Dynamics of topsoil carbon stocks after prescribed burning for pasture restoration in shrublands of the Central Pyrenees (NE-Spain)

Antonio Girona-García*, Oriol Ortiz-Perpiñá, David Badía-Villas

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

*Corresponding author: antoniogironagarcia@gmail.com

Full bibliographical details: Girona-García, A.; Ortiz-Perpiñá, O.; Badía-Villas, D. 2019. Dynamics of topsoil carbon stocks after prescribed burning for pasture restoration in shrublands of the Central Pyrenees (NE-Spain). Journal of Environmental Management 233: 695-705. DOI: 10.1016/j.jenvman.2018.12.057

Highlights

- Prescribed burning is used to remove shrubs and restore pastures
- The effect on soil C stocks and related biological properties is studied
- Slow prescribed burning severely affected soil properties at 0-1 cm depth
- Soil properties were not affected at sites where the fire spread rapidly
- Soil biological activity decrease occurred in the short- and mid-term

Abstract

Prescribed burning has been recently readopted as a management practice in the Central Pyrenees (NE-Spain) to stop shrub encroachment processes and recover pasturelands. The immediate effects of prescribed burning on soil C stocks and related biological properties and their evolution in the short- to mid-term after burning were assessed. The study was conducted during three autumnal prescribed burnings in the Central Pyrenees in the municipalities of Buisán, Asín de Broto and Yebra de Basa. At each site, the topsoil Ah horizon was sampled at soil depths of 0-1, 1-2 and 2-3 cm immediately before and immediately after burning. Additionally, seasonal samplings were conducted every 6 months up to one year in the case of the Asín and Yebra sites and up to 24 months at the Buisán site. The total soil organic C stock (SOCS) total N stock (NS), microbial biomass C (MBC), soil basal respiration (SR) and β-D-glucosidase activity were analyzed. The maximum temperatures recorded at the soil surface were 438 ºC (Buisán), 768 ºC (Asín) and 595 ºC (Yebra). At the Buisán site, burning significantly decreased the SOCS (-52 %), NS (-44 %), MBC (-57 %), SR (-72 %) and glucosidase activity (-66 %) at 0-1 cm depth, whereas fire had no direct effects on soil at the Asín and Yebra sites. The contrasting effects of burning on soil that were observed among sites were found to be related to differences in fire residence time. The prescribed fire at the Buisán site was on a plain slope under slow winds (<8 km h^{-1}) at a burning rate of 0.64 ha h^{-1}, which produced greater impacts on the soil properties than the burnings at the Asín and Yebra sites, where fire spread rapidly (2.72 and 1.43 ha h^{-1}, respectively). At the Buisán site, the SOCS and NS recovered to the unburned values 24 months after burning. One year after burning, the SOCS at Asín were 60 % higher than those of the unburned soils at 0-1 cm depth. At all sites a decreasing trend in soil biological activity in the short- and mid-term was observed. From the results it can be concluded that: 1) the direct effects of burning on soil are highly dependent on the environmental conditions, 2) in the mid-term, the reduction in soil biological activity and the incorporation of ashes and charred plant remains led to an increase in the SOCS of the burned soils.

Keywords: shrub encroachment, grazing lands, soil properties, prescribed fire, carbon

sequestration

1. Introduction

Grasslands are rich and diverse ecosystems that provide considerable environmental, economic and social services (Follett & Reed, 2010; Nadal-Romero et al., 2018a). Temperate grasslands present high natural soil fertility due to their high soil organic matter (SOM) content (Jones & Donelly, 2004; Conant, 2010; García-Pausas et al., 2017) and it is estimated that 176 to 295 billion tons of soil organic C (SOC) are stored in these ecosystems (Lal, 2004). Therefore, the sustainable management of these lands is of vital importance due to their potential for carbon sequestration and greenhouse gases emission regulation (Conant, 2010; Farley et al., 2013).

In the Central Pyrenees (NE-Spain), grazing lands were established for pastoral purposes below the potential tree line by removing the pre-existing vegetation such as shrubs and forests (Gartzia et al., 2014). In these areas, pasturelands have been traditionally maintained through livestock grazing and the recurrent elimination of shrubs by either fire or mechanical procedures (Gartzia et al., 2014; Nadal-Romero et al., 2018a). As a consequence of socioeconomic changes (i.e., rural exodus and the decrease of grazing activity) and the fire suppression policies that were enacted in the 20th century, these habitats have suffered from shrub encroachment (Komac et al., 2013; Nadal-Romero et al., 2018b). In the grazing lands of the Central Pyrenees, one of the main species that has led the ecological succession towards shrubs has been the thorny cushion dwarf, *Echinospartum horridum* (Vahl) Rothm (Komac et al., 2013; Nuche et al., 2018). This shrub forms large and dense monospecific patches that limit the establishment of herbaceous species (Komac et al., 2011) hence posing a threat to biodiversity and an increased flammability risk (Caballero et al., 2010; Gartzia et al., 2014).

Prescribed burnings, defined as the planned use of fire to achieve precise and clearly defined objectives (Fernandes et al., 2013), represent a suitable procedure for the elimination of shrubs from grazing lands (Goldammer & Montiel, 2010). Since fire can affect most of the soil physical, chemical and biological properties (González-Pérez et al., 2004; Certini, 2005), prescribed burnings are conducted under specific environmental conditions (i.e., high soil and fuel moisture, low temperature, moderate winds and favorable topography) to limit the severity of the fire (Vega et al., 2005). These conditions can vary widely, so previous studies on prescribed burnings showed heterogeneous outcomes, as recently reviewed in Alcañiz et al. (2018). Due to the low intensity that usually characterizes prescribed fires, it is common to find increases in

SOM contents after burning (Úbeda et al., 2005; Alcañiz et al., 2016), which are related to the incorporation of ashes and partially charred vegetal remains (González-Pérez et al., 2004). Additionally, some studies note that prescribed burning may have no effects on SOM (Alexis et al., 2007; Goberna et al., 2012; Fultz et al., 2016). Nevertheless, the effects of the fire management of shrublands on SOC stocks and dynamics are still uncertain (García-Pausas et al., 2017) and few studies have covered the effects of this practice on soils of montane and subalpine environments (San Emeterio et al., 2014, 2016; Armas-Herrera et al., 2016, 2018; Girona-García et al., 2018a, 2018b, 2018c). Previous works conducted in the Central Pyrenees showed that *Echinospartum horridum* burnings may have a severe impact on SOM and soil biological activity, limited to a thin layer of the topsoil, that persevere in the short- to mid-term (Armas-Herrera et al., 2018; Girona-García et al., 2018a). SOM turnover rates are mostly driven by soil microbial biomass so changes in its activity due to fire can induce variations in the C transfer between the soil and the atmosphere (Knicker, 2007; Dooley & Treseder, 2012). Therefore, it is of special interest to study the role that prescribed burnings play in the C cycle of mountain environments in the context of global change.

In Girona-García et al. (2018a), the effects of prescribed burning on soil organic matter and related biological properties were studied at one site throughout a year. From that work, it could be concluded that in order to extrapolate the results and assess the sustainability of this practice, it was needed to monitor the site further in time and to study a higher number of sites. For this reason, in the present work, the previously published dataset is further developed in time and two more study sites were added. This new study aimed to assess the immediate effects of *Echinospartum horridum* prescribed burning on topsoil SOC stocks and related biological properties, in the short- and mid-term at three locations of the Central Pyrenees (NE-Spain). The main hypothesis of this work are: 1) prescribed burning will have reduced or neutral effects on the selected soil properties, 2) the effects of fire will be confined to the uppermost soil cm, 3) those effects will be dependent on the site characteristics and environmental conditions under which the burning is conducted, 4) in the short- to mid-term the SOC and N stocks, if affected by fire, will recover to the unburned conditions and soil biological properties will respond to the new postfire environmental conditions.

2. Materials and methods

2.1. Study sites

The study was conducted in three mountain pasture areas encroached by *Echinospartum horridum* that were subjected to prescribed burning. The experimental plots were located in the municipalities of Buisán, Asín de Broto and Yebra de Basa (Central Pyrenees, Huesca province, NE-Spain, Fig. 1); these sites are referred to as Buisán, Asín and Yebra in the remainder of the text. The general site characteristics are provided in Table 1. The elevation, mean annual precipitation and mean annual temperature of the study sites are 1480 m a.s.l., 1015 mm and 8.3 ºC for Yebra; 1650 m a.s.l., 1120 mm and 8.8 ºC for Asín; and 1760 m a.s.l., 1500 mm, 6 ºC for Buisán. The soils are classified as Eutric Cambisol (Buisán), Calcaric Cambisol (Asín) and Leptic Cambisol (Yebra) according to the IUSS Working Group WRB (2014) and are characterized by high organic matter contents, high aggregate stability and neutral pH values; the textures are silty loam (Buisán and Asín) and sandy loam (Yebra). The parent material in Buisán consists of Eocene detritic sediments over clayey limestones alternated with marls; in Asín, the parent material is composed of Eocene marls and sandstones, while in Yebra, it consists of Eocene conglomerates. The Buisán plots were established at the bottom of a gentle slope (10 %); the Asín plots were located at the top of a steep slope (35 %) and the Yebra plots were positioned in the middle of a flat slope (5 %). The vegetation was constituted by a predominant thorny shrub (*Echinospartum horridum*) mosaic surrounded by herbaceous species such as *Bromus erectus* Huds., *Festuca nigrescens* Lam., *Agrostis capillaris* L., *Briza media* L., *Onobrychis pyrenaica* (Sennen) Sirj., *Trifolium pratense* L. and *Trifolium repens* L.

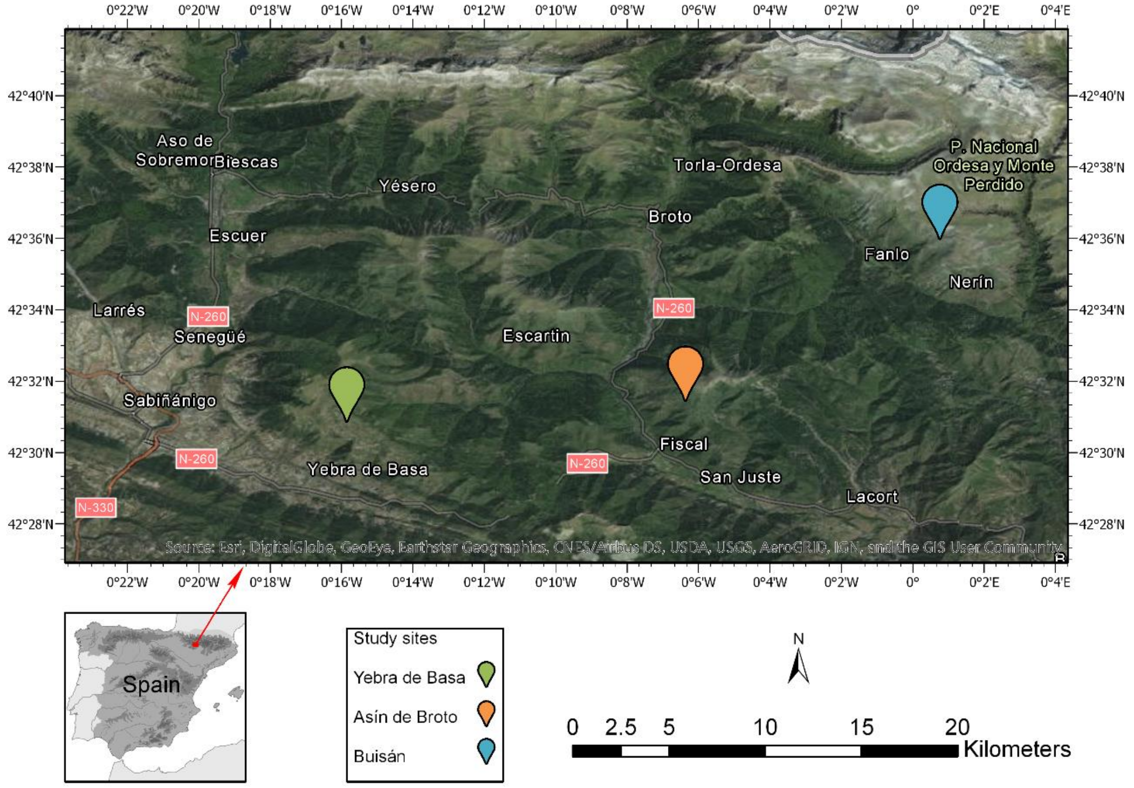


Fig. 1. Locations of the study sites.

2.2. Prescribed burning characteristics

The prescribed fires were conducted in November 2015 (Buisán), November 2016 (Asín) and December 2016 (Yebra) by firefighters of the EPRIF (Wildfire Prevention Team) of Huesca and BRIF (Reinforcement Brigades against Wildfires) of Daroca units. In all cases, the prescribed burnings were performed when the environmental conditions met the prescribed parameters for *Echinospartum horridum*: no heavy rainfall occurred prior to burning, the temperature range was between 5 and 15 ºC, the air relative humidity was of 35-70 % and the wind speed of 5-10 km h^{-1}. At the Buisán site, approximately 3.8 ha were burned by point source fires at a rate of 0.64 ha h^{-1}; in Asín, backing fires were applied downslope in a 7.4 ha area at a burning rate of 2.72 ha h^{-1}, and in Yebra, 2.2 ha were burned at 1.43 ha h^{-1} by a head fire favored by wind. The detailed description of each burning and the temperatures recorded at soil surface using type-K thermocouples are provided in Table 1.

Table 1. General characteristics of the study sites and prescribed burnings. The temperature analysis comprises the elapsed time since a temperature increase was detected until it stabilized during the cooling stage.

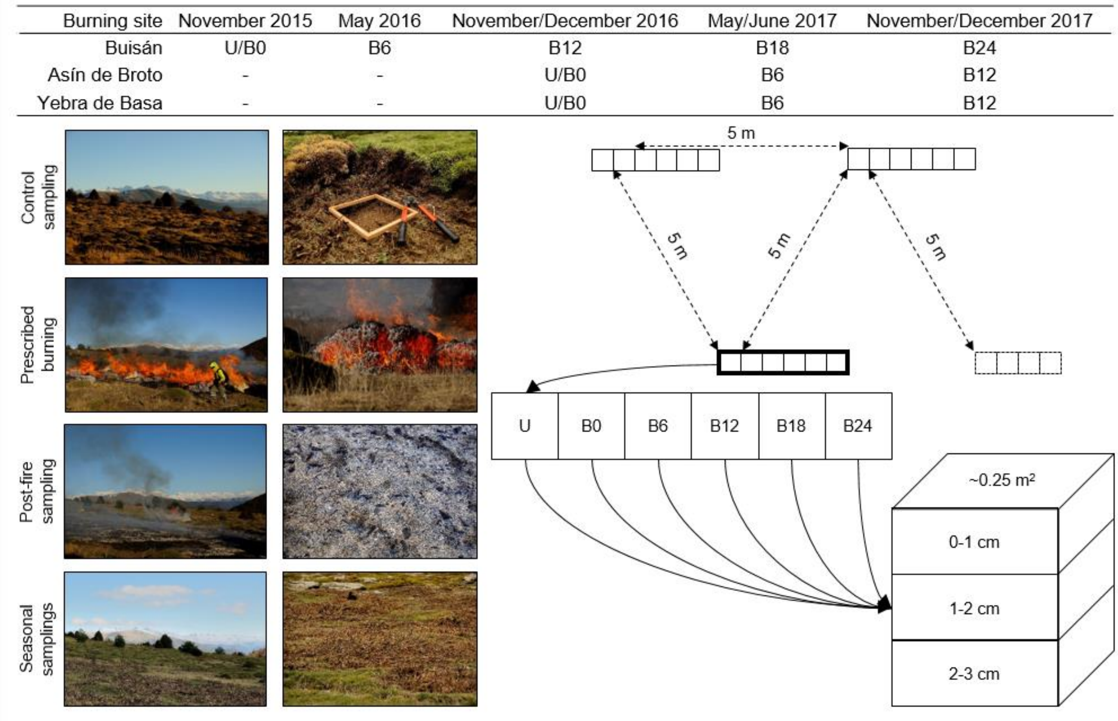
Study Site	**Buisán**	**Asín de Broto**	**Yebra de Basa**
Coordinates	42º 36' 04.4" N	42º 31' 12.3" N	42º 30' 55.0" N
	0º 00' 43.3" E	0º 06' 02.4" W	0º 15' 47.9" W
Elevation (m a.s.l.)	1760	1650	1480
Mean annual temperature (ºC)	6	8.8	8.3
Mean annual precipitation (mm)	1500	1120	1015
Aspect	S	W	S
Mean slope (%)	10	35	5
Soil classification (IUSS WRB 2014)	Eutric Cambisol	Calcaric Cambisol	Leptic Cambisol
Echinospartum horridum cover (%)	75	95	75
Estimated fuel loads (kg m^{-2}):			
Aerial biomass	9.24	11.71	9.24
Litter (OL + OF)	1.62	2.05	1.62
Burning Date	November 2015	November 2016	December 2016
Burned surface (ha)	3.8	7.4	2.2
Wind speed (km h^{-1})	<8	10-15	10-15
Firing technique	Point source fire	Backing fire	Head fire
Flame height (m)	1	0.7-1	1-3
Flame length (m)	1.5	0.65-1	1.5-3
Burning rate (ha h^{-1})	0.64	2.72	1.43
Temperature at soil surface:			
Maximum temperature (ºC)	438	768	595
Initial temperature (ºC)	13.1	7.5	4.9
Final temperature (ºC)	27.5	24.3	10.2
Temperature residence time (min):			
< 60 ºC	2.50	15.0	24.9
60-100 ºC	15.0	5.75	1.33
100 - 200 ºC	6.00	2.50	1.50

200 - 300 ºC	4.00	1.42	0.83
300 - 400 ºC	2.00	0.58	1.25
> 400 ºC	0.50	4.83	0.25
Pre-fire soil water content (%, 0-1 cm)	147 ± 17	37.0 ± 3.4	29.0 ± 3.3
Post-fire soil water content (%, 0-1 cm)	82.1 ± 37.4	44.0 ± 7.8	27.5 ± 10.2

Note: soil water contents are expressed as mean ± standard deviation of three (Buisán and Asín) or four (Yebra) field replicates that correspond to the sampling plots.

2.3 Soil sampling

For each site, three (Buisán and Asín) or four (Yebra) representative spots that were covered by *Echinospartum horridum* were selected prior to burning. After removing shrubs and the litter layer, the topsoil Ah horizons at each sampling point were meticulously scrapped using a spatula over surface areas of approximately 0.25 m^2 at 0-1, 1-2 and 2-3 cm depths. The soil samplings were conducted early in the morning immediately before burning to obtain unburned (U) samples that were considered controls. As soon as the soil cooled after burning, points adjacent to U were sampled after removing ashes and charred remains to assess the immediate effect of fire (B0). Afterwards, to monitor the evolution of soil after burning, seasonal samplings at points contiguous to U and B0 were conducted every 6 months up to 24 months in the case of Buisán (B6, B12, B18 and B24) and up to 12 months in the case of Asín and Yebra (B6, B12) (Fig. 2). Only the uppermost layer of the soil was sampled, as previous studies conducted under this kind of prescribed burning detected no fire effects below 3 cm depth (Armas-Herrera et al., 2016, 2018; Girona-García et al., 2018a, 2018b, 2018c). Additionally, a laboratory study that produced a temperature residence time that was similar to that in the present study showed that fire effects may be confined to the first 3 cm, especially when burning in wet conditions (Badía et al., 2017). All samples were collected in plastic bags to avoid desiccation and were rapidly transported and stored at 4 ºC to maintain the fresh conditions. Additionally, at each study site, 8 undisturbed soil samples were collected using steel cylinders (5 cm x 5 cm) for bulk density determination.



Burning site	November 2015	May 2016	November/December 2016	May/June 2017	November/December 2017
Buisán	U/B0	B6	B12	B18	B24
Asín de Broto	-	-	U/B0	B6	B12
Yebra de Basa	-	-	U/B0	B6	B12

Fig. 2. Sampling design followed throughout the study period for each study site. U: unburned samples; B0: immediate postfire samples; B6, B12, B18, B24: seasonal samplings every 6 months after burning.

2.4 Sample preparation and analysis

The fresh (not-dry) samples were sieved to fine earth (2 mm) and kept at 4 ºC for subsequent biological analysis. A portion of each sample was separated, air-dried until constant weight and ground to fine powder for total C and N, oxidizable C and carbonate content determination. The soil water content was obtained by gravimetric measurements to express all results based on 105 ºC dried soil. Each of the first 3 centimeters of the undisturbed soil samples obtained with the steel cylinders was carefully separated, dried at 105 ºC and weighed individually to determine bulk density.

The soil total carbon (STC) and nitrogen (N) were obtained by dry combustion using an elemental analyzer (Vario Max CN Macro Elemental Analyser, Germany). The soils at the Buisán site contained carbonates; thus, equivalent $CaCO_3$ was determined by the Bernard calcimeter method to determine the C in the form of $CaCO_3$, and this value was subtracted from the STC to obtain the total soil organic C (SOC). In the case of Asín and Yebra, STC corresponds to SOC, as soils showed no carbonate contents. From these data, the SOC and N stocks (SOCS and NS, respectively) contained in each soil layer, expressed in Mg ha^{-1}, were calculated as follows:

$$SOCS = SOC \times BD \times th \times (1 - CF) \times 0.01$$

where SOC is the concentration (g kg^{-1}) in the fine earth (<2 mm), BD is the soil bulk density (kg m^{-3}), *th* is the thickness (m) of each soil layer, and CF is the coarse fraction (>2 mm) expressed as CF per total soil (kg kg^{-1}). NS were calculated by the same equation but instead using the N concentrations.

The microbial biomass C (MBC) was determined following the chloroform fumigation-extraction method detailed in Vance et al. (1987) and an extraction factor of K_c=0.38 was applied. From these data, the MBC/SOC ratio was also calculated. Incubation assays of 28 days were conducted under controlled conditions of 25 ± 1 ºC and darkness, and the soil water content was maintained at values of 50 % (w w^{-1}) of field capacity. The emitted CO_2 was captured by NaOH traps and titrated with HCl, following the procedure described in Anderson (1982) on specified days of the incubation (days 1, 2, 4, 7, 10, 14, 18, 23 and 28). From these experiments, the C-CO_2 efflux (soil basal respiration, SR) was obtained, the C mineralization coefficient (CMC) was expressed as SR per oxidizable C unit and time, and the microbial metabolic quotient (qCO_2) was calculated as SR per MBC unit and time. For the determination of the oxidizable C, the wet-oxidation method with chromic acid (Nelson & Sommers, 1982) was followed. The soil β-D-glucosidase enzymatic activity was determined by the Eivazi and Tabatabai (1988) method.

2.5. Data analysis

To identify differences among the variables related to sampling time and soil depth, one-way ANOVA tests were performed because the interaction between time and depth was significant in most cases. The sampling time (U, B0, B6, B12, B18 and B24) was considered a categorical independent variable to analyze the effects of fire and time, and the data were split by soil depth (0-1, 1-2 and 2-3 cm). The variations in soil properties among soil depths were tested using soil depth as a categorical independent variable, and the data were split by sampling time (U, B0, B6, B12, B18 and B24). All the data met the assumptions of normality and homoscedasticity, and no further transformations were required. These analyses were performed using StatView for Windows version 5.0.1 (SAS Institute Inc. Cary, North Carolina, USA). A principal component analysis (PCA) was also performed to identify the relationships among the soil properties, using the Pearson correlation and a varimax rotation with Kaiser normalization (XLSTAT 2017. Addinsoft, Paris, France). The values reported in the text are expressed as the mean ± standard deviation unless otherwise noted.

3. Results

3.1. Intensity and severity of prescribed burning

The soil surface conditions observed immediately after the fire showed traits of low- to moderate-severity burning according to the indicators specified in Parsons et al. (2010). In the Asín and Yebra burnings, the fire consumed a small part of the uppermost litter layer, and there was no evidence of intense heat transfer into the soil. However, at the Buisán site, a high litter consumption was observed that resulted in scattered patches of bare soil, litter and ashes (black, gray and white). At the Yebra and Asín sites, thick accumulations of gray ashes over thick unburned litter layers were detected, indicating that ashes could come from the scorched canopy rather than from litter combustion. In addition, the soil structure remained unchanged and no aggregate weakening was observed at any site. At the Yebra and Asín sites, fine roots near the soil surface were not affected by burning, whereas some scorched fine roots were observed at Buisán site.

The data obtained using the type-K thermocouples placed on the soil surface showed maximum temperatures of 438 ºC (Buisán), 768 ºC (Asín) and 595 ºC (Yebra). Despite the lower maximum temperature registered in Buisán, a higher temperature residence time was observed compared to those in Asín and Yebra. In Buisán, temperatures were maintained over 60 ºC at the soil surface for ~27 min, whereas these temperatures were maintained for ~15 min in Asín and ~5 min in Yebra. This result could be due to the slow wind speed and the firing technique applied in Buisán, which resulted in a slow spread of fire and therefore, higher residence times. These differences were also reflected in the variations in soil water content after burning (Table 1). At the Buisán site, the soil water content at 0-1 cm in U was 147 ± 17 % and was significantly reduced by burning (B0) to 82.1 ± 37.4 %. On the other hand, in Asín and Yebra, the soil water contents remained unaltered during burning. Given the low number of observations, the temperature measurements can be considered only approximations of the fire intensity reached during the burnings.

3.2. Ground cover evolution after burning

At the Buisán site, ashes mixed with litter were still observed up to one year after burning (B12) and some integration into the soil of these materials was visually detected in the samplings that followed. At the Asín and Yebra sites, high amounts of ashes were still present between the soil surface and the litter layer in B6 while in B12, ashes were mixed with the uppermost topsoil layers. Although the sampling plots in Asín were located on a steep slope (35 %), no traits of fire-induced erosion were detected during the study period. Approximately 8 months after the fire, the burned plots in Buisán were mainly colonized by *Carex flacca* Schreb., *Carex humilis* Leyss., *Euphorbia cyparissias* L., *Iris latifolia* (Mill.) Voss, *Teucrium chamaedrys* L. and *Viola* cf. *rupestris*. Although the vegetation was not inventoried in B24, it was observed that the burned areas were covered to greater extents by herbaceous species and some incipient *Echinospartum* *horridum* seedlings. Nine months after burning, Badía et al. (2017a) described that vegetation represented the 29.2 % of the burned plot surface in Yebra. At this site, vegetation recovery was driven by resprouter species such as *Brachypodium pinnatum* (L.) Beauv., *Bromus erectus* Huds., *Agrostis capillaris* L., *Carex flacca* Schreb., *Sanguisorba minor* Scop., *Galium verum L., Teucrium chamaedrys* L., *Onopordum acaulon L.* and *Cirsium vulgare* (Savi) Ten. Vegetation recovery in Asín was scarce and slow compared to that in Buisán and Yebra. The burned surface in B12 was mainly covered by stones and litter, and few scattered *Buxus sempervirens* L. and *Onopordum acaulon* L. individuals were found. At all sites, it was observed that the burned *Echinospartum horridum* started to regenerate approximately one year after the fire.

3.3. Impacts of prescribed burning on soil organic C and N stocks

The total organic C stocks (SOCS) in the U soils varied among the study sites (Fig. 3a). The SOCS of U at Buisán were 12.8 ± 0.5 Mg ha^{-1} at 0-1 cm depth and gradually decreased to 4.74 ± 0.85 Mg ha^{-1} at 2-3 cm depth. At the Asín and Yebra sites, lower SOCS were detected in U compared to those at the Buisán site, and the values were similar for all studied soil depths. The SOCS in the topsoil of Asín ranged from 1.91 ± 0.27 to 2.6 ± 0.55 Mg ha^{-1}, whereas at Yebra, they varied from 3.54 ± 0.39 to 4.29 ± 0.51 Mg ha^{-1}. The soil N stocks (NS) showed the same trends observed in the SOCS for U soils (Fig. 3b). In Buisán, NS were of 0.769 ± 0.036 Mg ha^{-1} at the 0-1 cm soil depth and decreased to 0.369 ± 0.055 Mg ha^{-1} at 2-3 cm depth. On the other hand, NS ranged from 0.139 ± 0.028 to 0.185 ± 0.037 Mg ha^{-1} in Asín and 0.306 ± 0.024 to 0.362 ± 0.067 Mg ha^{-1} in Yebra at all studied soil depths.

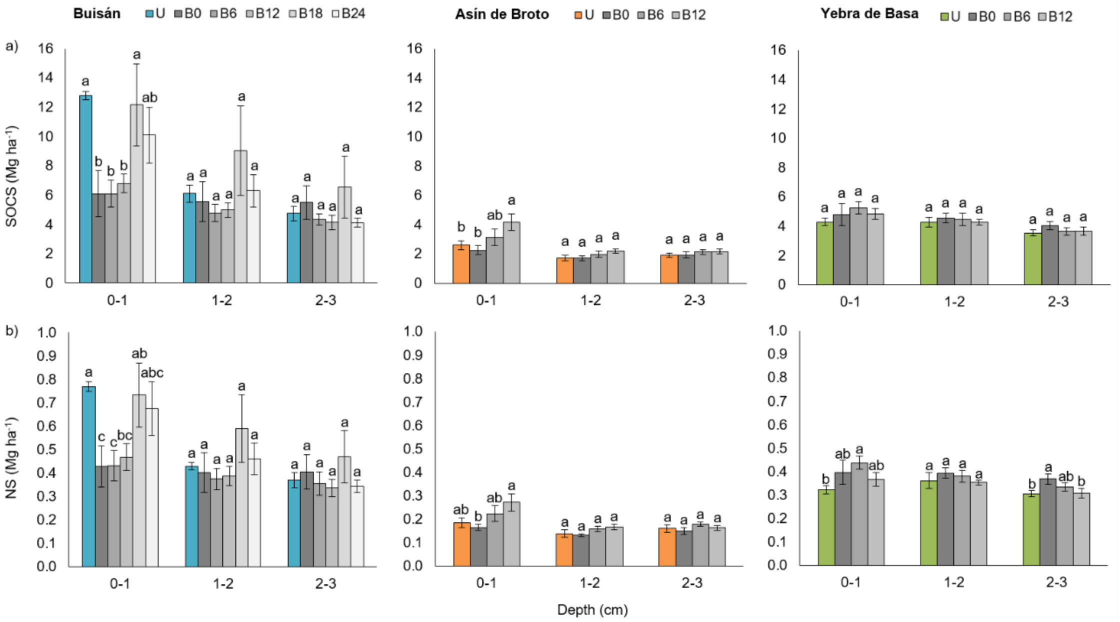


Fig. 3. Soil total organic carbon stocks (SOCS) and total nitrogen stocks (NS) for each study site, sampling time and soil depth (mean ± standard error). U: unburned samples; B0: immediate postfire samples; B6, B12, B18, B24: seasonal

samplings every 6 months after burning. Lowercase letters indicate significant differences at $p<0.05$ among sampling times for each studied soil depth and site.

The prescribed fires had contrasting effects on SOCS and NS among the study sites. At the Buisán site, burning (B0) significantly decreased SOCS (-52 %) and NS (-44 %) at the 0-1 cm depth compared to U, although these values remained unchanged in deeper soil layers. In Asín and Yebra, fire had no effects on topsoil SOCS and NS, whose B0 values were similar to those of U at all studied soil depths. The effects of fire detected on SOCS and NS at the Buisán site were still present in B6 and B12, and no differences compared to B0 were observed. Nevertheless, in the B18 and B24 samplings, the effects of fire on SOCS and NS were not as evident, as their values resembled the values of the U samplings. At the Yebra site, soils showed no variations in SOCS and NS in the short term, as reflected in the B6 and B12 values, which were statistically similar to the values from U and B0; however, at the Asín site, the SOCS values at B12 for 0-1 cm were significantly higher than those of U and B0. The SOM quality, as observed in the SOC/N ratio (Table 2), varied according to these results, and it significantly decreased after the Buisán burning at 0-1 cm and showed an increasing trend towards the U values over time. Consequently, the SOC/N ratio remained unchanged in all samplings at the Asín and Yebra sites.

3.4. Effects of prescribed burning on soil biological properties

The microbial biomass C (MBC) content in the studied U soils of Buisán was 35.6 ± 4.8 g kg^{-1} at 0-1 cm depth and gradually decreased to 14.6 ± 7.0 g kg^{-1} at 2-3 cm depth (Fig. 4a). The MBC content of the Asín U samples showed no differences among depths and ranged from 12.5 ± 6 to 18.2 ± 5.7 g kg^{-1}. In Yebra, the lowest MBC values in the U samples of the studied sites were found, and varied from 4.01 ± 0.86 to 5.73 ± 1.55 g kg^{-1} and no differences were detected among depths. In Buisán, the burning caused a significant decrease in the MBC content (-57 %) at the 0-1 cm depth, whereas no changes were detected in deeper layers. This behavior is different from that found for Asín and Yebra, where the MBC contents showed no differences between U and B0 samplings. A severe impact of fire on the MBC in Buisán was detected throughout the study period, and no differences were observed among the B0 and B6-to-B24 samplings, although an increasing trend was observed over time. Despite the lack of changes in the MBC content after fire in Asín, the values decreased significantly in B6 and B12 at all studied soil depths. On the other hand, at the Yebra site, the MBC values showed a transient pulse in B6 at 0 to 3 cm depth that recovered to U and B0 values one year after burning. The MBC/SOC ratio (Table 3) in the Buisán samples showed no changes among samplings because the MBC and SOC values varied equally throughout the study period. On the other hand, at the Asín and Yebra sites, where SOC did not change by burning or over time, the MBC/SOC ratio changed according to the MBC content.

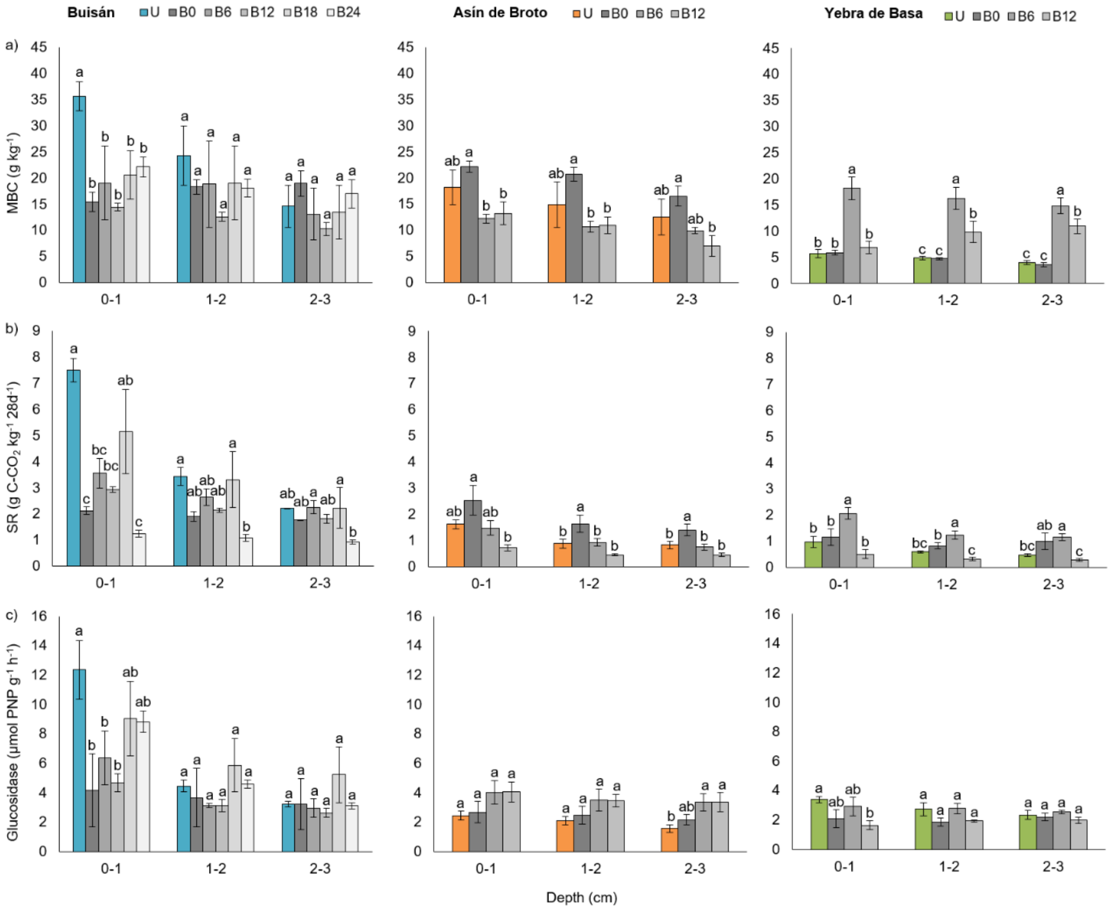


Fig. 4. Microbial biomass carbon (MBC), soil basal respiration (SR) and β-D-glucosidase activity (Glucosidase) for each study site, sampling time and soil depth (mean ± standard error). U: unburned samples; B0: immediate postfire samples; B6, B12, B18, B24: seasonal samplings every 6 months after burning. Lowercase letters indicate significant differences at p<0.05 among sampling times for each studied soil depth and site.

The soil basal respiration (SR) in the U samples from the Buisán site was significantly higher than those from the Asín and Yebra sites (Fig. 4b), and it showed a steep decreasing gradient with depth. In the Buisán burning, the SR was 72 % lower in B0 than that recorded in U at 0-1 cm depth and although not significant, there was a decreasing trend in deeper layers. On the other hand, at the Asín and Yebra sites, the SR values in B0 remained almost unchanged compared to those of U. The SR showed transient pulses in the spring samplings from Buisán (B6 and B18) and Yebra (B6) although no seasonal variations were observed in Asín. Nevertheless, in the B24 samples collected in Buisán and the B12 samples collected in Asín B12, the SR significantly decreased at all studied depths compared to B0, and the pulse observed in Yebra-B6 disappeared in B12.

The changes in the SR were reflected in the C mineralization coefficient (CMC) which was directly reduced by burning at 0-1 cm depth and kept decreasing at all studied soil depths up to B24 in Buisán (Table 3). In Asín, the CMC significantly increased at all studied depths in B0, although it decreased over time, and the lowest CMC values for this site were observed in B12 at the 0-1 and 1-2 cm depths. At the Yebra site, the CMC was not affected by burning, and in the same way as SR, it showed a transient pulse in B6 at 0 to 2 cm that was not detected in B12.

The microbial metabolic quotient (qCO_2) in Buisán showed slight variations with fire and sampling time up to B24, when it significantly decreased compared to the previous samplings (Table 3). In Asín, the qCO_2 did not change at any sampling time or depth during the studied period. In contrast, the qCO_2 of Yebra showed slight changes over time that followed a decreasing trend at all studied depths.

The β-D-glucosidase activity in the Buisán U samples was three-fold higher than those in the Asín and Yebra U samples (Fig. 4c). The β-D-glucosidase suffered from a 66 % decrease at 0-1 cm in Buisán, which was still evident in the B6 and B12 samplings. However, in B18 and B24, the fire-affected layer showed intermediate β-D-glucosidase values between U and B0, suggesting signs of recovery. In the Yebra site, β-D-glucosidase was not affected by burning, although it significantly decreased in B12 at 0-1 cm depth compared to U. On the other hand, β-D-glucosidase remained unchanged in Asín over time.

The obtained results are well summarized by the PCA (Fig. 5). Axis D1, which accounted for 49.03 % of the variation, distributed the samples by SOC, N and MBC contents as well as parameters related to biological activity such as the CMC and β-D-glucosidase. The Buisán samples showed higher positive loadings, especially in the U samples at 0-1 cm depth. The effects of fire at this site could be clearly observed, as burned samples showed lower positive loadings similar to those of the deeper soil layers in the U samples. The distribution of the Buisán samples on axis D1 also showed recovery in the soil properties of B18 and B24 because they were located near the U samples. However, axis D1 did not show any clear trend for Asín and Yebra, as the values of their soil properties were similar. On axis D2 (22.71 %), the Asín samples showed higher positive loadings due to the MBC/SOC ratio and SR, whereas the Yebra samples were distributed by their higher qCO_2. The effects of fire and time in Asín and Yebra showed opposite trends compared to Buisán, as indicated by the arrows in Fig. 5a. This result occurred as a consequence of the Yebra and Asín values being separated by biological parameters that progressively decreased in B6 and B12. Nevertheless, most of these variations can be considered only trends because no significant differences were detected among them.

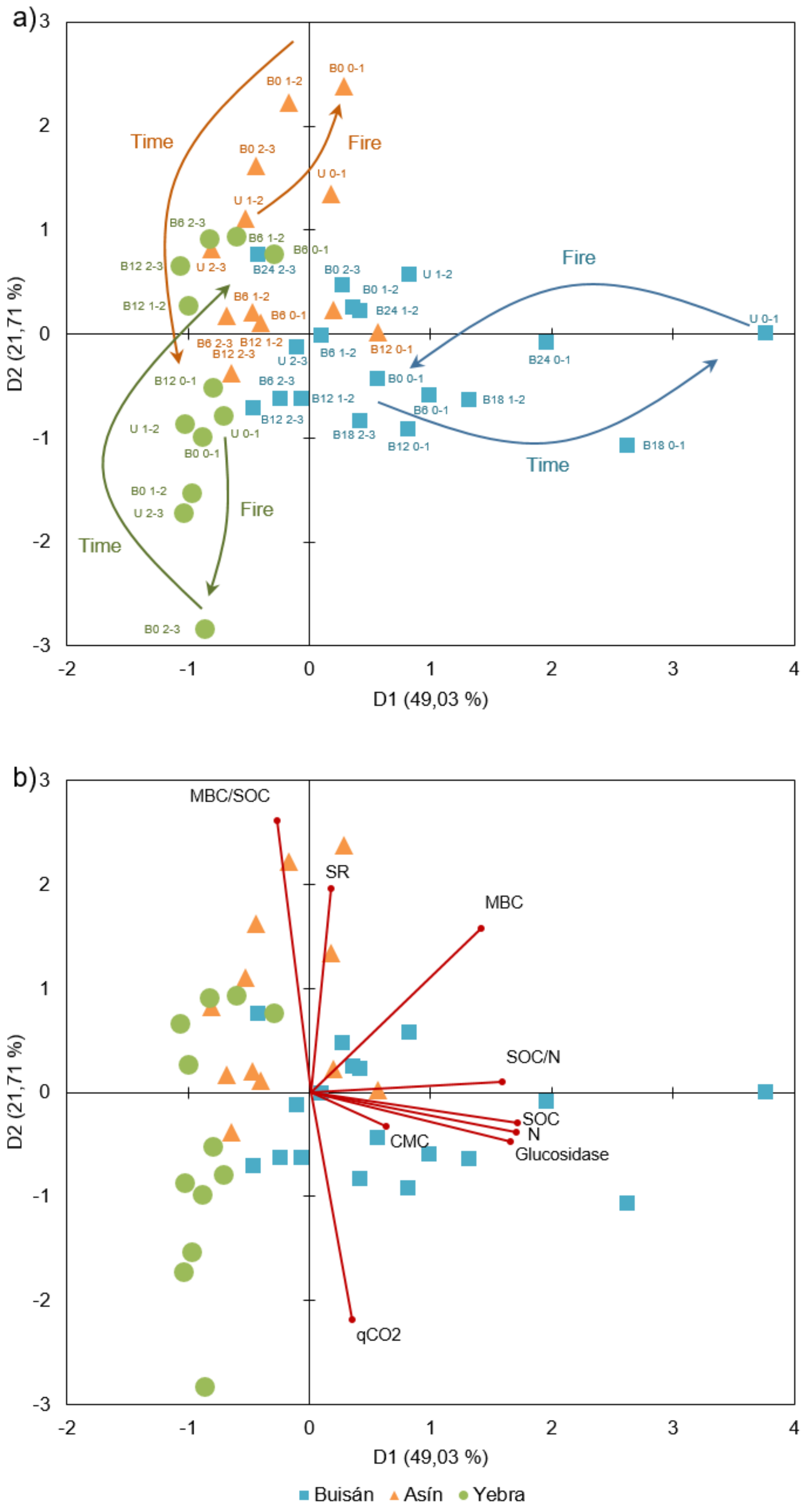


Fig. 5. Principal component analysis (PCA) after varimax rotation for the observations (a) and the variables (b). In figure a) abbreviations refer to unburned samples (U), immediate postfire samples (B0) and seasonal samplings every 6 months after burning (B6, B12, B18, B24) for each soil depth. Abbreviations in figure b) show soil total organic carbon (TOC), total

nitrogen (N), microbial biomass carbon (MBC), soil basal respiration (SR), carbon mineralization coefficient (CMC), microbial metabolic quotient (qCO_2) and β-D-glucosidase activity (Glucosidase).

4. Discussion

4.1. Contrasting effects of prescribed burning on SOC and N stocks and dynamics

The effects that heat may have on soil properties depend not only on the temperatures reached but also on their residence time. This phenomenon is clearly reflected in the contrasting results that were obtained in this study, which are well correlated with the temperature observations. Immediately after burning, significant effects were detected only in Buisán, where the lowest maximum temperature among sites was found. Nevertheless, temperatures were maintained over 60 ºC for approximately 27 min which was a higher heat residence time than those at Asín (~15 min) and Yebra (~5 min). The SOM can be substantially reduced when temperatures increase to a range of 200-250 ºC over a certain amount of time (Certini, 2005), which explains the remarkable decrease of SOCS and NS observed at 0-1 cm depth in Buisán. On the other hand, the high soil moisture content in the U soils of Buisán could have limited the transfer of heat to deeper soil layers because the latent heat of water prevents sudden temperature increases until it is completely vaporized (Campbell et al., 1995; Badía et al., 2017b). The fire-affected depths also showed changes in the SOC/N ratio, suggesting that N was immobilized into more recalcitrant forms or that fire had a greater effect on SOC (González-Pérez et al., 2004; Knicker, 2007). Severe reductions in SOC and N were also observed in Armas-Herrera et al. (2016) after a similar prescribed burning of *Echinospartum horridum* in the Central Pyrenees, and this impact was attributed to the high intensity of fire. In contrast, prescribed burning had no effects on SOCS and NS in Asín and Yebra, which agrees with the results of previous studies (Alexis et al., 2007; Marcos et al., 2009; Fontúrbel et al., 2012, 2016), and in our case, this result was probably a consequence of the rapid propagation of the fire. However, our data contrast the increases in SOC and N shortly after burning that are commonly reported in the literature (Úbeda et al., 2005; González-Pelayo et al., 2015; Alcañiz et al., 2016; San Emeterio et al., 2016), which are related to the contributions of ashes and charred vegetal remain. Nevertheless, because the soil was sampled hours after burning and the ashes were removed in the present study, this effect could not be detected. At the Yebra and Buisán sites, neither SOCS nor NS showed changes up to B12, indicating a poor integration of ashes and charred remains into the soil; nevertheless, this process could have occurred at the Asín site in B12 because the SOCS values were higher than those in the U and B0 soils. In Buisán, this integration could have occurred over a longer term, as can be observed in the results from B18 and B24, in which the SOCS and NS recovered to values similar to those of U. This recovery diverges from the results obtained in Armas-Herrera et al. (2018) after *Echinospartum horridum* prescribed fire in a nearby site, which indicated that the SOC and N contents did not recover even 5 years after the burning, which was related to the fire intensity and the occurrence of soil erosion processes.

The burning of soils with high water contents can limit the maximum temperatures reached (Campbell et al., 1995; Badía et al., 2017b), but it can also induce increased mortality of microorganisms in the uppermost soil layers as a consequence of moist heat (Choromanska & DeLuca, 2002); therefore, the effects of fire on soil biology can be marked when temperatures increase to 50-210 ºC (Mataix-Solera et al., 2009). For this reason, a severe decrease was observed in the MBC at Buisán in B0 because it is a sensitive soil property that can be altered at temperatures over 50 ºC (Bárcenas-Moreno & Bååth, 2009). This result is in accordance with previous works that showed that MBC decreased after prescribed burning in the uppermost topsoil layers (Fontúrbel et al., 2012; Armas-Herrera et al., 2016). The MBC/SOC ratio showed only slight variations after burning in Buisán and Asín, which suggests that the microbial population fluctuated in the same way as the available C. Therefore, the transient pulse observed in the MBC in Yebra as well as the MBC/SOC in B6 might be related to an increase in nutrient availability (Fontúrbel et al., 2016). The remarkable effect of fire on MBC observed at Buisán in B0 is clearly reflected in the SR reduction, which is a probable effect usually reported in the literature (Choromanska & Deluca, 2001; Hamman et al., 2008; Armas-Herrera et al. 2016). After burning, the SR showed a trend towards recovery up to B18, but it dramatically decreased in B24. Because MBC remained virtually unchanged after B0, changes in the SR and CMC could be related to shifts in microbial communities as well as a reduction in labile C because its availability determines C mineralization rates (Hamman et al., 2008). For this reason, despite the SOCS increase at Buisán in B24, the CMC plummeted, which suggests the contribution of C forms that are more resistant to microbial attack. The decrease in β-D-glucosidase immediately after burning at Buisán could be related to enzyme denaturation by heat (Knicker, 2007) and have also been observed in previous works conducted on prescribed fires (López-Poma & Bautista, 2014; Armas-Herrera et al., 2016). However, this effect was not detected in Asín or Yebra, which is also in line with the studies that indicated that burning had no effect on this property (Boerner et al., 2008; Fontúrbel et al., 2016). Nevertheless, the β-D-glucosidase activity over time differed among the study sites. In Buisán, an increasing trend towards recovery could be observed in the β-D-glucosidase values of B18 and B24. Similar results were obtained in Barreiro et al. (2010) and López-Poma & Bautista

(2014), where the reduction in the β-D-glucosidase activity observed after burning was still present one year later but started to recover from that point. In the case of the Yebra burning, although fire had no immediate effects on the β-D-glucosidase, it showed a decreasing trend over time, and the B12 values were lower than those of the U at 0-1 cm. This reduction could be a consequence of low labile C content, as hypothesized for SR and CMC, because β-D-glucosidase is regulated by substrate availability (Barreiro et al., 2016). In this way, the results suggest that the variations in the biological properties observed after burning may be a direct consequence of not only fire but also changes in SOM quality and microbial communities (Hart et al., 2005; Fontúrbel et al. 2016). Additionally, variations in soil microbiology are tightly related to changes in the vegetation, which can be a more predominant factor than the direct effects of fire itself in the recovery of microbial activity (Hart et al., 2005). Therefore, the slow recovery of vegetation might have had a negative impact on soil biological activity. On the other hand, the high soil biological activity observed prior to burning in the U soils could have been related to the favorable environment developed under the *Echinospartum horridum* canopy, where temperatures are softened and high levels of soil moisture are maintained (Cavieres et al., 2007).

It is noteworthy that the differences observed in our study compared to the results obtained after other prescribed burnings can be related to many variability-inducing factors, such as 1) the intensity, duration and distribution of fire (Granged et al., 2011); 2) moisture and amount of fuel loads (Neary et al., 1999); 3) weather conditions (Fernandes et al., 2013); 4) soil water content (Massman, 2012); 5) the sampled soil thickness (Badía-Villas, et al., 2014) and 6) whether the ashes were completely removed prior to sampling (Girona-García et al., 2018b).

4.2. Sustainability of utilizing prescribed burning for pastureland management

The results obtained in the present study provide valuable information regarding the sustainability of utilizing prescribed burning as a pastureland management tool in mountain environments. The studied soils stored large amounts of SOCS and NS so fire-induced changes even within the uppermost few centimeters of soil may account for high SOM losses. For this reason, it is of importance the contrasting effect that prescribed burning may have on soil depending on topography, wind speed and the ignition technique applied when burning shrubs that accumulate high biomass loads. The Buisán results indicated that burning might not be appropriate in plain areas under slow wind conditions. Nevertheless, the application of a head fire for burning in a plain area, as in the case of Yebra, where there was a moderate wind speed, resulted in no immediate changes to soil properties, and most of the litter remained unburned. Although the Asín burning was performed downslope and high temperatures were registered at the soil surface, the immediate effects of fire on soil were almost negligible due to the rapid spread of fire.

The results also suggest that burning in autumn may have a positive effect in avoiding material losses because the snowfall that followed the burnings could have stabilized the ashes and litter remains (Hamman et al., 2008). The maintenance of the litter layer is also important to prevent further C losses by soil erosion and to favor litter retention and incorporation into the soil. In addition, the decrease in soil biological activity may allow for higher C sequestration, which is probably favored by the incorporation of partially charred remains. However, these results indicate the need for further research on whether the substantial C losses produced by above-ground biomass combustion could be balanced by the improved C sequestration that is expected in the soil and the development of pastures. Some authors advise against the use of burning as a tool for shrub removal due to its effects on soil and suggest mechanical clearing as a better management practice (Nadal-Romero et al., 2018b; Nuche et al., 2018). In contrast, other authors claim that recurrent burning combined with grazing is the best practice for avoiding plant succession towards shrubs (Bartolomé et al., 2005; Komac et al., 2011). However, the literature agrees with the fact that grazing alone is not enough for shrub control (Nadal-Romero et al., 2018b), especially in the Pyrenees, where species such as *Echinospartum horridum* rapidly recover due to undergrazing (Montserrat et al., 1984; Badía et al., 2017a). In our study sites, the density of the livestock that grazed after burning did not seem to be enough to control the recovery of *Echinospartum horridum* because its seedlings quickly developed in the burned areas. Because either clearing and prescribed burning have not always had satisfactory outcomes (Gartzia et al., 2014), further research based on a comparative approach is needed to assess the suitability and sustainability of both practices.

5. Conclusions

The study shows that the prescribed burning of shrubs may have contrasting effects on SOCS and related biological properties. The use of prescribed fire on plain slopes under slow wind conditions exerted severe negative effects on SOCS and soil biological activity at 0-1 cm depth. These results were related to the long fire residence time that caused the partial consumption of litter, which allowed a higher amount of heat transfer to the soil surface. It is important to acknowledge this effect on soil because even changes in a small soil thickness may account for high C losses in ecosystems that store large amounts of SOM, such as pasturelands. However, when the site and environmental conditions favored the rapid spread of fire, the soil properties remained virtually unchanged. Apart from the direct impact of fire, variations in the C dynamics over time were observed as a consequence of the new postfire environmental conditions. At the site where burning remarkably reduced SOCS and biological properties at 0-1 cm, SOCS recovered to values similar to those of the unburned soils 2 years after burning. The results obtained at the three sites for the short- and mid-term showed a decreasing trend in C mineralization, which was probably due to the changes in the quality of new SOM inputs and the variations in cycling rates. Therefore, it is essential to study whether the increase in soil organic C content and pasture development could balance the losses produced by the burning of biomass over the long term.

Acknowledgments

This study is part of the results of the FUEGONEO (CGL2016-76620-R) project funded by the Spanish “Ministerio de Economía y Competitividad”. A. Girona-García is funded by an 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. We also thank Clara Martí, Juan Luis Mora, Cecilia M. Armas-Herrera, Juan Esteban, Asunción Callizo and Belén Aguado for their assistance in the field and laboratory during the study. We also thank the anonymous reviewers for their valuable comments and suggestions.

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 (Montgrí Massif, Catalonia, Spain). Sci. Total Environ. 572: 1329-1335. DOI: 10.1016/j.scitotenv.2016.01.115

Alcañiz, M., Outeiro, L., Francos, M., Úbeda, X., 2018. Effects of prescribed fire on soil properties: A review. Sci. Total Environ 613.614: 944:957. DOI: 10.1016/j.scitotenv.2017.09.144

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, J.P., 1982. Soil respiration. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.). Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Agronomy Series, nº 9. Madison, pp. 831-871.

Armas-Herrera, C.M., 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

Armas-Herrera, C.M., Martí, C., Badía, D., Ortiz-Perpiñá, O., Girona-García, A., Mora, J.L., 2018. Short- and mid-term evolution of topsoil organic matter and biological properties after prescribed burning for pasture recovery (Tella, Central Pyrenees, Spain). Land Degrad. Develop. 29: 1545-1554. DOI: 10.1002/ldr.2937

Badía-Villas, D., González-Pérez, J.A., Aznar, J.M., 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., Armas, C., Mora, J.L., Gómez, D., Montserrat, G., Palacios, S., 2017a. ¿Podemos controlar la expansión del erizón mediante quemas? Lucas Mallada 19:69-94.

Badía, D., López-García, S., Martí, C., Ortiz-Perpiñá, O., Girona-García, A., Casanova-Gascón, J., 2017b. Burn effects on soil properties associated to heat transfer under contrasting moisture content. Sci. Total Environ. 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 temperatures to simulate a range of fire intensities. Soil Biol. Biochem. 41: 2517-2526. DOI: 10.1016/j.soilbio.2009.09.010

Barreiro, A., Carballas, M.T., Díaz-Raviña, M., 2010. Response of soil microbial communities to fire and fire-fighting chemicals. Sci. Total Environ. 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. Biol. Fertil. Soils 52: 963-975. DOI: 10.1007/s00374-016-1133-5

Bartolomé, J., Plaixats, J., Fanlo, R., Boada, M., 2005. Conservation of isolated Atlantic heathlands in the Mediterranean region: effects on land-use changes in the Montseny biosphere reserve (Spain). Biol. Conserv. 122: 81-88. DOI: 10.1016/j.biocon.2004.05.024

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

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

Campbell, G.S., Jungbauer, J.D., Bristow, K.L., Hungerford, R.D., 1995. Soil temperature and water content beneath a surface fire. Soil Sci. 159: 363-374. DOI: 10.1097/00010694-199506000-00001

Cavieres, L.A., Badano, E.I., Sierra-Almeida, A., Molina-Montenegro, M.A., 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. Arct. Antarct. Alp. Res. 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, T.H., 2001. Prescribed Fire alters the Impact of Wildfire on Soil Biochemical Properties in a Ponderosa Pine Forest. Soil Sci. Soc. Am. J. 65: 232-238. DOI: 10.2136/sssaj2001.651232x

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

Conant, R.T., 2010. Challenges and opportunities for carbon sequestration in grassland systems: A technical report on grassland management and climate change mitigation. Integr. Crop Manage. 9, FAO, Rome.

Dooley, S.R., Treseder, K.K., 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, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. and Biochem. 20 (5): 601-606. DOI: 10.1016/0038-0717(88)90141-1

Farley, K.A., Bremer, L.L., Harden, C.P., Hartsig, J., 2013. Changes in carbon storage under alternative land uses in biodiverse Andean grasslands: implications for payment for ecosystem services. Conserv. Lett. 6: 21-27. DOI: 10.1111/j.1755-263X.2012.00267.x

Fernandes, P.M., Davies, G.M., Ascoli, D., Fernández, C., Moreira, F., Rigolot, E., Stoof, C.R., Vega, J.A., Molina, D., 2013. Prescribed burning in southern Europe: developing fire management in a dynamic landscape. Front. Ecol. Environ. 11: 4-14. DOI: 10.1890/120298

Follett, R.F., Reed, D.A., 2010. Soil Carbon Sequestration in Grazing lands: Societal Benefits and Policy Implications. Rangeland Ecol. & Manag. 63(1): 4-15. DOI: 10.2111/08-225.1

Fontúrbel, M.T., Barreiro, A., Vega, J.A., 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, M.T., Fernández, C., Vega, J.A., 2016. Prescribed burning versus mechanical treatments as shrubland management options in NW Spain: Mid-term soil microbial response. Appl. Soil Ecol. 107: 334-346. DOI: 10.1016/j.apsoil.2016.07.008

Fultz, L.M., Moore-Kucera, J., Dathe, J., Davinic, M., Perry, G., Wester, D., Schwilk, D.W., 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. Appl. Soil Ecol. 99: 118-128. DOI: 10.1016/j.apsoil.2015.10.023

García-Pausas, J., Romanyà, J., Montané, F., Rios, A.I., Taull, M., Rovira, P., Casals, P., 2017. Chapter 9: Are Soil Carbon Stocks in Mountain Grasslands Compromised by Land-Use Changes?. In: Catalan, L., Ninot, J., Aniz, M. (eds.). High Mountain Conservation in a Changing World. Advances in Global Change Research 62, Springer, Cham. DOI: 10.1007/978-3-319-55982-7_9

Gartzia, M., Alados, C.L., Pérez-Cabello, F., 2014. Assessment of the effects of biophysical and anthropogenic factors on woody plant encroachment in dense and sparse mountain grasslands based on remote sensing data. Prog. Phys. Geogr. 38: 201. DOI: 10.1177/0309133314524429

Girona-García, A., Badía-Villas, D., Martí-Dalmau, C., Ortiz-Perpiñá, O., Mora, J.L., Armas-Herrera, C.M, 2018a. Effects of prescribed fire for pasture management on soil organic matter and biological properties: a 1-year study case in the Central Pyrenees. Sci. Total Environ. 618: 1079-1087. DOI: 10.1016/j.scitotenv.2017.09.127

Girona-García, A., Zufiaurre Galarza, R., Mora, J.L., Armas-Herrera, C.M., Martí, C., Ortiz-Perpiñá, O., Badía-Villas, D., 2018b. Effects of prescribed burning for pasture reclamation on soil chemical properties in subalpine shrublands of the Central Pyrenees (NE-Spain). Sci. Total Environ. 644: 583-593. DOI: 10.1016/j.scitotenv.2018.06.363

Girona-García, A., Ortiz-Perpiñá, O., Badía-Villas, D., Martí-Dalmau, C., 2018c. Effects of prescribed burning on soil organic C, aggregate stability and water repellency in a subalpine shrubland: variations among sieve fractions and depth. Catena 166: 68-77. DOI: 10.1016/j.catena.2018.03.018

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

Goldammer, J.G., 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, C.C.S., Ferreira, A.J.D., Keizer, J.J., Andreu, V., Rubio, J.L., 2015. Water repellency of air-dried and sieved samples from limestone soils in central Portugal collected before and after prescribed fire. Plant Soil 394: 199-214. DOI: 10.1007/s11104-015-2515-4

González-Pérez, J.A., González-Vila, F.J., Almendros, G., Knicker, H., 2004. The effect of fire on soil organic matter – a review. Environ. Int. 30 (6): 855-870. DOI: 10.1016/j.envint.2004.02.003

Granged, A.J.P., Jordán, A., Zavala, L.M., Muñoz-Rojas, M., Mataix-Solera, J., 2011. 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

Hamman, S.T., Burke, I.C., Knapp, E.E., 2008. Soil nutrients and microbial activity after early and late season prescribed burns in a Sierra Nevada mixed conifer forest. Forest Ecol. Manag. 256: 367-374. DOI: 10.1016/j.foreco.2008.04.030

Hart, S.C., DeLuca, T.H., Newman, G.S., MacKenzie, M.D., Boyle, S.I., 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. Forest Ecol. Manage. 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, M.B., Donelly, A., 2004. Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO_2. New Phytol. 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, C.L., Camarero, J.J., 2011. Influence of topography on the colonization of subalpine grasslands by the thorny cushion dwarf Echinospartum horridum. Arct. Antarct. Alp. Res. 43 (4); 601-611. DOI: 10.1657/1938-4246-43.4.601

Komac, B., Sefi, S., Nuche, P., Escós, J., Alados, C.L., 2013. Modeling shrub encroachment in subalpine grasslands under different environmental and management scenarios. J. Environ. Manag. 121:160-169. DOI: 10.1016/j.jenvman.2013.01.038

Lal, R., 2004. Soil carbon sequestration to mitigate climate change. Geoderma 123: 1-22. DOI: 10.1016/j.geoderma.2004.01.032

López-Poma, R., Bautista, S., 2014. Plant regeneration functional groups modulate the response to fire of soil enzyme activities in a Mediterranean shrubland. Soil Biol. Biochem. 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. Ecol. Eng. 35: 820-828. DOI: 10.1016/j.ecoleng.2008.12.011

Massman, W.J., 2012. Modeling soil heating and moisture transport under extreme conditions: forest fires and slash pile burns. Water Resour. Res. 48, W10548. DOI: 10.1029/2011WR011710

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

Montserrat, P., Montserrat, J.M., Montserrat, G., 1984. Estudio de las comunidades de *Echinospartum horridum* en el Pirineo Español. Acta Biol. Mont. 4: 249-257.

Nadal-Romero, E., Otal-Laín, I., Lasanta, T., Sánchez-Navarrete, P., Errea, P., Cammeraat, E., 2018a. Woody encroachment and soil carbon stocks in subalpine areas in the Central Spanish Pyrenees. Sci. Total Environ. 636: 727-736. DOI: 10.1016/j.scitotenv.2018.04.324

Nadal-Romero, E., Lasanta, T., Cerdà, A., 2018b. Integrating extensive livestock and soil conservation policies in Mediterranean mountain areas for recovery of abandoned lands in the Central Spanish Pyrenees. A long-term research assessment. Land Degrad. Develop. 29(2): 263-273. DOI: 10.1002/ldr.2542

Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F., 1999. Fire effects on belowground sustainability: a review and synthesis. Forest Ecol. Manage. 122: 51-71. DOI: 10.1016/S0378-1127(99)00032-8

Nelson, R.E., Sommers, L.E., 1982. Total carbon and organic matter. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.). Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties, second ed. American Society of Agronomy, Madison, Wisconsin, pp. 539-557.

Nuche, P., Komac, B., Gartzia, M., Villellas, J., Reiné, R., Alados, C.L., 2018. Assessment of prescribed fire and cutting as means of controlling the invasion of sub-alpine grasslands by *Echinospartum horridum*. Appl. Veg. Sci. DOI: 10.1111/avsc.12354

Parsons, A., Robichaud, P.R., Lewis, S.A., Napper, C., Clark, J.T, 2010. Field guide for mapping post-fire soil burn severity. Gen. Tech. Rep. RMRS-GTR243. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 49 p.

San Emeterio, L., Múgica, L., Gutiérrez, R., Juaristi, A., Pedro, J., Canals, R.M., 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, M.D., Goicoa, T., Canals, R.M., 2016. Sustainability of traditional pastoral fires in highlands under global change: Effects of soil function and nutrient cycling. Agric. Ecosyst. Environ. 235: 155-163. DOI: 10.1016/j.agee.2016.10.009

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

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19 (6): 703-707. DOI: 10.1016/0038-0717(87)90052-6

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

Table 2. Soil total organic carbon (SOC), total N (N) and SOC/N ratio for each site, sampling time and depth. Lowercase letters indicate differences at $p<0.05$ among sampling times for each studied soil depth and site.

		SOC ($g\ kg^{-1}$)						N ($g\ kg^{-1}$)						SOC/N					
	Depth (cm)	0-1		1-2		2-3		0-1		1-2		2-3		0-1		1-2		2-3	
Buisán	U	243 ± 10	a	108 ± 18	a	78.8 ± 14.1	a	14.6 ± 0.7	a	7.57 ± 0.50	a	6.15 ± 0.91	a	16.6 ± 0.7	a	14.2 ± 1.8	a	12.9 ± 1.8	a
	B0	116 ± 52	b	97.6 ± 41.4	a	91.4 ± 32.7	a	8.14 ± 2.87	c	7.07 ± 2.58	a	6.75 ± 2.12	a	13.9 ± 1.3	c	13.6 ± 0.9	a	13.5 ± 0.9	a
	B6	116 ± 30	b	84.0 ± 17.9	a	72.4 ± 11.2	a	8.18 ± 2.09	c	6.61 ± 1.39	a	5.92 ± 1.47	a	14.1 ± 1.0	c	12.7 ± 0.5	a	12.5 ± 1.6	a
	B12	129 ± 20	b	87.8 ± 15.2	a	68.9 ± 14.3	a	8.89 ± 1.86	bc	6.81 ± 1.23	a	5.61 ± 1.06	a	14.6 ± 1.0	bc	12.9 ± 0.3	a	12.3 ± 0.3	a
	B18	231 ± 92	a	159 ± 93	a	109 ± 61	a	13.9 ± 4.5	ab	10.4 ± 4.4	a	7.81 ± 3.19	a	16.3 ± 1.5	ab	14.7 ± 2.3	a	13.4 ± 2.0	a
	B24	192 ± 62	ab	111 ± 34	a	68.6 ± 8.4	a	12.8 ± 3.8	abc	8.10 ± 2.05	a	5.71 ± 0.74	a	14.9 ± 0.8	abc	13.6 ± 0.9	a	12.0 ± 0.2	a
Asín	U	91.5 ± 19.4	b	65.0 ± 11.9	a	58.3 ± 8.2	a	6.51 ± 1.30	ab	5.20 ± 1.06	a	4.94 ± 0.86	a	14.1 ± 1.3	a	12.5 ± 0.5	a	11.9 ± 0.9	a
	B0	79.5 ± 17.7	b	64.1 ± 11.2	a	58.8 ± 10.8	a	5.80 ± 0.81	b	4.94 ± 0.40	a	4.59 ± 0.70	a	13.6 ± 1.2	a	12.9 ± 1.3	a	12.8 ± 0.4	a
	B6	111 ± 34	ab	73.9 ± 13.5	a	65.4 ± 8.3	a	7.90 ± 2.06	ab	5.96 ± 0.71	a	5.48 ± 0.48	a	13.9 ± 1.0	a	12.4 ± 0.9	a	11.9 ± 0.7	a
	B12	147 ± 34	a	82.3 ± 10.3	a	66.1 ± 9.8	a	9.56 ± 2.29	a	6.23 ± 0.73	a	5.01 ± 0.54	a	15.4 ± 0.1	a	13.2 ± 0.4	a	13.2 ± 0.3	a
Yebra	U	54.1 ± 6.5	a	51.4 ± 7.9	a	45.5 ± 5.1	a	4.07 ± 0.42	b	4.35 ± 0.81	a	3.94 ± 0.31	b	13.3 ± 0.5	a	11.9 ± 0.9	a	11.6 ± 0.7	a
	B0	60.3 ± 18.9	a	54.6 ± 7.9	a	52.0 ± 6.9	a	5.00 ± 1.28	ab	4.74 ± 0.55	a	4.74 ± 0.57	a	12.0 ± 0.9	a	11.5 ± 0.9	a	11.0 ± 0.9	a
	B6	66.2 ± 10.5	a	53.5 ± 10.0	a	46.9 ± 6.7	a	5.53 ± 0.70	a	4.57 ± 0.59	a	4.29 ± 0.42	ab	11.9 ± 0.6	a	11.7 ± 0.9	a	10.9 ± 1.0	a
	B12	61.1 ± 9.5	a	51.6 ± 4.6	a	47.1 ± 7.2	a	4.62 ± 0.74	ab	4.25 ± 0.25	a	3.96 ± 0.55	b	13.3 ± 1.4	a	12.1 ± 0.7	a	11.9 ± 0.6	a

U: unburned samples; B0: immediate post-fire samples; B6, B12, B18, B24: seasonal samplings every 6 months after burning.

Table 3. Microbial biomass carbon to total organic carbon ratio (MBC/SOC), carbon mineralization coefficient (CMC) and microbial metabolic quotient (qCO_2) for each site, sampling time and depth. Lowercase letters indicate differences at $p<0.05$ among sampling times for each studied soil depth and site.

		MBC/SOC						CMC (g $C\text{-}CO_2$ g^{-1} Cox y^{-1})						qCO_2 (g $C\text{-}CO_2$ g^{-1} MBC y^{-1})					
	Depth (cm)	0-1		1-2		2-3		0-1		1-2		2-3		0-1		1-2		2-3	
Buisán	U	0.147 ± 0.025	a	0.219 ± 0.059	a	0.179 ± 0.058	a	0.699 ± 0.083	a	0.527 ± 0.135	a	0.421 ± 0.097	abc	2.59 ± 0.49	a	1.50 ± 0.11	ab	1.64 ± 0.42	ab
	B0	0.146 ± 0.050	a	0.210 ± 1.091	a	0.227 ± 0.092	a	0.454 ± 0.139	bc	0.486 ± 0.098	ab	0.549 ± 0.093	a	1.85 ± 0.34	ab	1.41 ± 0.48	ab	1.15 ± 0.28	ab
	B6	0.156 ± 0.064	a	0.208 ± 0.113	a	0.174 ± 0.089	a	0.491 ± 0.099	b	0.481 ± 0.009	ab	0.453 ± 0.043	ab	2.89 ± 1.18	a	2.32 ± 1.00	a	2.77 ± 1.34	a
	B12	0.114 ± 0.026	a	0.146 ± 0.025	a	0.156 ± 0.052	a	0.361 ± 0.072	bc	0.370 ± 0.049	bc	0.403 ± 0.092	a	2.66 ± 0.25	a	2.23 ± 0.21	a	2.37 ± 0.59	a
	B18	0.089 ± 0.001	a	0.118 ± 0.011	a	0.120 ± 0.024	a	0.305 ± 0.062	c	0.306 ± 0.086	cd	0.307 ± 0.033	a	3.09 ± 0.77	a	2.39 ± 0.94	a	2.34 ± 1.01	a
	B24	0.121 ± 0.031	a	0.172 ± 0.058	a	0.254 ± 0.099	a	0.124 ± 0.043	d	0.175 ± 0.053	d	0.209 ± 0.049	b	0.756 ± 0.252	b	0.819 ± 0.319	b	0.758 ± 0.301	b
Asín	U	0.209 ± 0.093	ab	0.242 ± 0.146	ab	0.223 ± 0.117	ab	0.232 ± 0.036	b	0.161 ± 0.032	bc	0.172 ± 0.041	b	1.20 ± 0.34	a	0.865 ± 0.445	a	0.977 ± 0.504	a
	B0	0.285 ± 0.042	a	0.325 ± 0.020	a	0.285 ± 0.067	a	0.366 ± 0.125	a	0.297 ± 0.096	a	0.339 ± 0.103	a	1.49 ± 0.57	a	1.03 ± 0.35	a	1.15 ± 0.45	a
	B6	0.116 ± 0.027	b	0.146 ± 0.025	b	0.155 ± 0.037	ab	0.261 ± 0.017	ab	0.212 ± 0.025	ab	0.195 ± 0.006	b	1.54 ± 0.35	a	1.12 ± 0.24	a	1.00 ± 0.34	a
	B12	0.096 ± 0.045	b	0.135 ± 0.040	b	0.103 ± 0.038	b	0.098 ± 0.014	c	0.100 ± 0.019	c	0.113 ± 0.033	b	0.767 ± 0.37	a	0.565 ± 0.216	a	0.980 ± 0.504	a
Yebra	U	0.108 ± 0.033	b	0.095 ± 0.010	c	0.089 ± 0.021	b	0.166 ± 0.016	b	0.143 ± 0.044	bc	0.358 ± 0.158	a	1.43 ± 0.42	ab	1.23 ± 0.17	b	3.28 ± 0.78	b
	B0	0.103 ± 0.026	b	0.089 ± 0.017	c	0.070 ± 0.017	b	0.235 ± 0.036	b	0.355 ± 0.238	a	0.568 ± 0.219	a	1.82 ± 0.33	a	2.84 ± 1.95	a	5.82 ± 3.01	a
	B6	0.281 ± 0.086	a	0.305 ± 0.067	a	0.321 ± 0.084	a	0.500 ± 0.091	a	0.443 ± 0.022	a	0.483 ± 0.032	a	1.29 ± 0.48	ab	1.00 ± 0.17	b	1.11 ± 0.20	bc
	B12	0.119 ± 0.054	b	0.195 ± 0.088	b	0.244 ± 0.102	a	0.149 ± 0.123	b	0.098 ± 0.026	bc	0.097 ± 0.032	b	1.05 ± 0.68	b	0.460 ± 0.161	b	0.336 ± 0.095	c

U: unburned samples; B0: immediate post-fire samples; B6, B12, B18, B24: seasonal samplings every 6 months after burning.