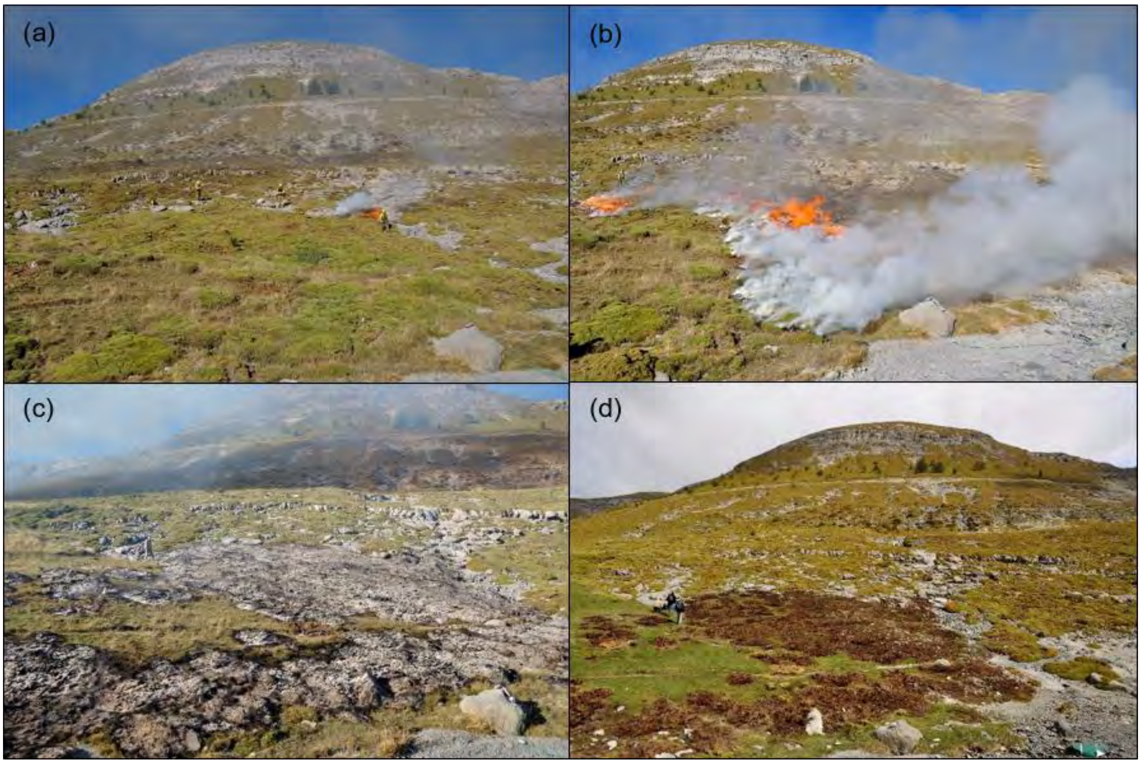


Figure 2. View of the study site before (a), during (b), immediately after (c) and one year after (d) prescribed burning

2.4. Sample preparation and laboratory methods

Samples were fresh sieved through a 2 mm mesh and kept in a refrigerator at 4 ºC for later biological analysis. Sub-samples were air dried until constant weight at room temperature and grounded for total C and N, oxidizable C and carbonates determination.

Soil moisture content was determined by the gravimetric method, drying until stable weight and it was used to calculate all the results on a 105 °C dried soil basis. Total C (TC) and nitrogen (TN) were determined by elemental analysis (Vario Max CN Macro Elemental Analyser, Germany). Equivalent $CaCO_3$ was obtained by the Bernard Calcimeter method in order to determine the C in form of $CaCO_3$ and then deducted to the TC for computing the total organic C (TOC). Microbial biomass C (C_{mic}) was determined through the chloroform fumigation-extraction method (Vance et al., 1987) using a calibration factor of $K_c = 0.38$. The C_{mic}/C ratio was calculated based on this data.

Before fumigation, K_2SO_4-extractable C (DOC) was obtained, which is also considered as a labile SOC fraction. SOC mineralisation was measured through incubation assays (28 days) under optimal conditions of 25 °C and 50 % water holding capacity moisture content. The emitted CO_2 was captured by NaOH traps and determined by HCl titration (Anderson, 1982) in selected days during the incubation: 1, 2, 4, 7, 10, 14, 18, 23 and 28. From these essays we calculated the cumulative C-CO_2 efflux over 28 days (soil basal respiration, SR); the C mineralisation coefficient (CMC) as SR per oxidizable C unit and time; and the microbial metabolic quotient (qCO_2) as SR per C_{mic} and time. Oxidizable C, determined by the wet-oxidation method with chromic acid (Nelson & Sommers, 1982), was used for the calculation of CMC since in normal conditions, it represents the C fraction that can be degraded by soil microorganisms. The soil β-D-glucosidase enzymatic activity was determined by the Eivazi and Tabatabai (1988) method.

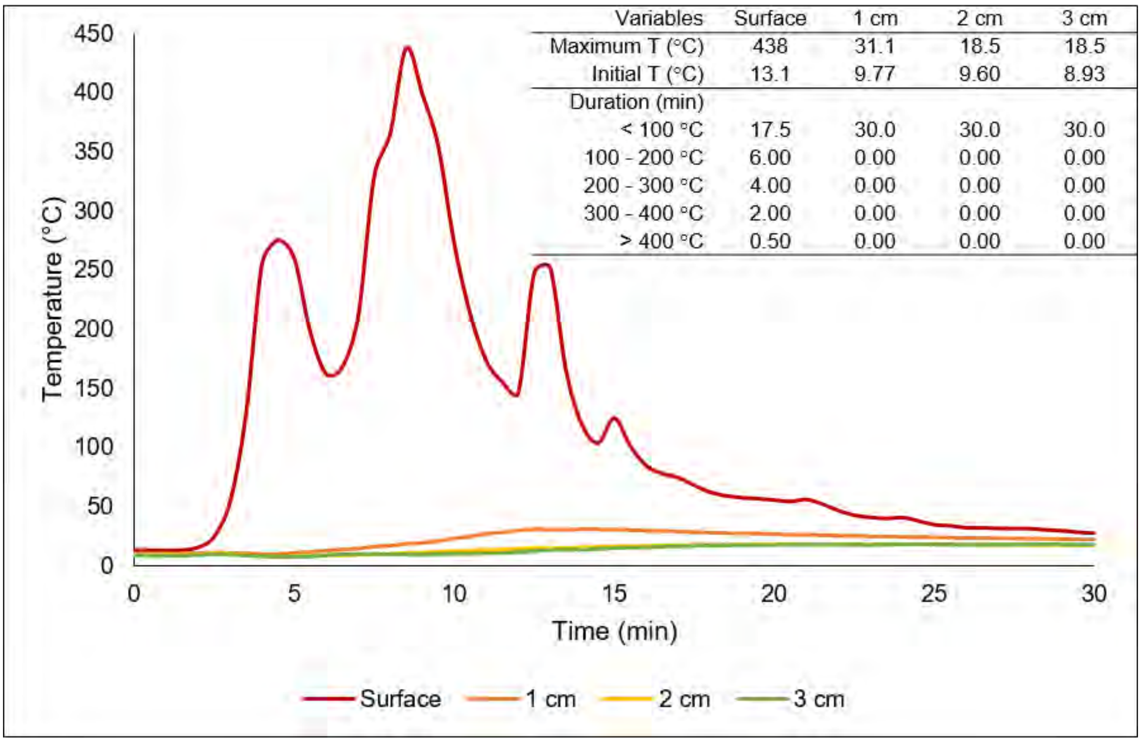
2.5. Statistical analysis

In order to identify the differences in the studied soil properties surrogated to burning and post-fire elapsed time as well as soil depth, one-way ANOVA tests were used since the interaction between time and depth was significant. Sampling time (U, B0, B6, B12) was considered as fixed factor to analyse the effect of fire and time, splitting data by soil depth (0-1, 1-2 and 2-3 cm). Additionally, changes in soil properties with depth were checked using soil depth (0-1, 1-2 and 2-3 cm) as fixed factor, splitting data by sampling time (U, B0, B6, B12). All data met the assumptions of normality and homoscedasticity so no transformations were required. These statistical analyses were carried out using StatView for Windows version 5.0.1 (SAS Institute Inc, Cary, North Carolina, USA). Data presented in the text are reported as mean ± standard deviation of the mean unless otherwise stated.

3. Results and discussion

3.1. Temperature reached during the prescribed fire and variations in soil water content

The data gathered via type-K thermocouples indicated a maximum temperature of 438 °C at soil surface while temperature at 1 cm depth only increased to 31 °C (Figure 3). Below 1 cm soil depth, temperatures remained almost unaltered during the prescribed fire. A high soil moisture (Figure 4) was observed in the unburned soil mainly at 0-1 cm depth since *E. horridum* morphology creates a microhabitat under its canopy in which temperatures are softened and soil water content is high (Cavieres et al., 2007). This high soil moisture content observed probably limited heating as it is slowed down by water content in soil until its complete vaporisation (Campbell et al. 1995; Badía et al. 2017). This affirmation is supported by the fact that water contained in the soil was not totally vaporised by fire at 0-1 cm soil depth in B0 samples. In addition, the lower soil moisture content observed in U at 1-2 cm depth as compared to the overlying layer may have also limited heat diffusivity since air is a worse heat conductor than water.



Variables	Surface	1 cm	2 cm	3 cm
Maximum T (°C)	438	31.1	18.5	18.5
Initial T (°C)	13.1	9.77	9.60	8.93
Duration (min)				
< 100 °C	17.5	30.0	30.0	30.0
100 - 200 °C	6.00	0.00	0.00	0.00
200 - 300 °C	4.00	0.00	0.00	0.00
300 - 400 °C	2.00	0.00	0.00	0.00
> 400 °C	0.50	0.00	0.00	0.00

Figure 3. Recorded temperature during prescribed burning via type-K thermocouples placed at soil surface, 1, 2 and 3 cm depth. The temperature analysis is presented in the upper-right corner

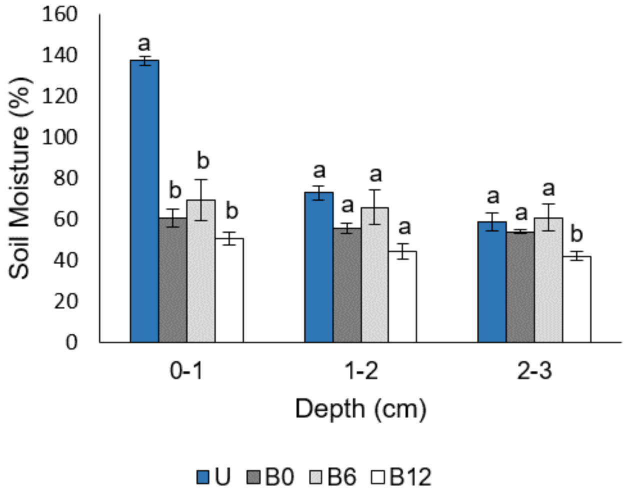


Figure 4. Soil water content in unburned (U), immediate post-fire samples (B0) and samples taken six months (B6) and one year (B12) after burning for each studied soil depth (mean value ± SE of three field replicates). For same sampling depth different letters indicate significant differences among sampling times ($P < 0.05$)

3.2. Fire effects on soil organic matter

The unburned (U) studied topsoil stores large amounts of SOM mainly at 0-1 cm, observing a steep decreasing gradient with depth (Figures 5a, 5b). Burning caused a decrease in TOC and TN at B0 that was only significant at 0-1 cm depth (-52 and -44 %, respectively) due to the substantial organic matter combustion that is initiated when temperatures reach a range of 200-250 °C (Badía & Martí, 2003; Certini, 2005; Santín & Doerr, 2016). The 1-3 cm topsoil depth remained virtually unaffected, not observing differences in TOC and TN values between U and B0. These results match those of a previous study carried out in the Central Pyrenees under similar experimental design (Armas-Herrera et al., 2016) where soil TOC decreased an average of 42 % with fire at 0-3 cm depth while TN was reduced a 24 % at 0-1 cm depth. The detected affection in SOM contrasts the data traditionally reported by literature regarding prescribed fires that point null or even positive effects in soil organic C and N content. Several studies show neutral effects of prescribed burning on soil C (Alexis et al., 2007; Fontúrbel et al*.,* 2012, 2016) and N (Marcos et al., 2009; Fultz et al., 2016). Furthermore, some authors indicate increases in soil C (Úbeda et al., 2005; Goberna et al., 2012; González-Pelayo et al., 2015) or N (Alcañiz et al., 2016; San Emeterio, 2016) after prescribed fires. Nevertheless, these prescribed burnings were conducted under different vegetation type and reached lower temperatures. Additionally, in some of them it is not clear if ashes and vegetal remains were removed prior to soil sampling as in the present study, so the positive effects might be linked to the incorporation of charred material. At B6, soil TOC and TN values showed no significant differences as compared to B0. This result may be related to the snow accumulation during the elapsed months between samplings as it can slow down SOM mineralisation and soil biological activity in the topsoil (Yi et al., 2015). A slight, not statistically significant, increase in TOC and TN content can be observed in B12 at 0-1 cm depth indicating a trend to recovery, although the determined values are still far from those of the U samples. The absence of significant variations with time could be explained by the lack of ash and charred materials incorporation into soil since most of them were still present on its surface in B6 and B12. On the other hand, N losses could have been higher than the inputs by ashes along this period of time (San Emeterio et al., 2016).

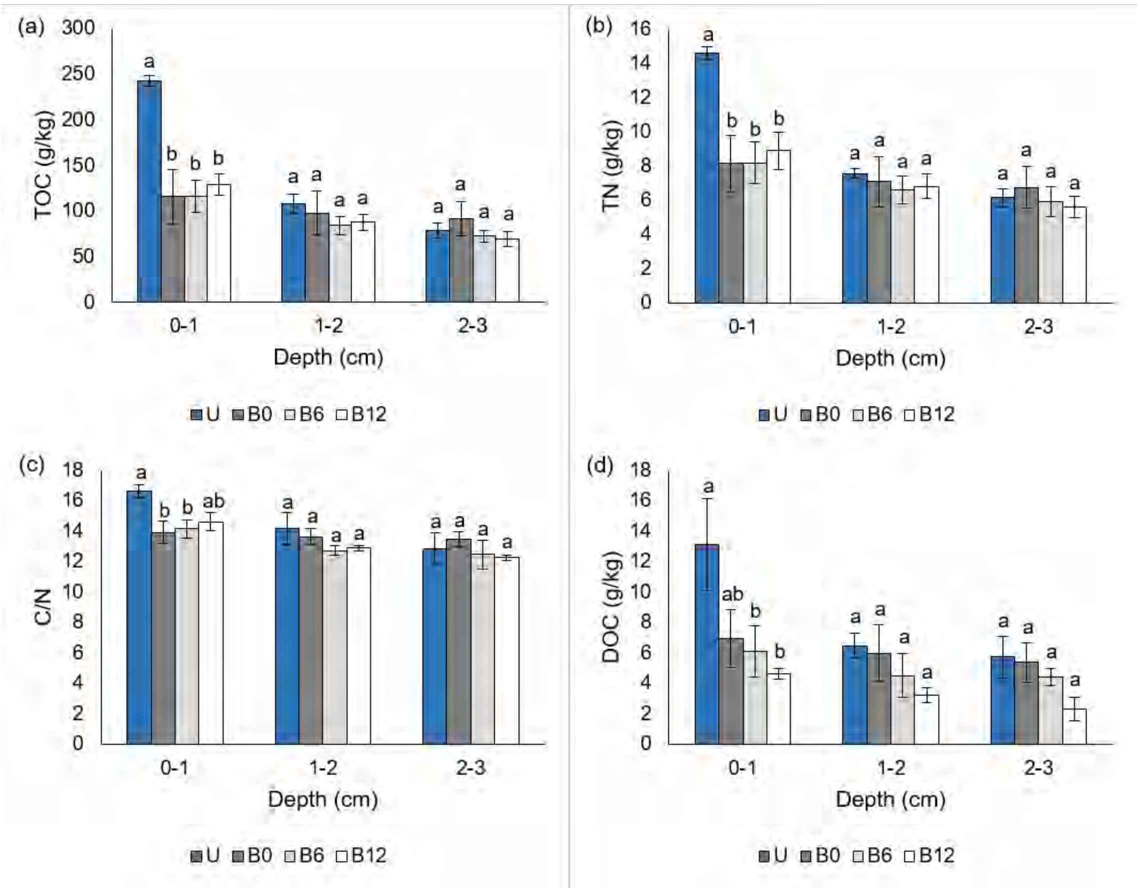


Figure 5. Fire effects on: a) total organic C (TOC); b) total N (TN); c) C/N ratio and d) K_2SO_4-extractable C (DOC) in unburned (U), immediate post-fire samples (B0) and samples taken six months (B6) and one year (B12) after burning for each studied soil depth (mean value ± SE of three field replicates). For same sampling depth different letters indicate significant differences among sampling times ($P < 0.05$)

Fire also affected organic matter turnover and quality by reducing soil C/N ratio at 0-1 cm depth in B0 as compared to U (Figure 5c). This result is a common effect of fire and might be related to N stabilisation or higher C losses (Badía & Martí, 2003; González-Pérez et al., 2004). At B6, C/N values presented no significant differences as compared to B0; nevertheless, B12 C/N ratio showed a trend towards recovery with intermediate values between U and B0 at 0-1 cm depth.

Soil DOC content values at 0-1 cm depth showed a trend to decrease in B0 as compared to U samples although it was not significant (Figure 5d). However, 6 months after the fire (B6), its content kept decreasing at 0-1 cm depth, being this reduction steeper one year later (B12) in relation to the U values. This result may indicate that the water-soluble, labile C is still being degraded by the remaining active microorganisms (Choromanska & DeLuca, 2001). Furthermore, soil DOC content highly depends on the inputs by the organic layers (Muqaddas et al., 2016) so its combustion eliminates the main DOC source. Additionally, these compounds are easily leached and lost by runoff (Michalzik and Martin, 2013).

3.3. Fire effects on soil biological properties and C cycling

The estimated soil microbial biomass C (C_{mic}) showed high values in the unburned soil decreasing dramatically along the studied soil depth (Figure 6a) since soil microbial biomass declines rapidly with depth and is concentrated in the more surficial soil 2.5 cm (Knicker, 2007). Immediately after burning, C_{mic} was severely affected, detecting a 57 % decrease at 0-1 cm depth, since it is a very sensitive soil property that can be notably altered at temperatures over 50 °C (Bárcenas-Moreno & Bååth, 2009) although no effects were observed in deeper layers. Armas-Herrera et al. (2016) obtained the same results where C_{mic} decreased remarkably with fire at 0-1 cm depth. Fontúrbel et al. (2012) in a prescribed fire carried out in a Galician shrubland dominated mainly by *Ulex europaeus* L. also detected a decrease in C_{mic} after burning although the effect was not as severe as in the present study. On the other hand, authors report an increase in C_{mic} shortly after prescribed fires due to the increase of nutrient availability (Goberna et al., 2012). Additionally, the impact of fire on soil microorganisms depends highly on soil water content as it prevents sudden increases in soil temperatures during fire; however, moist heat can produce a higher mortality than dry heat at 50-210 °C (Mataix-Solera et al., 2009). This might explain the differences in the prescribed fire effects observed in this study as compared to literature since soil moisture was considerably higher than commonly reported. At B6 and B12, no changes in C_{mic} were detected as compared to B0, which could be induced by the direct effect of fire or the elimination and slow recovery of vegetation. Hart et al. (2005) stated that the relation between some plant species and soil microbial communities might be the most important driving factor of soil microbiology dynamics to the extent that changes in the vegetation after fire can dominate over the fire effect itself. Furthermore, several years are required before the original unburned C_{mic} values recover (Bárcenas-Moreno et al., 2011).

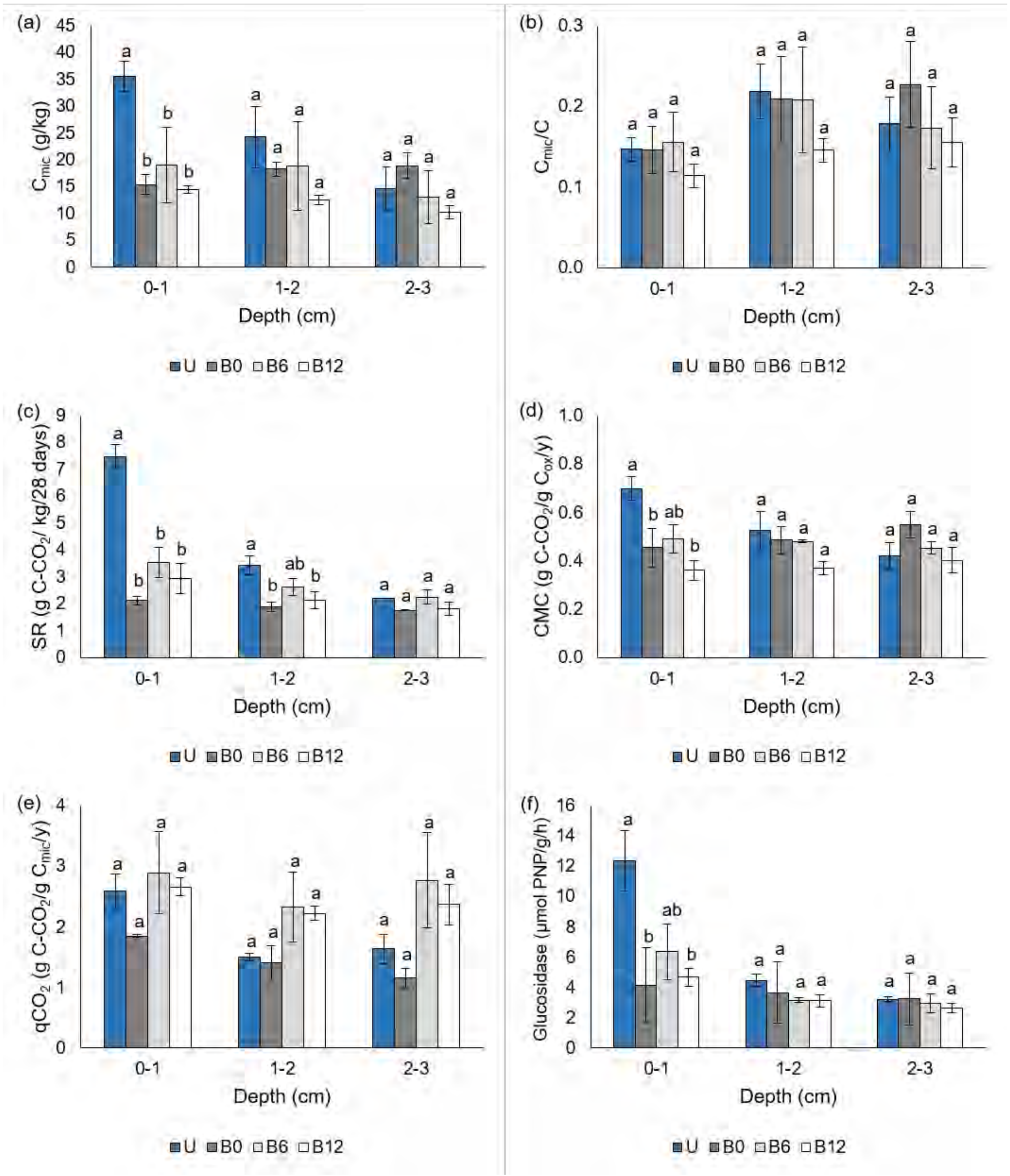


Figure 6. Fire effects on: a) microbial biomass C (C_{mic}); b) C_{mic}/C ratio; c) soil respiration (SR); d) C mineralisation coefficient (CMC); e) microbial metabolic quotient (qCO_2) and f) β-D-glucosidase activity (Glucosidase) in unburned (U), immediate post-fire samples (B0) and samples taken six months (B6) and

one year (B12) after burning for each studied soil depth (mean value ± SE of three field replicates). For same sampling depth different letters indicate significant differences among sampling times ($P < 0.05$)

No significant changes were observed in the C_{mic}/C ratio with neither fire, time nor depth because both parameters evolved following the same trends (Figure 6b). Soil C_{mic}/C ratio can be used as a soil quality indicator since in soils under degradative processes, such as fires, C_{mic} declines faster than organic matter (Zhao et al., 2012). Nevertheless, the lack of differences along the studied period of time might be related to the microbial populations adapting to the limited available C content since some authors claim that C_{mic}/C increases 1-3 months after fire due to higher nutrient availability (Fontúrbel et al., 2016).

Soil respiration (SR) in U, was significantly higher in the upper 0-1 cm as compared to 1-3 cm depth (Figure 6c). Burning (B0) had a remarkable effect on SR, suffering a 72 % decrease at 0-1 cm depth and a 45 % reduction at 1-2 cm depth**.** The cumulative C-CO_2 emitted during the incubation assays is represented in Figure 7 for the fire-affected soil depths (0-2 cm). SR was the most affected of all the studied soil properties by burning and the only one that significantly changed at 1-2 cm depth. SR reduction is a common effect surrogated to prescribed fires as reported by many authors (Choromanska & DeLuca, 2001; Hamman et al., 2008; Armas-Herrera et al., 2016). The marked affection on SR observed in the present study could be related to an increased mortality of soil microorganisms by fire due to wet heating (Choromanska & DeLuca, 2002; Mataix-Solera et al., 2009). In B6 and B12, fire effects were still detectable at 0-2 cm depth and no signs of recovery were observed. This contrasts the results found by Fontúrbel et al. (2012) in which the slight prescribed burning affection on SR recovered 180 days after fire to the unburned values although a thicker soil depth (0-5 cm) was sampled as compared to our study.

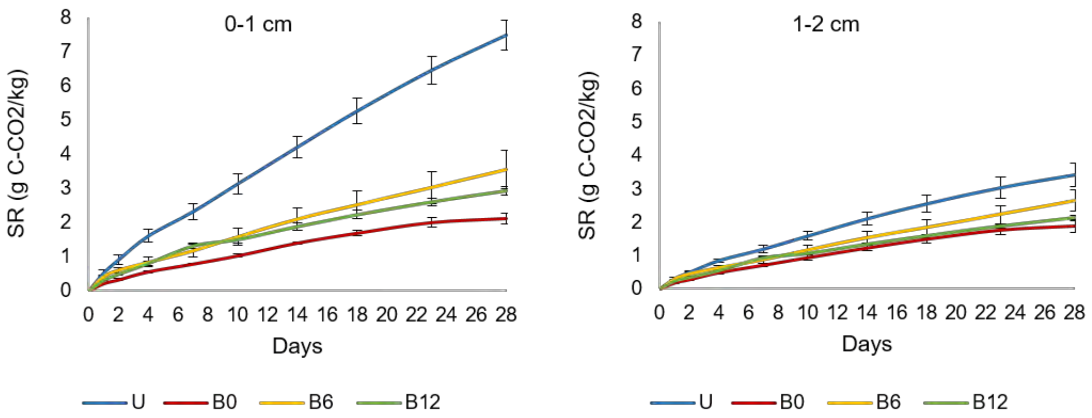


Figure 7. Soil respiration (SR) expressed as cumulative C-CO_2 emitted during the incubation assays (28 days) in unburned (U), immediate post-fire samples (B0) and samples taken six months (B6) and one year (B12) after burning for the fire-affected soil depths (0-1 and 1-2 cm)

A statistically significant reduction in the C mineralisation coefficient (CMC) was observed with fire (B0) at 0-1 cm depth while no further changes were detected with depth (Figure 6d). CMC remained stable along the study period with no significant differences in B6 and B12 as compared to B0 values. The observed CMC behaviour might be related to the loss of labile C, as was previously stated since the reduction of labile soil C can limit the C availability for heterotrophic microbes, decreasing temporarily mineralization rates (Hamman et al., 2008). Furthermore, García-Pausas et al. (2008) claimed that in Pyrenean grasslands, C mineralisation in soil surficial layers is related to the amounts of labile C which may allow higher C use by soil microorganisms.

We found no significant changes in the microbial metabolic quotient (qCO_2) neither with fire, time or depth (Figure 6e). The qCO_2 is an indicator of ecosystem stress so this is an unexpected result since the fire itself or the new situation surrogated to the elimination of vegetation cover, i.e. decrease in soil water content, can exert changes in qCO_2 (Zornoza et al., 2007). Nevertheless, a not statistically significant trend to decrease was observed at 0-1 depth immediately after fire (B0) which could indicate a slight affection on this parameter.

In the U soils, the β-D-glucosidase activity (Figure 6f) showed a steep gradient with depth and immediately after prescribed burning (B0), suffered a significant reduction of 66 % at 0-1 cm. This severe effect was also reported by Armas-Herrera et al. (2016) in which an average decrease of 49 % was detected at 0-3 cm depth after prescribed fire application. López-Poma & Bautista (2014) in an experimental burning carried out in a *Rosmarinus-Erica* L. Mediterranean shrubland, also observed a remarkable decrease in β-D-glucosidase two weeks after the fire under similar temperatures during burning as the present study (492 °C at soil surface). This immediate reduction after fire of β-D-glucosidase might be explained by denaturation of the enzymes due to high temperatures (Knicker, 2007; Goberna et al., 2012; López-Poma & Bautista, 2014). Nevertheless, some studies indicate that prescribed and experimental shrub burning have no effects on of β-D-glucosidase (Boerner et al., 2008; Fontúrbel et al., 2016). In B6, a transient pulse was detected in β-D-glucosidase activity with an intermediate situation between U and B0 values at 0-1 cm depth. This stationary situation was also observed in CMC values and could be explained by seasonal variations (Fontúrbel et al, 2016) as this effect disappears one year later. The behaviour observed in β-D-glucosidase might be also related to the variations in the labile C fraction as it was earlier mentioned, since it is highly regulated by substrate availability (Barreiro et al., 2016). Barreiro et al. (2010) and López-Poma & Bautista (2014) also observed no recovery in β-D-glucosidase activity one year after experimental burning although after this period of time it started to increase.

3.4. Vegetation evolution after prescribed burning

In the following summer after burning, an incipient vegetation occupation was observed in the burned plots. They were mainly colonised by resprouter species such as *Carex* *flaca*, *Carex humilis*, *Euphorbia cyparissus*, *Iris latifolia*, *Teucrium chamaedrys* and *Viola cf. rupestris*. Additionally, reseeding species i.e. the burned *Echinospartum horridum* were also found with a germination gradient. While some seedlings only had two cotyledons others were 1-4 cm tall but not thorny yet. Vegetation represented only a small surface of the burned plots, still covered by necromass such as burned leaves, litter and partially burned branches. The burned plots form a mosaic landscape as it can be seen in Figure 2d, surrounded by vegetal species of the *Bromion erecti* alliance, already present before burning. Since the objective of these prescribed burnings is the reduction of *Echinospartum horridum* cover it seems necessary the introduction of cattle in the burned plots in order to promote the consumption of its seedlings while are still edible, which would facilitate the colonisation of the pastoral species.

3.5. General considerations regarding prescribed burning

The results obtained in the present study indicate that the initial hypothesis is not completely fulfilled. Fire affection was indeed limited to the topsoil (0-2 cm) but it was much higher than expected. In addition, one year after burning and given the severe effect of fire, no recovery was observed in the studied soil properties. Furthermore, the grasses that have grown in the burned plots only represent a small percentage of its surface.

The decrease in C mineralisation detected after prescribed burning could allow for higher C sequestration in the long term, which is a positive effect from the point of view of climate change. Nevertheless, mountain shrubs store large amounts of C in its biomass and litter as a result of the slow decomposition derived from its low biochemical quality (Montané et al., 2007). Therefore, shrub burning entails substantial C losses not accounted for in this work. Additionally, this study has been carried out in the short term so it is risky to make any assumptions regarding this topic in the long term. This suggests that further research is needed in order to detect whether the decrease in C mineralisation could balance the C amount lost by burning at a larger time scale.

Comparing our results with those obtained by previous studies carried out in prescribed burnings might be a difficult task given the vast array of different sampling methodologies and ecosystems. The discordance between the data obtained and these reported by literature could be due to the variability in several factors as: 1) the intensity and duration of the prescribed burning as well as how it is distributed (Granged et al., 2011); 2) the vegetation type, its moisture and fuel loads (Neary et al., 1999); 3) weather conditions (Fernandes et al., 2013); 4) the presence and moisture content of the duff layer (Valette et al., 1994); 5) soil moisture, which could pause the temperature rise during the evaporative stage of soil drying (Massman, 2012); 6) a flawed soil sampling design, given the low intensity of prescribed fires, in which maybe too much soil thickness is sampled and a dilution effect is produced (Badía-Villas et al., 2014). This heterogeneity complicates the search for general patterns of prescribed fire effects on soils and suggests that those effects are highly site-dependant.

4. Conclusions

All the studied soil properties (TOC, TN, C/N, C_{mic}, SR, β-D-glucosidase) were significantly reduced by fire at 0-1 cm depth while only SR was also affected down to 1-2 cm depth. These results indicate a high affection of prescribed burning on SOM and biological activity although limited to a thin soil layer. Despite the moderate temperatures recorded during the burning, this affection can be explained by the slow fire spread and the effect of wet heating. The results of this research also indicated that none of the studied soil properties recover in the short-term (one year) so monitoring further in time is needed in order to assess the sustainability of this practice in relation to soil conservation and C cycle.

Acknowledgements

This study is part of the results of the FUEGOSOL project (CGL2013-43440-R) funded by the Spanish “Ministerio de Economía, Industria y Competitividad”. It was also partially supported by the European Regional Fund and Aragón Regional Government through the PaleoQ (E56) research group. A. Girona-García is funded by a FPI research grant (BES-2014-068072). We thank Rafael de Partearroyo and Raúl Vicente (EPRIF, Huesca) for allowing us to participate in the prescribed fire events and Daniel Gómez for the help identifying plant species. We also thank the comments and suggestions of the two anonymous reviewers that contributed to improve the manuscript.

REFERENCES

Alcañiz M, Outeiro L, Francos M, Farguell J, Úbeda X. 2016. Long-term dynamics of soil chemical properties after a prescribed fire in a Mediterranean forest (Mongrí Massif, Catalonia, Spain). Science of the Total Environment 572: 1329-1335. DOI: 10.1016/j.scitotenv.2016.01.115

Alexis M, Rasse D, Rumpel C, Bardoux G, Pechot N, Schmalzer P, Drake B, Mariotti A. 2007. Fire impact on C and N losses and charcoal production in a scrub oak ecosystem. Biogeochemistry 82 (2): 201-216. DOI: 10.1007/s10533-006-9063-1

Anderson JP. 1982. Soil respiration. In: Page AL, Miller RH, Keeney DR (Eds.). Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Agronomy Series, nº 9. Madison, pp. 831-871.

Armas-Herrera CM, Martí C, Badía D, Ortiz-Perpiñá O, Girona-García A, Porta J. 2016. Immediate effects of prescribed burning in the Central Pyrenees on the amount and stability of topsoil organic matter. Catena 147: 238-244. DOI: 10.1016/j.catena.2016.07.016

Badía D, Martí C. 2003. Effect of simulated fire on organic matter and selected microbiological properties of two contrasted soils. Arid Land Research and Management 17: 55-69. DOI: 10.1080/15324980390169064

Badía-Villas D, González-Pérez JA, Aznar JM, Arjona-Gracia B, Martí-Dalmau C. 2014. Changes in water repellency, aggregation and organic matter of a mollic horizon burned in laboratory: soil depth affected by fire. Geoderma 213: 400-407. DOI: 10.1016/j.geoderma.2013.08.038

Badía D, López-García S, Martí C, Ortiz-Perpiñá O, Girona-García A, Casanova-Gascón J. 2017. Burn effects on soil properties associated to heat transfer under contrasting moisture content. Science of the Total Environment 601-602: 1119-1128. DOI: 10.1016/j.scitotenv.2017.05.254

Bárcenas-Moreno G, Bååth E. 2009. Bacterial and fungal growth in soil heated at different temperaturas to simulate a range of fire intensities. Soil Biology & Biochemistry 41: 2517-2526. DOI: 10.1016/j.soilbio.2009.09.010

Bárcenas-Moreno G, García-Orenes F, Mataix-Solera J, Mataix-Beneyto J, Bååth E. 2011. Soil microbial recolonisation after a fire in a Mediterranean forest. Biology and Fertility of Soils 47: 262-272. DOI: 10.1007/s00374-010-0532-2

Barreiro A, Carballas MT, Díaz-Raviña M. 2010. Response of soil microbial communities to fire and fire-fighting chemicals. Science of the Total Environment 408: 6172-6178. DOI: 10.1016/j.scitotenv.2010.09.011

Barreiro A, Martín A, Carballas T, Díaz-Raviña M. 2016. Long-term response of soil microbial communities to fire and fire-fighting chemicals. Biology and Fertility of Soils 52: 963-975. DOI: 10.1007/s00374-016-1133-5

Boerner REJ, Giai C, Huang J, Miesel JR. 2008. Initial effects of fire and mechanical thinning on soil enzyme activity and nitrogen transformations in eight North American forest ecosystems. Soil Biology & Biochemistry 40: 3076-3085. DOI: 10.1016/j.soilbio.2008.09.008

Caballero R, Fernández González F, Pérez Badía R, Molle G, Roggero PP, Bagella S, D'Ottavio P, Papanastasis VP, Fotiadis G, Sidiropoulou A, Ispikoudis I. 2010. Grazing systems and biodiversity in Mediterranean areas: Spain, Italy and Greece. Pastos 39: 9-154.

Campbell GS, Jungbauer JD, Bristow KL, Hungerford RD. 1995. Soil temperature and water content beneath a surface fire. Soil Science 159: 363-374. DOI: 10.1097/00010694-199506000-00001

Cavieres LA, Badano EI, Sierra-Almeida A, Molina-Montenegro MA. 2007. Microclimatic Modifications of Cushion Plants and Their Consequences for Seedling Survival of Native and Non-native Herbaceous Species in the High Andes of Central Chile. Arctic, Antarctic, and Alpine Research 39 (2):229-236. DOI: 10.1657/1523-0430(2007)39[229:MMOCPA]2.0.CO;2

Certini G. 2005. Effects of fire on properties of forest soils: a review. Oecologia 143: 1-10. DOI: 10.1007/s00442-004-1788-8

Choromanska U, DeLuca TH. 2001. Prescribed Fire alters the Impact of Wildfire on Soil Biochemical Properties in a Ponderosa Pine Forest. Soil Science Society of America Journal 65: 232-238. DOI: 10.2136/sssaj2001.651232x

Choromaska U, DeLuca TH. 2002. Microbial activity and nitrogen mineralization in forest mineral soils following heating: evaluation of post-fire effects. Soil Biology & Biochemistry 34: 263-271. DOI: 10.1016/S0038-0717(01)00180-8

Dooley SR, Treseder KK. 2012. The effect of fire on microbial biomass: a meta-analysis of field studies. Biogeochemistry 109: 49-61. DOI: 10.1007/s10533-011-9633-8

Eivazi F, Tabatabai MA. 1988. Glucosidases and galactosidases in soils. Soil Biology and Biochemistry 20 (5): 601-606. DOI: 10.1016/0038-0717(88)90141-1

Eswaran H, Van Den Berg E, Reich P. 1993. Organic carbon in soils of the world. Soil Science Society of America Journal 57: 192-194. DOI: 10.2136/sssaj1993.03615995005700010034x

Fernandes PM, Davies GM, Ascoli D, Fernández C, Moreira F, Rigolot E, Stoof CR, Vega JA, Molina D. 2013. Prescribed burning in southern Europe: developing fire management in a dynamic landscape. Frontiers in Ecology and the Environment 11: 4-14. DOI: 10.1890/120298

Follett RF, Reed DA. 2010. Soil Carbon Sequestration in Grazing Lands: Societal Benefits and Policy Implications. Range Ecology & Management 63(1):4-15. DOI: 10.2111/08-225.1

Fontúrbel MT, Barreiro A, Vega JA, Martín A, Jiménez E, Carballas T, Fernández C, Díaz-Raviña M. 2012. Effects of an experimental fire and post-fire stabilization treatments on soil microbial communities. Geoderma 191: 51-60. DOI: 10.1016/j.geoderma.2012.01.037

Fontúrbel MT, Fernández C, Vega JA. 2016. Prescribed burning versus mechanical treatments as shrubland management options in NW Spain: Mid-term soil microbial response. Applied Soil Ecology 107: 334-346. DOI: 10.1016/j.apsoil.2016.07.008

Fultz LM, Moore-Kucera J, Dathe J, Davinic M, Perry G, Wester D, Schwilk DW, Rideout-Hanzak S. 2016. Forest wildfire and grassland prescribed fire effects on soil biogeochemical processes and microbial communities: two case studies in the semi-arid Southwest. Applied Soil Ecology 99: 118-128. DOI: 10.1016/j.apsoil.2015.10.023

García-Pausas J, Casals P, Camarero L, Huguet C, Thompson R, Sebastià MT, Romanyà J. 2008. Factors regulating carbon mineralization in the Surface and subsurface soils of Pyrenean mountain grasslands. Soil Biology & Biochemistry 40: 2803-2810. DOI: 10.1016/j.soilbio.2008.08.001

Goberna M, García C, Insam H, Hernández MT, Verdú M. 2012. Burning Fire-Prone Mediterranean Shrublands: Immediate Changes in Soil Microbial Community Structure and Ecosystem Functions. Microbial Ecology 64 (1): 242-255. DOI: 10.1007/s00248-011-9995-4

Goldammer JG, Montiel C. 2010. Identifying good practices and programme examples for prescribed and suppression fire. In: Montiel C, Kraus D (Eds.). Best Practices of Fire Use – Prescribed Burning and Supression Fire Programmes in Selected Case-Study Regions in Europe. European Forest Institute, Joensuu, pp. 35-44.

González-Pelayo O, Gimeno-García E, Ferreira CCS, Ferreira AJD, Keizer JJ, Andreu V, Rubio JL. 2015. Water repellency of air-dried and sieved samples from limestone soils in central Portugal collected before and after prescribed fire. Plant and Soil 394: 199-214. DOI: 10.1007/s11104-015-2515-4

González-Pérez JA, González-Vila FJ, Almendros G, Knicker H. 2004. The effect of fire on soil organic matter – a review. Environmental International 30 (6): 855-870. DOI: 10.1016/j.envint.2004.02.003

Granged AJP, Jordán A, Zavala LM, Muñoz-Rojas M, Mataix-Solera J. Short-term effects of experimental fire for a soil under eucaliptus forest (SE Australia). 2011. Geoderma 167-168: 125-134. DOI: 10.1016/j.geoderma.2011.09.011

Halada L, Evans D, Romão C, Petersen JE. 2011. Which habitats of European importance depend on agricultural practices? Biodiversity and Conservation 20: 2365-2378. DOI: 10.1007/s10531-011-9989-z

Hamman ST, Burke IC, Knapp EE. 2008. Soil nutrients and microbial activity after early and late season prescribed burns in a Sierra Nevada mixed conifer forest. Forest Ecology and Management 256: 367-374. DOI: 10.1016/j.foreco.2008.04.030

Hart SC, DeLuca TH, Newman GS, MacKenzie MD, Boyle SI. 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. Forest Ecology and Management 220: 166-184. DOI: 10.1016/j.foreco.2005.08.012

IUSS Working Group WRB. 2014. World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome

Jones MB, Donelly A. 2004. Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO_2. New Phytologist 164: 423-439. DOI: 10.1111/j.1469-8137.2004.01201.x

Knicker H. 2007. How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. Biogeochemistry 85: 91-118. DOI: 10.1007/s10533-007-9104-4

Komac B, Alados CL, Camarero JJ. 2011. Influence of topography on the colonization of subalpine grasslands by the thorny cushion dwarf Echinospartum horridum. Arctic, Antarctic and Alpine Research 43 (4); 601-611. DOI: 10.1657/1938-4246-43.4.601

Komac B, Sefi S, Nuche P, Escós J, Alados CL. 2013. Modeling shrub encroachment in subalpine grasslands under different environmental and management scenarios. Journal of Environmental Management 121:160-169. DOI: 10.1016/j.jenvman.2013.01.038

Lasanta T. 2010. Grazing in mountain areas: management strategies and territorial impacts. Estudios Geográficos 268: 203-233. DOI: 10.3989/estgeogr.0459

López-Poma R, Bautista S. 2014. Plant regeneration functional groups modulate the response to fire of soil enzyme activities in a Mediterranean shrubland. Soil Biology & Biochemistry 79: 5-13. DOI: 10.1016/j.soilbio.2014.08.016

Marcos E, Villalón C, Calvo L, Luis-Calabuig E. 2009. Short-term effects of experimental burning on soil nutrients in the Cantabrian heathlands. Ecological Engineering 35: 820-828. DOI: 10.1016/j.ecoleng.2008.12.011

Massman WJ. 2012. Modeling soil heating and moisture transport under extreme conditions: forest fires and slash pile burns. Water Resources Research 48, W10548. DOI: 10.1029/2011WR011710

Mataix-Solera J, Guerrero C, García-Orenes F, Bárcenas GM, Torres MP. 2009. Forest fire effects on soil microbiology. In: Cerdà A, Robichaud PR (Eds.). Fire Effects on Soils and Restoration Strategies. Science Publishers, Enfield, NH, USA, pp. 133-175. DOI: 10.1201/9781439843338-c5

Mataix-Solera J, Cerdà A, Arcenegui V, Jordán A, Zavala LM. 2011. Fire effects on soil aggregation: a review. Earth Science Reviews 109: 44-60. DOI: 10.1016/j.earscirev.2011.08.002

Michalzik B, Martin S. 2013. Effects of experimental duff fires on C, N and P fluxes into the mineral soil at a coniferous and broadleaf forest site. Geoderma 197: 169-176. DOI: 10.1016/j.geoderma.2013.01.010

Molina D. 2009. Fuego prescrito y planes de quema. In: Vélez R (Coord.). La defensa contra incendios forestales: fundamentos y experiencias. 2nd Ed. McGraw-Hill. Madrid.

Montané F, Rovira P, Casals P. 2007. Shrub encroachment into mesic mountain grasslands in the Iberian Peninsula: Effects of plant quality and temperature on soil C and N stocks. Global Biogeochemical Cycles 21, GB4016. DOI: 10.1029/2006GB002853

Muqaddas B, Chen C, Lewis T, Wild C. 2016. Temporal dynamics of carbon and nitrogen in the surface soil and forest floor under different prescribed burning regimes. Forest Ecology and Management 382: 110-119. DOI: 10.1016/j.foreco.2016.10.010

Neary DG, Klopatek CC, DeBano LF, Ffolliott PF. 1999. Fire effects on belowground sustainability: a review and synthesis. Forest Ecology and Management 122: 51-71. DOI: 10.1016/S0378-1127(99)00032-8

Nelson RE, Sommers LE. 1982. Total carbon and organic matter. In: Page AL, Miller RH, Keeney DR (Eds.). Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties, second ed. American Society of Agronomy, Madison, Wisconsin, pp. 539-557.

Saha MC, Butler TJ. 2017. Grassland. In: Thomas B, Murray BG, Murphy DJ (Eds.). Encyclopedia of Applied Plant Sciences (Second Edition). Academic Press, Oxford, pp. 180-185. DOI: 10.1016/B978-0-12-394807-6.00168-4.

San Emeterio L, Múgica L, Gutiérrez R, Juaristi A, Pedro J, Canals RM. 2014. Changes in the soil nitrogen content of Pyrennean grasslands after controlled burnings for ammelioration purposes. Pastos 43 (2): 44-53.

San Emeterio L, Múgica L, Ugarte MD, Goicoa T, Canals RM. 2016. Sustainability of traditional pastoral fires in highlands under global change: Effects of soil function and nutrient cycling. Agriculture, Ecosystems and Environment 235: 155-163. DOI: 10.1016/j.agee.2016.10.009

Santín C, Doerr SH. 2016. Fire effects on soils: the human dimension. Philosophical Transactions of the Royal Society B: Biological Sciences 371: 20150171. DOI: 10.1098/rstb.2015.0171

Úbeda X, Lorca M, Outeiro LR, Bernia S, Castellnou M. 2005. Effects of prescribed fire on soil quality in Mediterranean grassland (Prades Mountains, north-east Spain). International Journal of Wildland Fire 14 (4): 379-384. DOI: 10.1071/WF05040

Valette JC, Gomendy V, Maréchal J, Houssard C, Gillon D. 1994. Heat transfer in the soil during very low-intensity experimental fires: the role of duff and soil moisture content. International Journal of Wildland Fire 4 (4): 225-237. DOI: 10.1071/WF9940225

Vance ED, Brookes PC, Jenkinson DS. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19 (6): 703-707. DOI: 10.1016/0038-0717(87)90052-6

Vega JA, Fernández C, Fontúrbel T. 2005. Throughfall, runoff and soil erosion after prescribed burning in gorse shubland in Galicia (NW Spain). Land Degradation & Development 16 (1): 37-51. DOI: 10.1002/ldr.643

Yi Y, Kimball JS, Rawlins MA, Moghaddam M, Euskirchen ES. 2015. The role of snow cover affecting boreal-arctic soil freeze-thaw and carbon dynamics. Biogeosciences 12: 5811-5829. DOI: 10.5194/bg-12-5811-2015

Zhao H, Tong DQ, Lin Q, Lu X, Wang G. 2012. Effects of fires on soil organic carbon pool and mineralization in a Northeastern China wetland. Geoderma 189-190: 532-539. DOI: 10.1016/j.geoderma.2012.05.013

Zornoza R, Guerrero C, Mataix-Solera J, Arcenegui V, García-Orenes F, Mataix-Beneyto J. 2007. Assessing the effects of air-drying and rewetting pre-treatment on soil microbial biomass, basal respiration, metabolic quotient and soluble carbon under Mediterranean conditions. European Journal of Soil Biology 43: 120-129. DOI: 10.1016/j.ejsobi.2006.11.004