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Antonio Girona García

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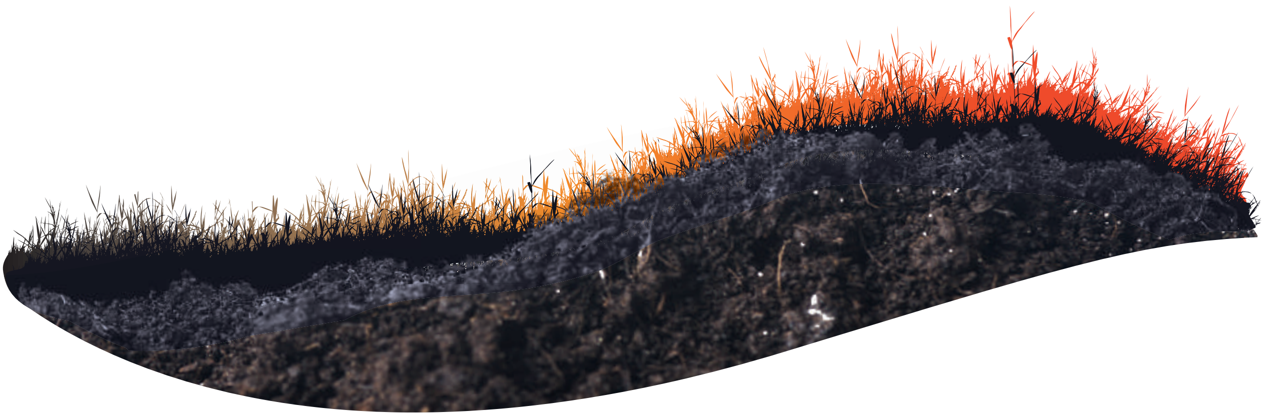

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El Dr. David Badía Villas, catedrático del área de Edafología y Química Agrícola de la Universidad de Zaragoza en la Escuela Politécnica Superior de Huesca

HACE CONSTAR:

Que Antonio Girona García ha realizado la tesis titulada “Changes in soil properties after prescribed burning for pasture restoration in shrublands of the Central Pyrenees (NE-Spain)“ para optar al Grado de Doctor por la Universidad de Zaragoza, y certifica que ha sido elaborada bajo su dirección en la Escuela Politécnica Superior de Huesca, Universidad de Zaragoza

Fdo. David Badía Villas

Huesca, febrero de 2019

LIST OF PUBLICATIONS

This doctoral dissertation is presented as a compendium of publications, as authorized by the supervisor and the doctoral committee of the department of Agricultural and Environmental Sciences of the University of Zaragoza. Antonio Girona García is the main author of the publications that structure this dissertation and all of them have been published in renowned international scientific journals. These publications are:

1. Girona-García, A., Ortiz-Perpiñá, O., Badía-Villas, D., Martí-Dalmau, C., 2018. 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. Journal impact factor (JCR): 3.256 (Q1, *Soil Sciences*).

2. Girona-García, A., Zufiaurre Galarza, R., Mora, J.L., Armas-Herrera, C.M., Martí, C., Ortiz-Perpiñá, O., Badía-Villas, D., 2018. Effects of prescribed fire for pasture reclamation on soil chemical properties in subalpine shrublands of the Central Pyrenees (NE-Spain). Science of the Total Environment 644: 583-593. DOI: 10.1016/j.scitotenv.2018.06.363. Journal impact factor (JCR): 4.610 (Q1, *Environmental Sciences*).

3. Girona-García, A., Badía-Villas, D., Martí-Dalmau, C., Ortiz-Perpiñá, O., Mora, J.L., Armas-Herrera, C.M., 2018. Effects of prescribed fire for pasture management on soil organic matter and biological properties: a 1-year study case in the Central Pyrenees. Science of the Total Environment 618: 1079-1087. DOI: 10.1016/j.scitotenv.2017.09.127. Journal impact factor (JCR): 4.610 (Q1, *Environmental Sciences*).

4. 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 Journal impact factor (JCR): 4.005 (Q1, *Environmental Sciences*).

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A mis padres

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LIST OF ABBREVIATIONS AND ACRONYMS

AS	aggregate stability
B0	soil samples obtained immediately after burning
B6	soil samples obtained 6 months after burning
B12	soil samples obtained 12 months after burning
B18	soil samples obtained 18 months after burning
B24	soil samples obtained 24 months after burning
BD	bulk density
CEC	cation exchange capacity
CF	coarse fraction
CMC	carbon mineralization coefficient
DOC	dissolved organic carbon
EC	electrical conductivity
E-cations	exchangeable cations
N	total nitrogen
NS	total nitrogen stocks
m a.s.l.	meters above sea level
MBC	microbial biomass carbon
MWD	mean weight diameter
qCO_2	microbial metabolic quotient
SOC	soil organic carbon
SOCS	soil organic carbon stocks
SOM	soil organic matter
SR	soil basal respiration
STC	soil total carbon
SWC	soil water content
SWR	soil water repellency
th	soil thickness
U	unburned samples
v	volume

w	weight
WDPT	water drop penetration time
WE-cations	water-extractable cations

ABSTRACT

Grazing lands are rich and diverse ecosystems that provide considerable environmental, economic and social services. The semi-natural grasslands of the Pyrenees require of human intervention for its conservation and have been traditionally maintained through livestock grazing and the recurrent elimination of shrubs. For this reason, since the late 20th century, as a consequence of the rural exodus and the decrease in livestock densities that it entailed, altogether with the fire suppression policies that were enacted, the pasturelands of the Central Pyrenees have suffered shrub encroachment by species such as the *Echinospartum horridum*. In the last decade, there have been sustained efforts to reverse the shrub encroachment of the pastures either for reducing fuel loads and recovering grazing lands. Prescribed burnings, defined as the planned use of fire to achieve precise and clearly defined objectives, are a suitable tool for the elimination of shrubs and represent a less risky practice than the non-regulated traditional burnings. However, fire can affect most of the soil physical, chemical and biological properties so prescribed burnings are conducted under favorable environmental conditions to limit their severity. However, the effect that this practice has on soil in humid mountain environments is still uncertain.

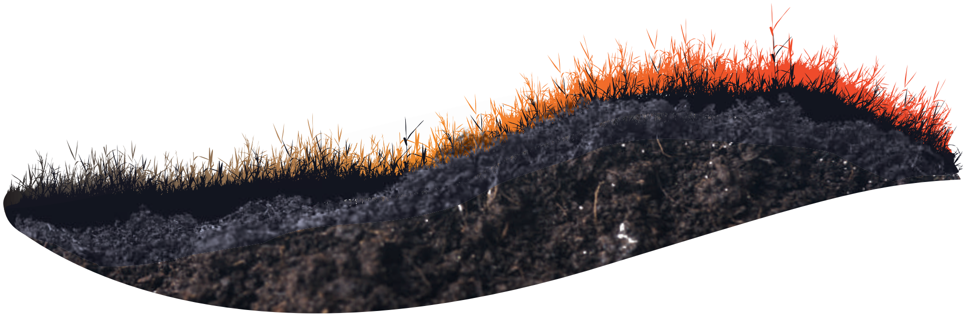
The objective of this thesis is to analyze the effects that the prescribed burnings of *Echinospartum horridum* for pasture restoration have on soil physical, chemical and biological properties in the immediate, short- and mid-term in the montane and subalpine belt of the Central Pyrenees (NE-Spain).

In Chapter 4, the effect of prescribed burning on the soil aggregate stability, organic carbon and water repellency of different soil depths and aggregate sizes was studied. Soil samples were collected from an area (Buisán, Huesca) treated by an autumnal low-intensity prescribed fire at 0-1, 1-2, 2-3 and 3-5 cm depths just before and ~1 hour, 6 months and 12 months after burning. Samples were separated as whole soil (<10 mm) and 6 sieve fractions, <0.25, 0.25-0.5, 0.5-1, 1-

2, 2-4 and 4-10 mm. We analyzed soil organic carbon (SOC), aggregate stability (AS) and soil water repellency (SWR). In the unburned samples, SOC and SWR were higher in the <0.25 to 2 mm sieve fractions than the 2 to 10 mm sieve fractions. Fire severely and significantly decreased the SOC content in the whole soil and the <0.25 mm fraction at 0-1 cm depth and in the 0.25-0.5 mm fraction at 0-2 cm depth. SWR was reduced by burning mainly at 0-1 cm depth for the whole soil and the <0.25 to 2 mm sieve fractions. Nevertheless, the AS of the 0.25-0.5 mm aggregates increased after fire, while the rest of the sieve fractions remained virtually unaffected. One year after the prescribed burning, SOC slightly increased and SWR recovered in the fire-affected fractions, while the AS for all aggregate sizes and depths showed a considerable decrease. The results suggest that the direct effects of burning are still present one year after burning, and the post-fire situation may pose an increased risk of soil loss. Furthermore, our results indicate that fine soil fractions are more likely to be affected by fire than coarser soil fractions and highly influence the whole soil behavior.

In Chapter 5, the effect that this practice may have on soil chemical properties such as SOC, N, pH, electrical conductivity, water-extractable and exchangeable cations (Ca^{2+}, Mg^{2+} and K^{+}), cation exchange capacity, inorganic N forms ($N\text{-}NH_4^{+}$ and $N\text{-}NO_3^{-}$) and available P was assessed. Two prescribed burnings conducted at the subalpine level of the Central Pyrenees in the municipalities of Tella-Sin (April 2015) and Buisán (November 2015) were studied. At each site, the topsoil was sampled in triplicate at soil depths of 0-1, 1-2 and 2-3 cm immediately before (U), immediately after (B0) and one year after (B12) burning, and litter and/or ashes were removed prior to sampling. The results indicate that in the B0 samples, burning significantly reduced the SOC and N contents as well as the exchangeable Ca^{2+} and Mg^{2+} at 0-1 cm, whereas the rest of the studied properties remained virtually unchanged. However, in the B12 samples we detected a decrease of nutrient content that was probably related to leaching and/or erosion processes.

In Chapters 6 and 7, 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 in triplicate 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 °C (Buisán), 768 °C (Asín) and 595 °C (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 ranged from neutral to severe at the topsoil (0-1 cm), which was related to the specific environmental conditions during the prescribed fires and 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 soil organic carbon content in the burned soils.



CHAPTER 1:
Background

1. GRAZING LANDS ECOSYSTEMS

Grazing lands are rich and diverse ecosystems that provide considerable environmental, economic and social services (Follett & Reed, 2010; Nadal-Romero et al., 2018a). Pastures are a valuable source of food for livestock and thus, play an important role in the economy of the mountain populations (Villagra et al., 2015). Pasturelands are also habitats of high animal and vegetal biodiversity and usually host species adapted to conditions near their distribution limits, which usually increases the number of endemisms (Canals & Sebastiá, 2000). Grazing lands also contribute to the prevention of wildfires because they maintain open landscapes with low fuel loads that act as firebreaks (Bernués et al., 2014; Reiné, 2017). 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 carbon (SOC) are stored in these ecosystems (Lal, 2004). Therefore, the sustainment 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).

2. GRAZING LANDS IN THE CENTRAL PYRENEES

In the Pyrenees (NE-Spain), grasslands 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). Nowadays, grazing lands occupy approximately 600,000 ha in the Central Pyrenees (Caballero et al., 2009). These semi-natural grasslands require of human intervention for its conservation and 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). For this reason, since the late 20th century, as a consequence of the rural exodus and the decrease in livestock densities that it entailed, altogether with the fire suppression policies that were enacted, the pasturelands of the Central Pyrenees have suffered shrub encroachment (Komac et al., 2013; Nadal-Romero et al., 2018a). The shrub encroachment of grasslands entails a remarkable reduction in plant species richness (Price & Morgan, 2008) and livestock production (Scholes & Archer, 1997). Therefore, this process may also have an impact on biodiversity and the ecosystem services provided by pasturelands (Harrison et al., 2010; Naito & Cairns, 2011) as well as an increased flammability risk (Caballero et al., 2009; Gartzia et al., 2014).

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 (**Figure 1**) that limit the establishment of herbaceous species (Komac et al., 2011). The *Echinospartum horridum* is a species of great ecological amplitude that develops at elevations that range from 650 to 2344 m a.s.l. (Badía et al., 2017a) mainly on the Spanish side of the Pyrenees (**Figure 2**).

Figure 1. View of an *Echinospartum horridum* shrub (Buisán, June 2016)

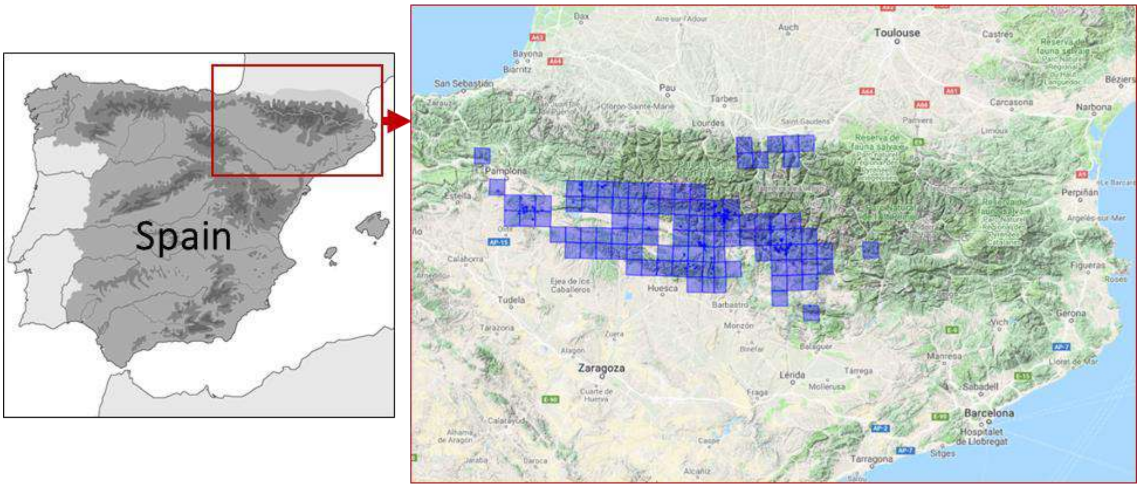


Figure 2. Distribution of *Echinospartum horridum* (blue squares) in the Pyrenees. Adapted from atlasflorapyrenaea.org

3. MANAGEMENT OF SHRUB ENCROACHMENT IN THE CENTRAL PYRENEES

Since the mid-90s, there have been sustained efforts to reverse the shrub encroachment of the pastures either for reducing fuel loads and thus the wildfire risk, or for recovering grazing lands. The most commonly used practices for the management of shrub encroachment are the prescribed burnings and the mechanical clearings. Prescribed burnings (**Figure 3**), defined as the planned use of fire to achieve precise and clearly defined objectives, are a suitable tool for the elimination of shrubs from grazing lands (Goldammer & Montiel, 2010) and represent a less risky practice than non-regulated traditional burnings (Fernandes et al., 2013). Furthermore, 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). 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) and that both practices may have environmental drawbacks. In flat areas with good accessibility, mechanical clearings could be a good choice for shrub removal (Lasanta et al., 2016) although this practice is more expensive than prescribed burnings, which could be the most convenient tool in hard-to-reach areas located at the subalpine and alpine belts (Lyet et al., 2009).

Figure 3. Prescribed burning of *Echinospartum horridum* conducted by the EPRIF of Huesca at the Sobás pass (Yebra de Basa, Spain, December 2016)

4. FIRE AND SOIL

Soils provide numerous ecosystem services because of their multiple functions: they support plant growth, regulate water supplies, act as nature's recycling system, participate in biogeochemical cycles and are the habitat of numerous organisms (Weil & Brady, 2017). Fires such as prescribed burnings can modify most of soil physical, chemical and biological properties both directly as a consequence of the heating (**Figure 4**) or indirectly due to the new post-fire environment in which vegetation has been removed (Santín & Doerr, 2016).

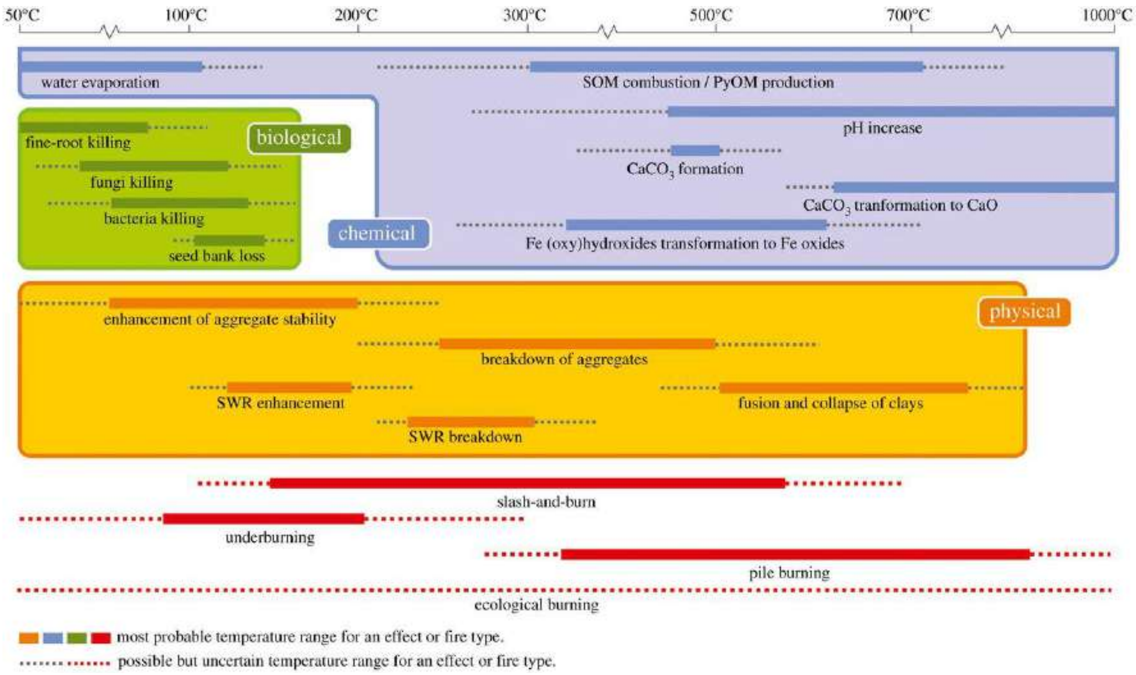


Figure 4. Changes in soil physical, chemical and biological properties associated to temperature ranges reached near the soil surface for different types of fires induced by humans. Source: Santín & Doerr (2016)

The extent of those changes in soil properties will be determined, among others, by the fire intensity (energy release rate) and severity (effect on the ecosystem components) (Neary et al., 2005). During burning, heat is mainly transferred into the atmosphere and it is estimated that only 10-15 % of the energy released by the combustion of aboveground fuels is transmitted into the soil (Debano & Neary, 2005). The fire intensity is not the only factor determining its effects on soils but also the duration of the burnings and the soil water content. Apart from the intrinsic insulating properties of soils, their water content also plays an important role in thermal conductivity. The high specific heat capacity of water prevents sudden temperature increases because heating in the soil is slowed down until water is completely vaporized (Campbell et al., 1995). Therefore, the depth to which heat is transmitted during fires is greatly influenced by this combination of factors. When burning, temperature increases are mainly detected near the soil surface whereas they are hardly noticeable downwards in the soil. For this reason, the probable effects of prescribed burning on soil are mainly confined to its uppermost layers (Badía et al. 2017b and references therein).

4.1. Fire effects on soil organic matter

The organic matter is considered to be the most essential constituent of soils as it virtually influences all soil physical, chemical and biological properties. The soil organic matter (SOM) contributes to soil aggregate formation and thus, enhances soil structure which is important in terms of soil aeration and water storage. The SOM participates in numerous soil chemical transformations, for example, by contributing to the storage and supply of available nutrients, which is of vital importance for plant development. Additionally, SOM acts as an energy source for the microorganisms that regulate the cycling of nutrients (Weil & Brady, 2017).

The SOM can undergo changes in its quantity and composition during fires, since its combustion is initiated when temperatures of 200-250 °C are reached (Badía & Martí, 2003; Certini, 2005; Santín & Doerr, 2016). 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 SOM amounts 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). 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).

4.2. Fire effects on soil physical properties

Soil organic matter (SOM), aggregation and water repellency (SWR) are relevant interrelated soil properties (Zheng et al., 2016) that can be affected by fire (Mataix-Solera et al., 2011). SOM plays a primary role in soil quality, influencing relevant physical properties such as soil aggregation and its stability since it can act as a binding agent during aggregate formation (Tisdall & Oades, 1982). SOM is also known to be linked to the occurrence of SWR, which is a natural property of soils that reduces infiltration and enhances surface runoff and erosion (Doerr et al., 2000; Zavala et al., 2014). SWR can be determined by SOM, among many other factors (Jordán et al., 2013 and references therein), as it contains organic hydrophobic substances that coat mineral particles or are present in the interstitial spaces of soil. However, the SOM amount is not always the most determinant factor in the development of SWR; its composition and distribution among the different soil aggregate sizes are also important (Jiménez-Morillo et al., 2016). Additionally, hydrophobic substances can coat soil aggregates, increasing their stability (Mataix-Solera et al., 2011). SWR is a soil property that can be affected by fire in different ways, induced or enhanced as a consequence of the partial combustion of SOM (Mataix-Solera et al., 2011) as well as removed by the oxidation or translocation of hydrophobic organic substances (Jordán et al., 2010).

Although fire effects on soil aggregation have been widely studied, contrasting results have been reported, as reviewed by Mataix-Solera et al. (2011), and there are still uncertainties about how this property is affected by heat (Jiménez-Pinilla et al., 2016). Low-intensity fires may have a neutral effect on soil aggregation or even increase it due to the stability of SOM and inorganic binding agents in temperature ranges below 200 °C. However, sudden heating can produce disaggregation even at low temperatures due to the forces exerted by escaping water steam (Albalasmeh et al., 2013 and references therein). On the other hand, high-intensity fires may produce remarkable changes in soil aggregation, as it can be degraded due to SOM combustion or increased as a consequence of particle fusion and the recrystallization of clay minerals (Mataix-Solera et al., 2011 and references therein). These effects may vary depending on the fire severity and main aggregate stabilizing agent, so the analysis of related parameters, i.e., SOM, soil aggregate size distribution, and water repellency, are required in order to understand how this property is affected by fire.

4.3. Fire effects on soil nutrient content

The combustion of SOM and vegetation may produce an increase in the available nutrients by either the mineralization of organic compounds or the production of ashes (González-Pérez et al., 2004; Knicker, 2007). Then, the incorporation of ashes into the soil can lead to increases in pH and electrical conductivity (EC) (Certini, 2005). The literature shows that the available concentrations of Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} are commonly increased after prescribed burning (Arocena & Opio, 2003; Lavoie et al., 2010; Alcañiz et al., 2016). Inorganic N forms can also increase after burning from either the contribution of ashes or the mineralization of soil organic N. For this reason, it is common to detect ammonium gains immediately after burning that will result in nitrates increases, via nitrification, over time (Gundale et al., 2015; San Emeterio et al., 2016). Fire may also boost the contents of available P in the soil via both the contributions of ashes as well as the mineralization of its organic forms that can occur even at relatively low temperatures (Úbeda et al., 2005; Badía-Villas et al., 2014; Larroulet et al., 2016; García-Oliva et al., 2018). This enrichment in nutrients produced by fire may promote the rapid establishment of herbaceous species. However, another consequence of SOM destruction is the loss of adsorption sites in the soil, thereby reducing the cation exchange capacity (CEC) (Badía & Martí, 2003). In this way, depending on the severity and recurrence of burning, these practices could also lead to nutrient losses (Wanthongchai et al., 2008). Nevertheless, the CEC usually remains unchanged after prescribed burning (Larroulet et al., 2016; Fonseca et al., 2017).

4.4. Fire effects on soil biological properties

Fire may have severe effects on soil microorganisms when temperatures reach a range of 50-210 °C (Mataix-Solera et al., 2009). Soil moisture plays an important role in the survival of microorganisms during fires. 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 an increased mortality of microorganisms in the uppermost soil layers as a consequence of moist heat (Choromanska & DeLuca, 2002). The reduction in soil microbial biomass is one of the direct effects that fire can exert on soil microbiology (Mataix et al., 2009), as it is a very sensitive soil component that can be notably altered at temperatures over 50 °C (Bárcenas-Moreno & Bååth, 2009). However, not only the amount of soil microbial biomass can be affected by fire but also its activity. Most of soil microorganisms require organic materials as a source of energy so any fire-induced quantitative or qualitative changes in SOM are going to have an impact on soil biological activity (Busse & DeBano, 2005). The SOM turnover rates are mostly driven by soil microbial biomass so changes in its activity due to fire can also induce variations in the carbon transfer between the soil and the atmosphere (Knicker, 2007; Dooley & Treseder, 2012). Nevertheless, the response of soil microbial activity to fire is not only going to depend on environmental factors (i.e. temperature, moisture and substrate availability) but also on the resilience of microbial populations. 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 according to Hart et al. (2005).

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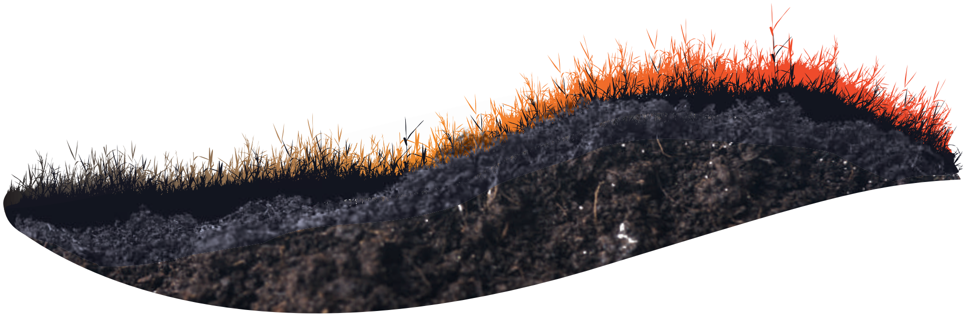


CHAPTER 2:

Justification, Objectives and Structure of the Dissertation

1. JUSTIFICATION

The idea of the present work arose from the need of assessing the sustainability of the *Echinospartum horridum* prescribed burnings in the Central Pyrenees from the point of view of soil. In light of the ideas elaborated in the previous chapter, depending on the environmental conditions under which the burnings are conducted, changes in soil physical, chemical and biological properties are a probable effect of fire. Therefore, it is of special interest to study the contrasted effects, both direct and indirect, that burning may have on soil properties depending on variability-inducing factors such as the ignition technique used, wind speed, burning season, site topography, etc.

Numerous studies have been conducted after prescribed burnings of forest understories and shrubs from the point of view of soil in the Mediterranean Basin. However, the literature covering the effects of this practice in humid mountain environments is scarce, and there are still uncertainties regarding the effects of fire applied for shrub encroachment and pasture restoration. This dissertation contributes to fill the gaps in the general knowledge of fire effects on soils in humid mountain environments such as the montane and subalpine belts of the Pyrenees. Furthermore, this study adds insight to the field of prescribed burnings applied for shrub encroachment reduction and pasture restoration, which provides valuable data for managers in the decision-making process.

2. OBJECTIVES

Previous things considered, the working hypothesis of this study are: 1) prescribed burning will have contrasted effects on the soil properties, 2) the effects of fire will be confined to the uppermost layers, 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 physical and chemical soil properties, if affected by fire, will recover to the unburned conditions and soil biological properties will respond to the new post fire environmental conditions.

Therefore, the general objective of this dissertation is to analyze the effects that the prescribed burnings of *Echinospartum horridum* for pasture restoration have on soil physical, chemical and biological properties in the immediate, short- and mid-term in the montane and subalpine belt of the Central Pyrenees (NE-Spain), specifically:

1. The effects of prescribed burning on soil physical properties, focusing on soil water repellency and soil aggregation.

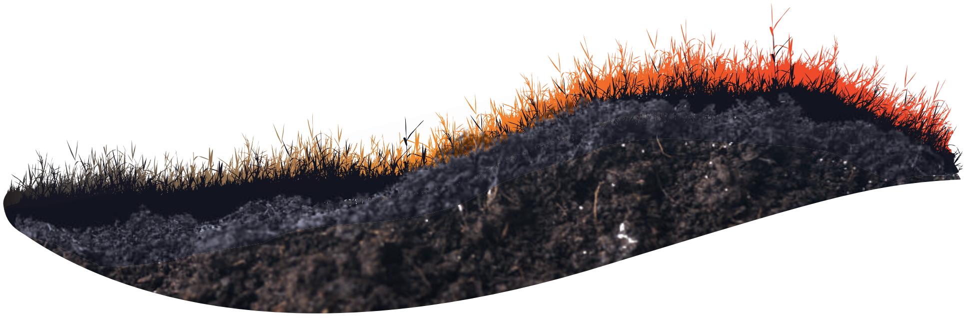
2. The effects of prescribed burning on soil nutrient content and related properties: pH, electrical conductivity, water-extractable and exchangeable cations, inorganic nitrogen forms and phosphorous.

3. The effects of prescribed burning on soil organic matter and biological properties: soil organic carbon, total nitrogen, microbial biomass carbon, soil respiration, carbon mineralization rates, microbial metabolic quotient and β-d-glucosidase activity.

4. Analyze the implications for management of the results obtained for objectives 1, 2 and 3 and identify the factors that could explain the probable contrasted effects of fire on soil among sites.

3. STRUCTURE OF THE DISSERTATION

Consequently, the published papers that structure the dissertation answer the specific objectives stated above. The objective 1 is covered in Chapter 4, where the effects of prescribed burning on soil organic carbon, aggregate stability, water repellency and mean weight diameter are studied over one year after burning in different soil aggregate sizes and depths. The objective 2 is answered in the Chapter 5, where the effects of prescribed burning on soil nutrients were assessed over a year in the two of the study sites. Chapter 6 deals with objective 3, where it is analyzed the effect that prescribed fires may have on soil organic matter and biological properties during one year. In Chapter 7, the objective 3 is further developed by studying the effects of prescribed burning on soil organic carbon stocks dynamics over a longer term and adding more study sites. The objective 4 is covered in all the chapters; however, in Chapter 7, the effect that burning may have on soil under different environmental conditions is discussed in more detail.



CHAPTER 3:
Materials and Methods

Although the methodology is already briefly explained in the materials and methods section of each of the following chapters, part of that information is further elaborated and supplemented with additional figures in the present chapter.

1. GENERAL CHARACTERISTICS OF THE STUDY SITES

The study was conducted in four mountain pasture areas in the municipalities of Buisán, Tella-Sin, Asín de Broto and Yebra de Basa (Central Pyrenees, Huesca province, NE-Spain, **Figure 1**), referred to as Buisán, Tella, Asín and Yebra throughout the text.

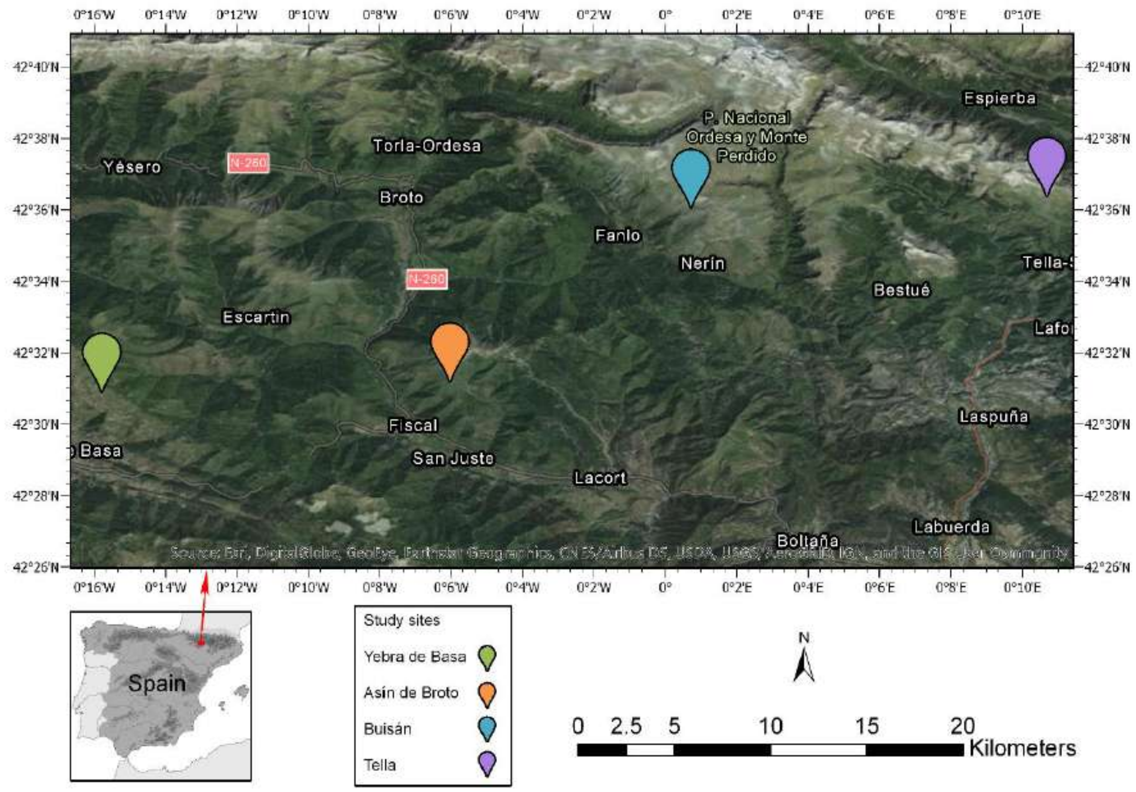


Figure 1. Location map of the study sites

These summer pastures have suffered shrub encroachment, resulting in a predominant *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 (**Figure 2**).

Figure 2. General view of the study sites: a) Buisán (November 2015), b) Tella (November 2016), c) Asín de Broto (November 2016) and d) Yebra de Basa (December 2016)

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 °C for Yebra; 1650 m a.s.l., 1120 mm and 8.8 °C for Asín; 1760 m a.s.l., 1500 mm, 6 °C for Buisán; and 1875 m a.s.l., 1700 mm, 5 °C for Tella. The soils are classified as Eutric Cambisol (Buisán), Calcaric Cambisol (Asín), Leptic Cambisol (Yebra) and Eutric Epileptic Cambisol (Tella) according to the IUSS Working Group WRB (2014) and are characterized by high organic matter contents, high aggregate stability and neutral (Buisán, Asín and Yebra) or acidic (Tella) pH values; the textures are silty loam (Buisán and Asín), sandy loam (Yebra) and silty clay loam (Tella). The parent material in Buisán and Tella consists of Eocene detritic sediments over clayey limestones alternated with marls; in Asín, the parent material is composed of Eocene marls and sandstones; 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

%), the Yebra plots were positioned in the middle of a flat slope (5 %) and the Tella plots were in the middle of a moderate slope (25 %).

Table 1. General characteristics of the study sites

Study Site	Tella	Buisán	Asín de Broto	Yebra de Basa
Coordinates	42º 36' 24.1 "N	42º 36' 04.4"N	42º 31' 12.3"N	42º 30' 55.0"N
	0º 10' 41.9"E	0º 00' 43.3"E	0º 06' 02.4"W	0º 15' 47.9"W
Elevation (m a.s.l.)	1875	1760	1650	1480
MAT (°C)	5	6	8.8	8.3
MAP (mm)	1700	1500	1120	1015
Aspect	S	S	W	S
Mean slope (%)	25	10	35	5
Soil classification (IUSS WRB 2014)	Eutric Epileptic Cambisol	Eutric Cambisol	Calcaric Cambisol	Leptic Cambisol

2. CHARACTERISTICS OF THE PRESCRIBED BURNINGS

2.1. Prescribed burning conditions

The prescribed burnings were conducted in April 2015 (Tella), 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. The general characteristics of each prescribed burning are provided in **Table 2.**

Table 2. General characteristics of the prescribed burnings

Study Site	Tella	Buisán	Asín de Broto	Yebra de Basa
Echinospartum horridum cover (%)	80	75	95	75
Estimated fuel loads (kg m^{-2}):				
Aerial biomass	9.86	9.24	11.71	9.24
Litter (OL + OF)	1.73	1.62	2.05	1.62
Burning Date	April 2015	November 2015	November 2016	December 2016
Burned surface (ha)	11.5	3.8	7.4	2.2
Wind speed (km h^{-1})	10-15	<8	10-15	10-15
Ignition technique	Backing fire	Point source fire	Backing fire	Head fire
Flame height (m)	0.1	1	0.7-1	1-3
Flame length (m)	1.5-2	1.5	0.65-1	1.5-3
Burning rate (ha h^{-1})	2.82	0.64	2.72	1.43
Pre-fire soil water content (%, 0-1 cm)	100 ± 32	147 ± 17	37.0 ± 3.4	29.0 ± 3.3
Post-fire soil water content (%, 0-1 cm)	74.5 ± 29.0	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, Asín and Tella) or four (Yebra) field replicates that correspond to the sampling plots

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 °C, 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 applying the point source fire technique and creating a grid of spot ignitions that burned from the east to the west flanks that followed a slow progression (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}, in Yebra, 2.2 ha were burned at 1.43 ha h^{-1} by a head fire favored by wind; and in Tella, a backing fire was ignited to spread against the wind and downslope, that burned at a rate of 2.82 ha h^{-1}.

2.2. Prescribed burning intensity and severity

2.2.1. Intensity of the prescribed burnings

To measure the temperatures reached during the prescribed burnings, type-K thermocouples were used. As the study went on, more thermocouples were being installed at each burning. At the Tella site, which was the first burning, a set of two thermocouples was inserted near one of the sampling points at 1 and 2 cm depth of the mineral horizon. At the Buisán site, a set of 4 thermocouples was placed in one of the sampling points at the mineral soil surface, 1, 2 and 3 cm depth. At the Asín and Yebra sites, two sets of 4 thermocouples each were placed in two of the sampling points in the same way as for Buisán. However, some mishaps occurred during the burnings. At the Asín site, one of the thermocouple sets moved during the burning so reliable data could only be obtained from one set. At the Yebra site, the cords of one of the thermocouple sets were burned whereas the thermocouples of the other set moved and registered temperatures only at the soil surface. At the Tella site, the temperatures recorded at 1 and 2 cm depth seem to be too high compared to the other prescribed burning, which suggests that the thermocouples were inserted closer to the soil surface. For this reasons, despite temperatures were measured at different soil depths, only the soil surface measurements were considered as reliable. Furthermore, because of the low number of temperature measurements achieved, the registered data can be considered only approximations of the fire intensity reached during the burnings.

The data recorded (**Table 3**) at the soil surface showed maximum temperatures of 438 °C (Buisán), 768 °C (Asín) and 595 °C (Yebra). On the other hand, a maximum temperature of 397 °C was registed at the Tella site for the 1 cm depth. 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 °C 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.

Table 3. Analysis of the temperatures registered during the prescribed burnings. The temperature analysis comprises the elapsed time since a temperature increase was detected until it stabilized during the cooling stage

Study Site	Tella	Buisán	Asín de Broto	Yebra de Basa
Temperature measured at:	1 cm depth	Soil surface	Soil surface	Soil surface
Maximum temperature (°C)	397	438	768	595
Initial temperature (°C)	16.0	13.1	7.5	4.9
Final temperature (°C)	25.5	27.5	24.3	10.2
Temperature residence time (min):				
< 60 °C	28.5	2.50	15.0	24.9
60-100 °C	4.50	15.0	5.75	1.33
100 - 200 °C	5.00	6.00	2.50	1.50
200 - 300 °C	9.50	4.00	1.42	0.83
300 - 400 °C	3.00	2.00	0.58	1.25
> 400 °C	0.00	0.50	4.83	0.25

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2.2.2. Severity of the prescribed burnings

At all sites, the aerial biomass of *Echinospartum horridum* was mostly eliminated by burning, resulting in burned trunks, partially charred litter and patches of black and gray ashes. 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 and Tella sites, 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 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.

3. SOIL SAMPLING

For each site, three (Tella, 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) and Tella (B12) (**Figure 3**). At the Buisán site in U, B0, B6 and B12 samplings, additional samples were obtained for the 0-1, 1-2, 2-3 and 3-5 cm depths and preserved in sealed containers to maintain the original soil structure for the study developed in Chapter 4. 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). Other studies conducted in the laboratory also indicated that fire effects may be confined to the first 3 cm of soil (Badía-Villas et al., 2014; Aznar et al., 2016), 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 °C 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	Spring 2015	Autumn 2015	Spring 2016	Autumn 2016	Spring 2017	Autumn 2017
Tella	U/B0	-	B12	-	-	-
Buisán	-	U/B0	B6	B12	B18	B24
Asín de Broto	-	-	-	U/B0	B6	B12
Yebra de Basa	-	-	-	U/B0	B6	B12

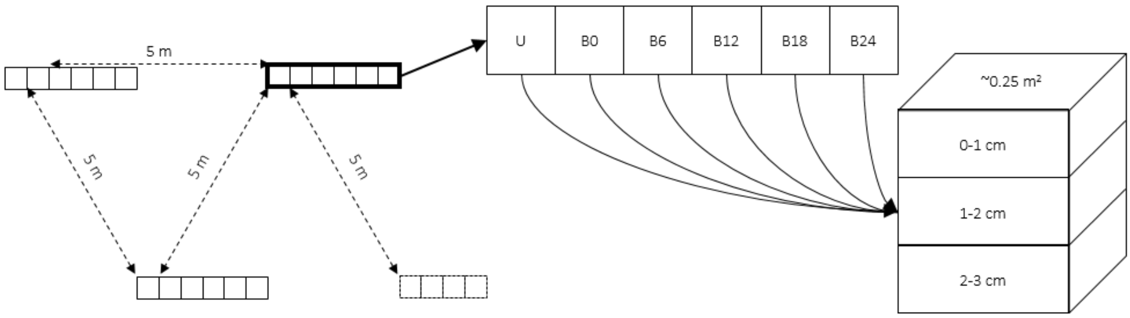

Figure 3. Timeframe and design of the samplings followed throughout the study period for each study site. U: unburned samples; B0: immediate post fire samples; B6, B12, B18, B24: seasonal samplings every 6 months after burning.

4. SOIL SAMPLES PREPARATION

The obtained soil samples were stored and prepared in different ways depending on the physical, chemical and biological properties that were going to be determined (**Figure 4**). The fresh samples were sieved to fine earth (2 mm) and kept at 4 °C for the biological analysis. A portion of each sample was separated and air-dried until constant weight. Additionally, a portion of the air-dried samples was taken and ground to fine powder. The unaltered soil samples obtained in Buisán for the study of Chapter 4 were separated in 6 different aggregate sizes (<0.25, 0.25-0.5, 0.5-1, 1-2, 2-4 and 4-10 mm) by manually shaking a nested column of sieves, avoiding aggregate destruction. In the samples obtained using the steel cylinders, the different depths (0-1, 1-2, 2-3 cm) were separated for the determination of bulk density.

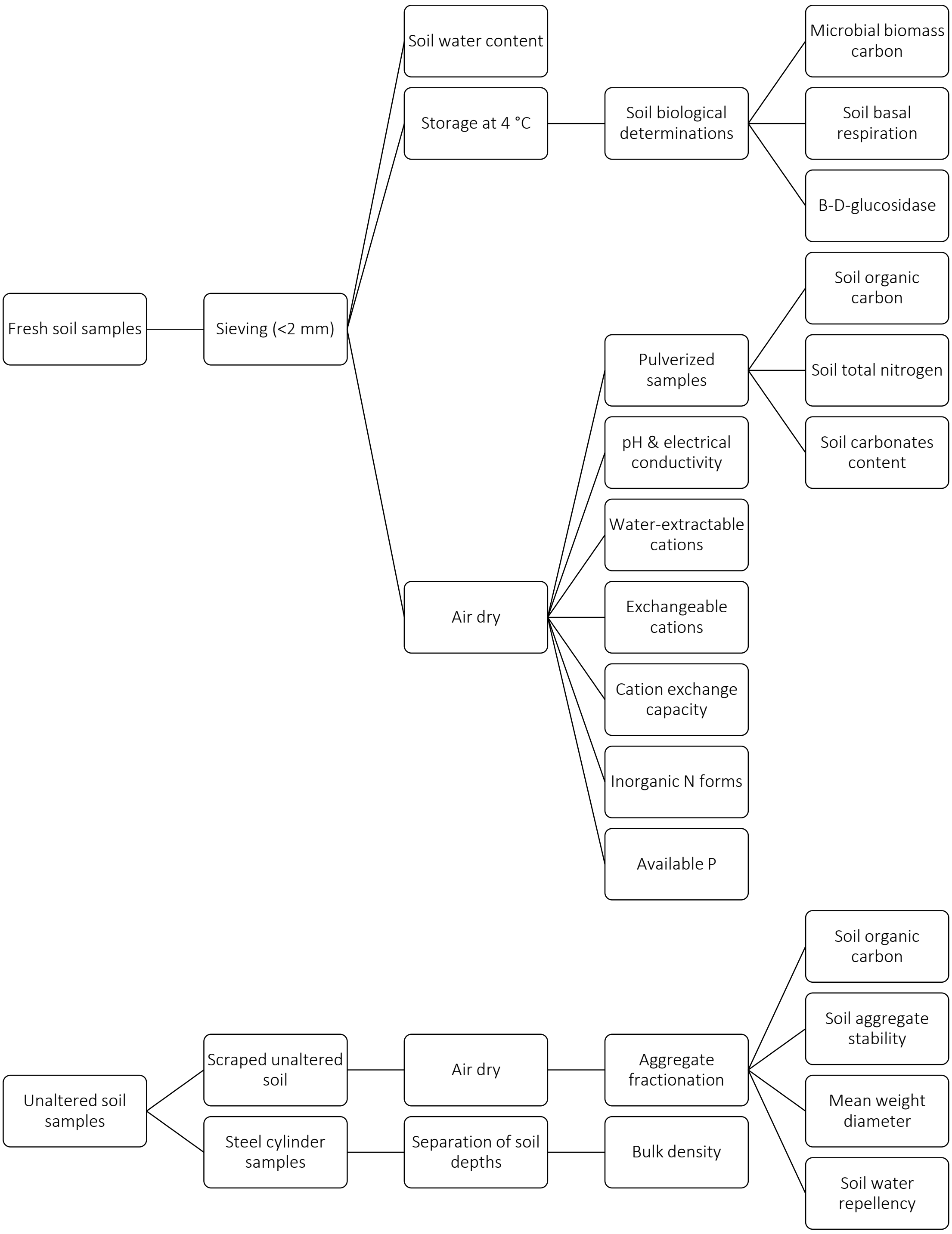

Figure 4. Diagram of the procedure followed for each type of soil sample

5. SOIL ANALYSIS

5.1. Determination of soil physical properties

5.1.1. Mean weight diameter

The mean weight diameter (MWD) was determined for the study of Chapter 4. The unaltered soil samples obtained in Buisán were separated in 6 different aggregate sizes (<0.25, 0.25-0.5, 0.5-1, 1-2, 2-4 and 4-10 mm) by manually shaking a nested column of sieves, avoiding aggregate destruction. Afterwards, stones were removed when they were present in the sieve fractions. Then, each aggregate size was weighed separately and its percentage respect the total weight was calculated to obtain the mean weight diameter (Van Bavel, 1949). The different aggregate fractions that resulted of this procedure were preserved separately for the determination of soil aggregate stability, water repellency and oxidizable organic carbon.

5.1.2. Aggregate stability

The soil aggregate stability (AS) was determined by the wet sieving method detailed by Kemper & Koch (1966) and revised by Schinner et al. (1996). This treatment emulates the forces exerted on soil by runoff or immersion conditions. The AS was determined for the Buisán samples that were sieved into different aggregate sizes (Chapter 4). Approximately 4 g of each sieve fraction (0.25-0.5, 0.5-1, 1-2, 2-4 and 4-10 mm) were placed in duplicate on 38 mm diameter sieves with 0.25 mm mesh size and then submerged and subjected to sieving action for 5 minutes. Afterwards, the remaining aggregates were carefully removed from the sieves, oven-dried at 105 °C and weighted in order to obtain the weight of the stable aggregates and large-sized sand (>0.25 mm). Then, each sample was submerged in 50 mL of 0.1 M sodium pyrophosphate decahydrate ($Na_4P_2O_7 \cdot 10H_2O$) for 2 hours to disperse the stable aggregates. Eventually, the samples were washed using distilled water, oven-dried at 105 °C and weighted, obtaining the weight of sand. The percentage of soil AS was determined using the expression:

$$AS\ (\%) = \frac{100\ (\text{weight of stable aggregates and sand}) - (\text{weight of sand})}{(\text{weight of sample}) - (\text{weight of sand})}$$

The AS could not be measured in the <0.25 mm sieve fraction, as we used a sieve with 0.25 apertures. The AS values obtained for the 1-2 mm aggregate size were considered representative of the whole soil, as suggested by the method.

5.1.3. Water repellency

The persistence of soil water repellency (SWR) was assessed through the water drop penetration time test (WDPT) consisting of applying droplets of distilled water on the soil surface and measuring the time until its complete infiltration (Wessel, 1988). The analysis was conducted under laboratory conditions with controlled temperature (20-25 °C) and relative humidity (50 %) in order to reduce sources of variability. Drops of distilled water (~0.05 mL/drop) were applied to the whole soil samples (<10 mm) and each sieve fraction (0.25-0.5, 0.5-1, 1-2, 2-4 and 4-10 mm), and the complete penetration time into the soil was measured. Given the wide array of values obtained via the WDPT, SWR was categorized into the 5 classes defined by Bisdom et al. (1993): wettable (<5 s), slightly water repellent (5-60 s), strongly water repellent (60-600 s), severely water repellent (600-3600 s) and extremely water repellent (>3600 s).

5.1.4. Water content

The soil water content was obtained from the fresh samples gravimetrically. A portion of each soil sample was weighed in fresh conditions and afterwards, it was oven-dried at 105 °C until constant weight. After drying, the soil samples were weighed again. The soil water content was calculated following the formula:

$$\text{Soil water content}\ (\%) = \frac{(\text{weight of the fresh sample}) - (\text{weight of the dried sample})}{(\text{weight of the dried sample})} \times 100$$

The soil water content data was used as a correction factor for all the analytical determinations to express the results on a 105 °C dried soil basis.

5.1.5. Bulk density

Each of the first 3 centimeters of the undisturbed soil samples obtained with the steel cylinders was carefully separated, dried at 105 °C until constant weight and weighed individually. Afterwards, the height and radius of the used steel cylinders was measured. The bulk density was then calculated as the mass of dried soil at 105 °C per volume unit.

5.2. Determination of soil chemical properties

5.2.1. Carbon and nitrogen

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 Tella, 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:

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

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 oxidizable organic carbon was also determined following the wet-oxidation method with chromic acid (Nelson & Sommers, 1982). This procedure was followed for the determination of SOC in the study of Chapter 4. The oxidizable organic carbon was also used for the determination of the carbon mineralization coefficient as it will be explained in section 5.3.2.

In Chapter 4, the SOC corresponds to the oxidizable organic carbon, whereas in Chapters 5, 6 and 7, the term SOC refers to total soil organic carbon.

5.2.2. pH and electrical conductivity

Soil pH was determined from potentiometric measurements of a 1:5 (w v^{-1}) suspension of soil and distilled water and the electrical conductivity (EC) was determined using an electrical conductivity meter in a 1:10 (w v^{-1}) suspension of soil and distilled water (McLean, 1982). The soil:water ratio was increased to be able to properly determine the soil properties because of the organic matter content and thus, low density of the samples.

5.2.3. Water-extractable cations

The water-extractable (WE) cations (Ca^{2+}, Mg^{2+} and K^+) in the soil samples were determined by atomic absorption (Mg^{2+}) and emission (Ca^{2+} and K^+) spectrometry (AAS/AAE Spectrometer Varian SpectrAA 110) in 1:10 (w v^{-1}) filtered extracts of soil and distilled water after 2 hours of shaking (Sharpley & Kampath, 1988).

5.2.4. Inorganic nitrogen forms

Ammoniacal ($N\text{-}NH_4^+$) and nitric ($N\text{-}NO_3^-$) forms of nitrogen were determined according to the methods in Bremner & Keeney (1965) in 1:5 (w v^{-1}) filtered extracts of soil and 1M KCl after 30 minutes of shaking. The ammonia was separated by steam distillation from an aliquote of the extract and collected in a boric acid solution; then, it was determined by titration using 0.005N H_2SO_4. In the same extract, Devarda alloy was added to reduce the remaining nitrate to ammonium and the same procedure was followed for its distillation and titration.

5.2.5. Available phosphorous

The available P was determined following the method of Olsen & Sommers (1982). P was extracted using 0.5M $NaHCO_3$ buffered at pH 8.5 (1:20 w v^{-1} of soil and extractant). Then, an aliquot of each sample was taken and its P content was determined colorimetrically by measuring the concentration of the complex formed by the reaction of phosphate with acid ammonium molybdate, using a UV/visible spectrophotometer (Cole-Parmer, Jenway 6300 Spectrophotometer, United Kingdom).

5.2.6. Exchangeable cations and cation exchange capacity

To determine the exchangeable Ca^{2+}, Mg^{2+} and K^{+} as well as the cation exchange capacity (CEC), a sequential extraction procedure was followed. Exchangeable cations were determined by atomic spectroscopy in the leachate obtained after three consecutive extractions (total shaking time of 15 minutes) with 1M CH_3COONa buffered at pH 8.2 (ratio 1:20, w v^{-1}). After that, samples were washed three times with ethanol (ratio 1:20, w v^{-1}) to remove the excess of the displacing solution without disturbing the adsorbed Na^{+}. Then, the adsorbed Na^{+} was displaced after three consecutive extractions (total shaking time of 15 minutes) with 1M CH_3COONH_4 buffered at pH 7 (ratio 1:20 w v^{-1}), and it was determined by atomic emission spectrometry, considering its value equal to that of the CEC (Bower et al., 1952; Rhoades, 1982). The use of sodium acetate instead of ammonium acetate as first extractant does not allow the determination of exchangeable Na^{+}; however, it avoids the overestimation of the other exchangeable cations due to the carbonate dissolution that can occur when using ammonium acetate (Bower et al., 1952).

5.3. Determination of soil biological properties

5.3.1. Microbial biomass carbon

The microbial biomass carbon (MBC) was determined following the chloroform fumigation-extraction method detailed in Vance et al. (1987). This method consists of soil fumigation with chloroform during 24 h, followed by an extraction with 0.5M K_2SO_4 (1:5 w v^{-1}). Meanwhile, an extraction with 0.5M K_2SO_4 (1:5 w v^{-1}) was also conducted in non-fumigated samples which was considered as dissolved organic carbon (DOC). Afterwards, the carbon contained in both fractions was determined by chromic acid oxidation. An extraction factor of K_c=0.38 was applied on the carbon value resulting of the difference of both fractions, obtaining the MBC.

5.3.2. Incubation assays

Incubation assays of 28 days were conducted under controlled conditions of 25 ± 1 °C and darkness, and the soil water content of the samples 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 carbon 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.

5.3.3. β-D-glucosidase activity

The soil β-D-glucosidase enzymatic activity was determined following the Eivazi and Tabatabai (1988) method. The fresh soil samples were incubated with substrate (β-D-glucopyranoside) for 1 h at 37 °C in a medium buffered at pH 6. Then, the product (*p*-nitrophenol) was extracted by filtration after the addition of $CaCl_2$ and tris(hydroxymethyl)aminomethane buffer at pH 12. Afterwards, the concentration of *p*-nitrophenol was determined by colorimetry at 400 nm.

5.4. Summary of the determined soil properties

The determinations explained in the previous section were not conducted for all the samplings of all the study sites. For this reason, in **Table 4**, it is specified which soil properties were analyzed at each study site and sampling time and depth as well as how they are distributed throughout the different chapters of the thesis.

Table 4. Soil properties determined for each site, sampling time and soil depth and chapters in which they are included

Chapter	Study site	Sampling times	Soil depths (cm)	Determined soil properties
4	Buisán	U, B0, B6, B12	0-1, 1-2, 2-3, 3-5	Mean weight diameter (MWD) Aggregate stability (SAS) Water repellency (SWR) Oxidizable organic carbon (SOC)
5	Buisán Tella	U, B0, B12	0-1, 1-2, 2-3	Total organic carbon (SOC) Total nitrogen (N) pH & electrical conductivity (EC) Water-extractable cations (WE-cations) Exchangeable cations (E-cations) Cation exchange capacity (CEC) Inorganic N species (N-NH_4^+, N-NO_3^-) Available phosphorous (P)
6	Buisán	U, B0, B6, B12	0-1, 1-2, 2-3	Total organic carbon (SOC) Total nitrogen (N) SOC/N ratio Dissolved organic carbon (DOC) Microbial biomass carbon (MBC) MBC/SOC ratio Soil basal respiration (SR) Carbon mineralization coefficient (CMC) Microbial metabolic quotient (qCO_2) Β-D-glucosidase activity
7	Buisán Asín Yebra	U, B0, B6, B12, B18, B24 U, B0, B6, B12 U, B0, B6, B12	0-1, 1-2, 2-3	Total organic carbon stocks (SOCS) Total nitrogen stocks (NS) SOC/N ratio Microbial biomass carbon (MBC) MBC/SOC ratio Soil basal respiration (SR) Carbon mineralization coefficient (CMC) Microbial metabolic quotient (qCO_2) Β-D-glucosidase activity

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

6. STATISTICAL 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). For the data in Chapter 4, the same procedure was followed but data was analyzed for all the aggregate sizes (<0.25, 0.25-0.5, 0.5-1, 1-2, 2-4, 4-10 and <10 mm). 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). Principal component analysis (PCA) were 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.

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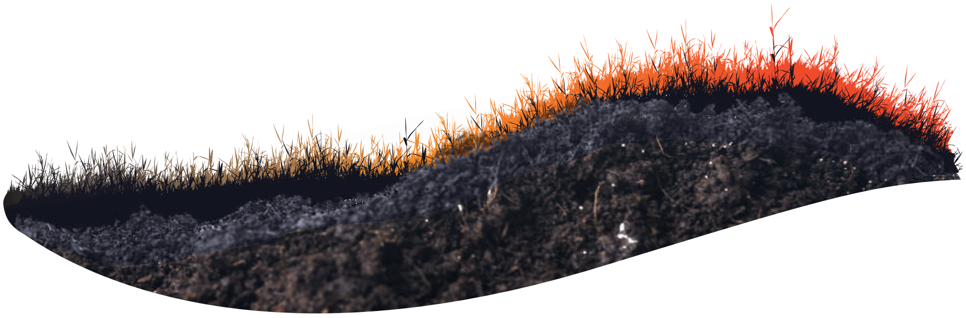

CHAPTER 4:

Effects of prescribed burning on soil organic C, aggregate stability and water repellency in a subalpine shrubland: variations among sieve fractions and depth

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ABSTRACT

Soil organic matter, aggregation and water repellency are relevant interrelated soil properties that can be affected by fire. The aim of this work was to analyze the effects of shrub prescribed burning for pasture reclamation on the soil aggregate stability, organic carbon and water repellency of different soil depths and aggregate sizes in a subalpine environment. Soil samples were collected from an area treated by an autumnal low-intensity prescribed fire in the Central Pyrenees (NE-Spain) at 0-1, 1-2, 2-3 and 3-5 cm depths just before and ~1 hour, 6 months and 12 months after burning. Samples were separated as whole soil (<10 mm) and 6 sieve fractions, <0.25, 0.25-0.5, 0.5-1, 1-2, 2-4 and 4-10 mm. We analyzed soil organic C (SOC), aggregate stability (AS) and soil water repellency (SWR). In the unburned samples, SOC and SWR were higher in the <0.25 to 2 mm sieve fractions than the 2 to 10 mm sieve fractions. Fire severely and significantly decreased the SOC content in the whole soil and the <0.25 mm fraction at 0-1 cm depth and in the 0.25-0.5 mm fraction at 0-2 cm depth. SWR was reduced by burning mainly at 0-1 cm depth for the whole soil and the <0.25 to 2 mm sieve fractions. Nevertheless, the AS of the 0.25-0.5 mm aggregates increased after fire, while the rest of the sieve fractions remained virtually unaffected. One year after the prescribed burning, SOC slightly increased and SWR recovered in the fire-affected fractions, while the AS for all aggregate sizes and depths showed a considerable decrease. The results suggest that the direct effects of burning are still present one year after burning, and the post-fire situation may pose an increased risk of soil loss. Furthermore, our results indicate that fine soil fractions are more likely to be affected by fire than coarser soil fractions and highly influence the whole soil behavior.

1. INTRODUCTION

Livestock grazing has played a primary role in the traditional management of pasturelands in the Central Pyrenees (NE-Spain) (Nadal-Romero et al., 2016). Nevertheless, as a consequence of socio-economic changes (i.e., rural exodus and reduction of stocking densities), this activity has considerably decreased in the past few decades (Komac et al., 2013). Currently, pasturelands cover a surface of approximately 600,000 ha in the Central Pyrenees (Caballero et al., 2010). The mesophytic Pyrenean pastures are composed of subclimax species that require the grazing of shrubs to survive (Halada et al., 2011). As a consequence of grazing reduction, the Pyrenees have suffered shrub encroachment processes, dominated by the thorny cushion dwarf (*Echinospartum horridum* (Vahl) Rothm), among others (Komac et al., 2013; Nuche et al., 2018). This species forms large and dense monospecific covers (Komac et al.,2011) that pose a threat to biodiversity and increase flammability risks (Caballero et al., 2010). Prescribed burning, defined as the planned use of fire to achieve precise and clearly defined objectives (Fernandes et al., 2013), serves as a practical and economical procedure for maintaining grazing lands and stopping shrub succession (Goldammer & Montiel, 2010). However, fire, depending on its severity, can affect most of the soil physical, chemical and biological properties (Certini, 2005). The intensity and duration of fires are highly influenced by the environmental conditions; for this reason, prescribed burnings are carried out under favorable conditions of soil and fuel moisture, temperature and topography (Molina, 2009) to limit their impact on the soil (Vega et al., 2005). Nevertheless, contrasting effects of prescribed burning on soil properties have been reported in the literature (Alcañiz et al., 2018).

Soil organic matter (SOM), aggregation and water repellency (SWR) are relevant interrelated soil properties (Zheng et al., 2016) that can be affected by fire (Mataix-Solera et al., 2011). SOM plays a primary role in soil quality, influencing relevant properties such as soil aggregation and its stability since it can act as a binding agent during aggregate formation (Tisdall & Oades, 1982).

SOM is also known to be linked to the occurrence of SWR, which is a natural property of soils that reduces infiltration and enhances surface runoff and erosion (Doerr et al., 2000; Zavala et al., 2014). SWR can be determined by SOM, among many other factors (Jordán et al., 2013 and references therein), as it contains organic hydrophobic substances that coat mineral particles or are present in the interstitial spaces of soil. However, the SOM amount is not always the most determinant factor in the development of SWR; its composition and distribution among the different soil aggregate sizes are also important (Jiménez-Morillo et al., 2016a). Additionally, hydrophobic substances can coat soil aggregates, increasing their stability (Mataix-Solera et al., 2011).

Fire can induce changes in SOM, since its combustion is initiated when temperatures of 200-250 °C are reached (Badía & Martí, 2003; Certini, 2005; Santín & Doerr, 2016). Several studies have reported that prescribed burning has no effects on SOM (Alexis et al., 2007; Goberna et al., 2012; Fultz et al., 2016), while others have observed increases in SOM content (Úbeda et al., 2005; Alcañiz et al., 2016) due to the incorporation of partly charred plant material or litter (González-Pérez et al., 2004). However, previous works investigating *Echinospartum horridum* prescribed fires in the Central Pyrenees have indicated a severe decrease in SOM immediately after burning (Armas-Herrera et al., 2016; Girona-García et al., 2018).

Although fire effects on soil aggregation have been widely studied, contrasting results have been reported, as reviewed by Mataix-Solera et al. (2011), and there are still uncertainties about how this property is affected by heat (Jiménez-Pinilla et al., 2016a). Low-intensity fires may have a neutral effect on soil aggregation or even increase it due to the stability of SOM and inorganic binding agents in temperature ranges below 200 °C. However, sudden heating can produce disaggregation even at low temperatures due to the forces exerted by escaping water steam (Albalasmeh et al., 2013 and references therein). On the other hand, high-intensity fires may produce remarkable changes in soil aggregation, as it can be degraded due to SOM combustion or increased as a consequence of particle fusion and the recrystallization of clay minerals (Mataix-Solera et al., 2011 and references therein). These effects may vary depending on the fire severity and main aggregate stabilizing agent, so the analysis of related parameters, i.e., SOM, soil aggregate size distribution, and water repellency, are required in order to understand how this property is affected by fire. Furthermore, there is a gap in knowledge on how prescribed burnings applied for vegetation management purposes affect aggregate stability (Alcañiz et al., 2018).

SWR is a soil property that can be affected by fire in different ways, induced or enhanced as a consequence of the partial combustion of SOM (Mataix-Solera et al., 2011) as well as removed by the oxidation or translocation of hydrophobic organic substances (Jordán et al., 2010).

Numerous studies have been carried out in Mediterranean environments involving the aforementioned soil properties after wildfires and prescribed and experimental burnings. However, to the author’s knowledge, no studies of this type have been conducted for prescribed burnings in subalpine environments.

The objective of this work was to study the effects of the prescribed burning of shrubs for pasture management in a subalpine environment on interrelated soil properties, such as SOC content, aggregate stability and SWR, among different aggregate sizes and topsoil depths during a one-year period. In this way, we also aimed to detect which soil aggregate sizes are more prone to be affected by fire and how those changes influenced the whole soil behavior.

2. MATERIAL AND METHODS

2.1. Study area and prescribed burning description

The study site is located in Buisán, Central Pyrenees (NE-Spain; 42°36’04.4” N 0°00’43.3” E), at 1760 m.a.s.l. The average slope ranges from 12 to 30 % and faces south. The mean annual temperature is 5.7 °C, and the mean annual precipitation 1270 mm. Due to fire exclusion after

1980 and the decay of grazing activity, the *Echinospartum horridum* population in this region has widely increased, considerably decreasing the grassland cover (Komac et al., 2011, 2013).

Soils are characterized by neutral pH values, high soil organic matter content, fine textures and variable carbonate content and are classified as an association of Eutric Cambisols and Calcaric Cambisols (IUSS Working Group WRB, 2014). The characteristics of a representative soil profile are shown in **Table 1.**

Table 1. Physical and chemical properties of the Eutric Cambisol at the study site

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^{-1})	115	80.5	50.5	36.4	32.3
CEC (cmol(+) kg^{-1})	33.1	24.2	19.9	17.9	14.3
OM (g kg^{-1})	173	89.3	53.2	39.1	27.7
C/N	12.9	10.1	9.1	8.1	7.6
Clay (g kg^{-1})	228	318	310	370	370
Silt (g kg^{-1})	661	602	612	550	554
Sand (g kg^{-1})	111	80	80	80	76
Textural class (USDA)	Silty loam	Silty clay loam	Silty clay loam	Silty clay loam	Silty clay loam
FC (g kg^{-1})	546	409	337	325	302
PWP (g kg^{-1})	394	252	202	189	174
AWC (g kg^{-1})	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

The prescribed burning was performed in November, 2015 by qualified firefighters of the EPRIF (Wildfire Prevention Team) of Huesca and BRIF (Reinforcement Brigades against Wildfires) of Daroca units when the environmental conditions met the established prescription parameters. It had not rained for 10 days prior to the burning, and the air relative humidity was 35-70 %, with a maximum temperature of 15 °C and a wind speed <8 km h^{-1}. The delimited burning area (3.8 ha) presented a rectangular shape, and approximately 75 % of its total surface was covered by *E. horridum* shrubs. The estimated aerial biomass was ~9.2 kg m^{-2}, and the amount of litter was ~1.6 kg m^{-2}. Fire was applied on *E. horridum* shrubs following the point source ignition technique from N to S, forming a fire line that spread from E to W at a rate of 0.64 ha h^{-1}. The average flame length and height were 1.5 and 1 m, respectively. Burning eliminated all the *E. horridum* shrubs in the area, leaving only burned trunks, ashes and partially charred litter.

An approximation of the temperatures reached during the prescribed burning (**Table 2**) was obtained via Type-K thermocouples placed at the mineral soil surface and at 1, 2 and 3 cm depths in one of the sampling sites. The recorded data show a maximum temperature of 438 °C at the soil surface, whereas the temperature remained almost unchanged below 1 cm depth. Data analysis also indicates that the uppermost soil layer was exposed to a temperature range of 100-400 °C for 12 minutes.

Table 2. Temperature recorded via type-K thermocouples placed at soil surface and at 1, 2 and 3 cm depth. Data analysis comprises the elapsed time since temperature increase was detected until it stabilized during cooling stage

Variables	Surface	1 cm	2 cm	3 cm
Maximum temperature (°C)	438	31.1	18.5	18.5
Initial temperature (°C)	13.1	9.77	9.60	8.93
Final temperature (°C)	27.5	22.2	17.6	18.2
Duration (min)				
<60 °C	2.50	30.0	30.0	30.0
60 - 100 °C	15.0	0.00	0.00	0.00
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

2.2. Soil sampling

We chose three representative sampling points covered by *E. horridum* shrubs separated by 5 m. At each point, soil was carefully scrapped from the topsoil Ah horizon at 0-1, 1-2, 2-3 and 3-5 cm depths in an approximate surface area of 0.25 m^2 (**Figure 1**) in the early morning to obtain unburned (U) control samples. Prior to sampling, the shrubs and organic layers were removed. Hours later, prescribed burning was conducted, and as soon as possible, points adjacent to the U points were sampled after ashes and charred plant remains were removed to study the immediate effects of fire (B0). Additionally, in order to assess short-term changes in the studied soil properties, the burned soils were sampled 6 months (B6) and one year (B12) after the prescribed burning. All samples were preserved in sealed containers in order to maintain the original soil structure. It should be noted that, for most of the period between the collection of B0 and B6 samples, the study site was covered by snow, and livestock (cows and goats) grazed the study site between 8 and 12 months after burning.

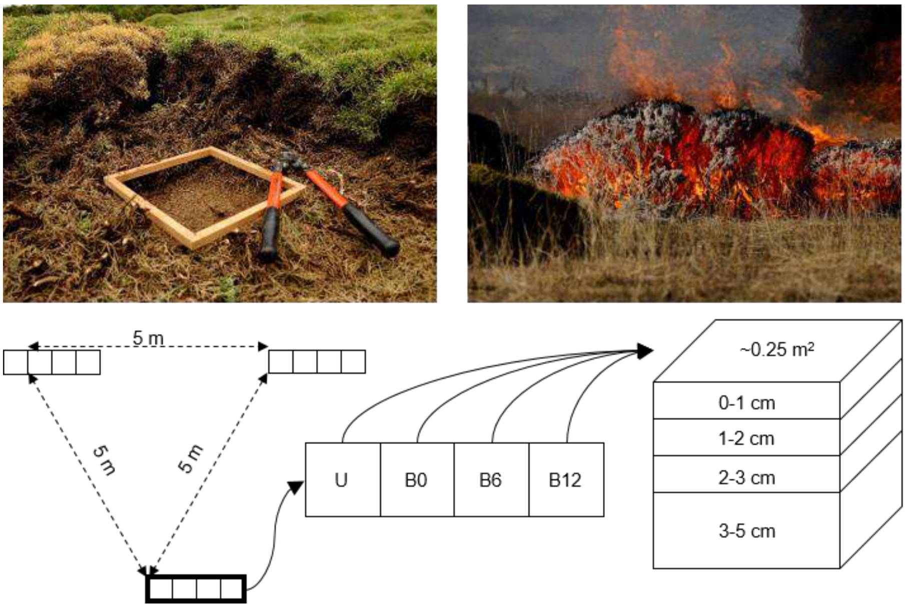

Figure 1. Design of sampling plots and burning of *Echinospartum horridum*. Unburned (U), immediately after (B0), 6 months (B6) and one year (B12) after burning sampling

2.3. Samples preparation and analysis

Soil samples were air-dried at room temperature until constant weight. A small portion was taken from all samples (n = 48) in order to analyze the whole soil (<10 mm). The remaining samples were separated in 6 different aggregate sizes (<0.25, 0.25-0.5, 0.5-1, 1-2, 2-4 and 4-10 mm) by manually shaking a nested column of sieves, avoiding aggregate destruction, to obtain 288 fractional samples (4 sampling times x 3 sampling points x 4 soil depths x 6 aggregate sizes). Afterwards, stones were removed when they were present in the sieve fractions. Then, each aggregate size was weighed separately to obtain the mean weight diameter (MWD, Van Bavel, 1949) and preserved to analyze the following properties.

The soil organic carbon (SOC) content was obtained for ground samples through the chromic acid wet oxidation method (Walkley-Black, 1934) for the whole soil (<10 mm) and each sieve fraction (0.25-0.5, 0.5-1, 1-2, 2-4 and 4-10 mm).

Soil aggregate stability (AS) was determined by the wet sieving method detailed by Kemper & Koch (1966) and revised by Schinner et al. (1996). This treatment emulates the forces exerted on soil by runoff or immersion conditions. Approximately 4 g of each sieve fraction (0.25-0.5, 0.5-1, 1-2, 2-4 and 4-10 mm) were placed in duplicate on 38 mm diameter sieves with 0.25 mm mesh size and then submerged and subjected to sieving action for 5 minutes. Afterwards, the remaining aggregates were carefully removed from the sieves, oven-dried at 105 °C and weighted in order to obtain the weight of the stable aggregates and large-sized sand (>0.25 mm). Then, each sample was submerged in 50 mL of 0.1 M sodium pyrophosphate decahydrate ($Na_4P_2O_7 \cdot 10H_2O$) for 2 hours to disperse the stable aggregates. Eventually, the samples were washed using distilled water, oven-dried at 105 °C and weighted, obtaining the weight of sand. The percentage of soil AS was determined using expression [1]

$$\text{AS (\%)}=\frac{100\ (\text{weight of stable aggregates and sand})\text{ - (weight of sand)}}{(\text{weight of sample})\text{ - (weight of sand)}} \qquad [1]$$

AS could not be measured in the <0.25 mm sieve fraction, as we used a sieve with 0.25 mm apertures. The AS values obtained for the 1-2 mm aggregate size were considered representative of the whole soil, as suggested by the method.

The persistence of soil water repellency (SWR) was assessed through the water drop penetration time test (WDPT) consisting of applying droplets of distilled water on the soil surface and measuring the time until its complete infiltration (Wessel, 1988). The analysis was conducted under laboratory conditions with controlled temperature (20-25 °C) and relative humidity (50 %) in order to reduce sources of variability. Drops of distilled water (~0.05 mL/drop) were applied to the whole soil samples and each sieve fraction, and the complete penetration time into the soil was measured (8 drops per sample; n = 2688). Given the wide array of values obtained via the WDPT, SWR was categorised into the 5 classes defined by Bisdom et al. (1993): wettable (<5 s), slightly water repellent (5-60 s), strongly water repellent (60-600 s), severely water repellent (600-3600 s) and extremely water repellent (>3600 s).

2.4. Statistical analysis

To identify the differences in the studied soil properties related to burning and post-fire elapsed time, as well as soil depth and aggregate size, one-way ANOVA tests were used, since the interaction between time and depth was significant in most cases. The sampling time (U, B0, B6, B12) was considered a fixed factor to analyze the effect of fire and time, and the data were split by soil depth (0-1, 1-2, 2-3 and 3-5 cm) for each soil fraction (<0.25, 0.25-0.5, 0.5-1, 1-2, 2-4, 4-10 and <10 mm). Additionally, changes in soil properties with depth were checked using soil depth (0-1, 1-2, 2-3, 3-5 cm) as a fixed factor, for which the data were split by sampling time (U, B0, B6, B12) for each soil fraction (<0.25, 0.25-0.5, 0.5-1, 1-2, 2-4, 4-10 and <10 mm). The correlations among variables for all sampling time and soil depth categories were also studied. These analyses were performed using StatView for Windows version 5.0.1 (SAS Institute Inc. Cary, North Carolina, USA). The homogeneous groups for the studied variables among the soil fractions within every soil depth and sampling time category were obtained using Statistica 8.0 (Stat Soft Inc. Tulsa, Oklahoma, USA). Data presented in the text are reported as the mean ± standard deviation of the mean unless otherwise stated.

3. RESULTS AND DISCUSSION

3.1. Soil Organic Carbon

The highest soil organic carbon (SOC) content, 182 ± 62 g kg^{-1}, was detected in the <0.25 mm aggregates of the unburned (U) samples at 0-1 cm depth (**Figure 2**). At 0-1 and 1-2 cm depths, the U SOC values showed a decreasing trend with increasing aggregate size, whereas in deeper soil layers, this trend was not so marked. In this way, SOC of all aggregate sizes consistently decreased with depth. A similar behavior was observed for SOC content of the U whole soil (<10 mm), which also decreased with depth from 161 ± 40 g kg^{-1} at 0-1 cm depth to 55.4 ± 5.1 g kg^{-1} at 3-5 cm depth.

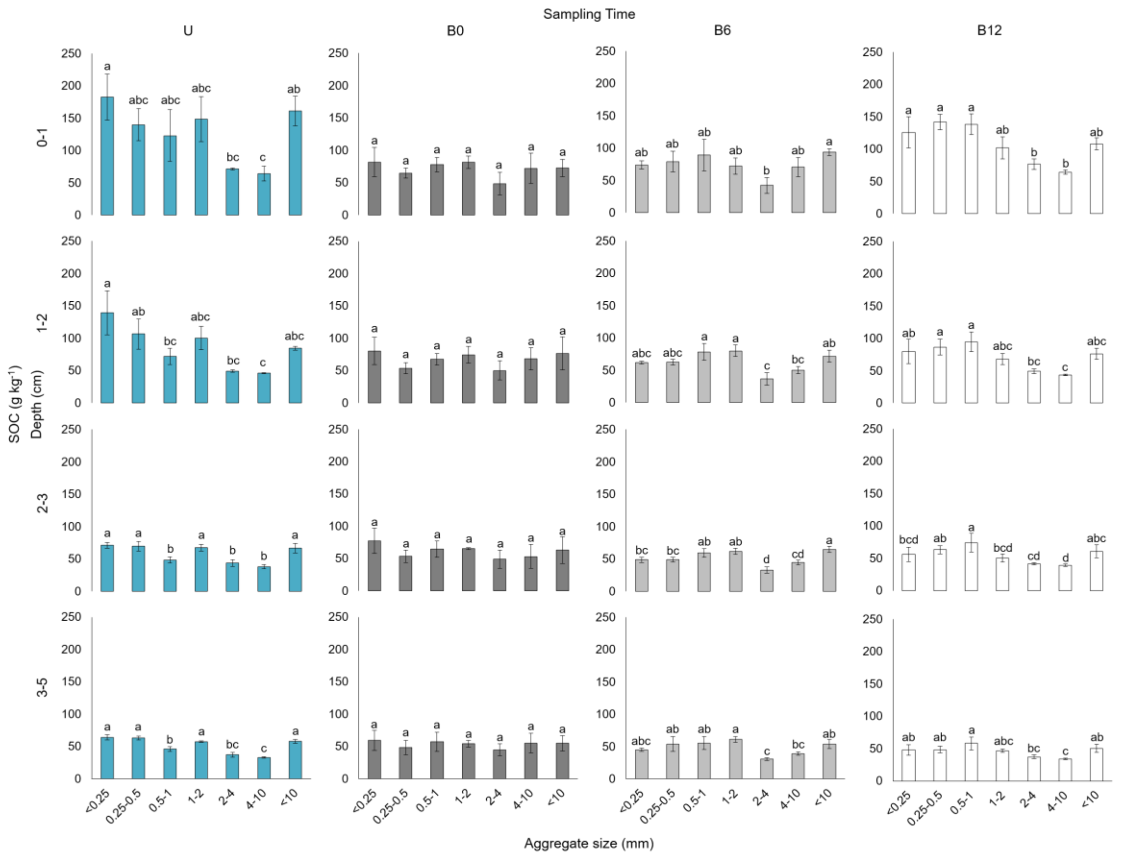

Figure 2. Soil Organic Carbon (SOC) content of unburned samples (U), immediate post-fire samples (B0), 6 months (B6) and one year (B12) post-fire samples for each sieve fraction (<0.25, 0.25-0.5, 0.5-1, 1-2, 2-4 and 4-10 mm) and whole soil (<10 mm) per sampling depth (0-1, 1-2, 2-3, 3-5 cm). Letters indicate homogeneous groups at p< 0.05 among sieve fractions and whole soil for each soil depth and sampling time. Mean ± SE

Compared to U, burning (B0) markedly decreased SOC content in the <0.25 mm fraction at 0-1 cm depth (81.8 ± 39.3 g kg^{-1}) and in the 0.25-0.5 mm fraction at 0-1 and 1-2 cm depth (64.7 ± 13.2 and 53.7 ± 15.3 g kg^{-1}, respectively). However, the rest of the studied soil sieve fractions and depths were not significantly affected by fire. The whole soil (<10 mm) had a similar response to fire as the <0.25 and 0.25-0.5 fractions, showing a significant decrease in SOC from 161 ± 40 (U) to 72.5 ± 23.2 g kg^{-1} (B0) at 0-1 cm depth. These results suggest that the SOC contents in the U and B0 whole soil are linked to those of the finer fractions, which appear to be more sensitive to fire than the coarser fractions. This result is supported by the variance analysis, which indicates that the SOC values in the finer sieve fractions are highly influenced by fire (**Table 3**).

Table 3. Variance analysis of Soil Organic Carbon (SOC), Aggregate Stability (AS) and Soil Water Repellency (SWR) for all studied depths (D) and treatments (T; burning and elapsed time since burning) in each sieve fraction (<0.25, 0.25-0.5, 0.5-1, 1-2, 2-4, 4-10 mm) and whole soil (<10 mm)

Fraction (mm)		<0.25		0.25-0.5		0.5-1		1-2		2-4		4-10		<10	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p
SOC															
	T	6.998	**0.0009**	9.753	**0.0001**	2.149	0.1135	4.118	**0.0144**	2.663	0.0653	2.065	0.1245	3.208	**0.0361**
	D	9.590	**0.0001**	15.40	**<0.0001**	8.843	**0.0002**	10.51	**<0.0001**	3.443	**0.0286**	4.907	**0.0064**	15.16	**<0.0001**
	T x D	1.614	0.1533	1.983	0.0749	0.818	0.6036	1.590	0.1618	0.352	0.9489	0.114	0.9991	2.073	0.0628
AS															
	T	-	-	30.47	**<0.0001**	87.15	**<0.0001**	71.97	**<0.0001**	68.13	**<0.0001**	70.61	**<0.0001**	71.97	**<0.0001**
	D	-	-	2.362	0.0954	3.552	**0.0251**	0.987	0.4113	1.433	0.2513	1.468	0.2423	0.987	0.4113
	T x D	-	-	1.479	0.2098	0.634	0.7595	0.905	0.5323	0.186	0.9942	0.997	0.4631	0.905	0.5323
SWR															
	T	9.371	**0.0002**	14.31	**<0.0001**	2.578	0.0709	5.097	**0.0054**	0.270	0.8467	0.064	0.9786	13.763	**<0.0001**
	D	7.424	**0.0008**	24.17	**<0.0001**	5.051	**0.0056**	6.562	**0.0014**	6.228	**0.0019**	3.748	**0.0205**	30.493	**<0.0001**
	T x D	3.844	**0.0029**	7.286	**<0.0001**	0.897	0.5392	2.800	**0.0152**	0.272	0.9778	0.300	0.9694	5.221	**0.0002**

Six months after fire (B6), the detected fire effects on SOC were still present and it decreased in the <0.25 mm fraction at 1-2 cm depth compared to U. One year after fire (B12), the SOC values of the <0.25 mm fraction showed a slight increase at 0-2 cm depth compared to those of B0 and B6. The statistical analysis showed that the SOC state of B12 was between that of B0-B6 and U, which suggests signs of recovery of the fire-affected fractions. For the 0.25-0.5 mm fraction at 0-1 cm depth, the B12 SOC values were similar to those of U, indicating a recovery for this aggregate size and depth; in addition, at 1-2 cm depth, SOC also showed a slight increase for the <0.25 mm fraction. No further changes related to fire or elapsed time were observed for the rest of the studied aggregate sizes and depths. The SOC increase detected in B12 for the smaller sieve fractions could be explained by the late incorporation of ashes and partially charred plant remains that become fine organic particles that are mixed with the soil after fire (González-Pérez et al., 2004). Nevertheless, these slight variations were not observed in the whole soil, which remained virtually unchanged compared to B0 during the studied period.

The SOC distribution among the U sieve fractions agrees with the results of Jiménez-Morillo et al. (2016a), which indicated that the SOC content was higher in the finer fractions than the coarser fractions of unburned soils under four different vegetation species (*Quercus suber*, *Pteridium aquilinum*, *Pinus pinea* and *Halimium halimifolium*) in the Doñana National Park (SE-Spain). However, Jiménez-Morillo et al. (2016b), in a different study carried out on soils under *Quercus suber*, detected higher C contents in the 0.5 to 2 mm sieve fractions than in the finer <0.5 mm sieve fractions. Nevertheless, the SOC values obtained in the sieve fractions after burning contrasted with those previously reported in the literature. Jiménez-Morillo et al. (2016b) observed that, after a wildfire, sieve fractions (<0.05 to 2 mm) generally showed higher C contents than unburned sieve fractions, which could be related to the incorporation of different sizes of charred materials (Skjemstad et al., 1996; Nocentini et al., 2010). Our results also contrast with those of Jordán et al. (2011), who suggested that the destruction of organic matter during fires affects mainly the coarse aggregates, as combustion can be more intense in this size range due to the oxygen present in macropores.

Our whole soil results contrast with the traditionally reported neutral or positive effects of prescribed burning on SOC, as reviewed by Alcañiz et al. (2018). However, these reductions in the SOC content after the prescribed burning of *E. horridum* were also detected by Armas-Herrera et al. (2016) and Girona-García et al. (2018). Immediately after fire, Armas-Herrera et al. (2016) reported an average SOC decrease of 43 % at 0-3 cm depth, while Girona-García et al. (2018) reported a SOC reduction of 54 % at 0-1 cm depth. A reduction in the SOC content is a common effect of fire, since SOM combustion is initiated when temperatures reach a range of 200-250 °C (Badía & Martí, 2003; Certini, 2005; Santín & Doerr, 2016). Temperature analysis showed that, in at least one of the studied points, the uppermost soil layer was exposed to temperatures between 200-400 °C for 6 minutes. In addition to this approximation, the slow spread of fire (0.64 ha/h) suggests high fire residence times. Additionally, *E. horridum* shrubs (**Figure 1**) form low and dense patches (Komac et al., 2011) with a homogeneous spatial distribution of fuel loads, which supports higher temperatures and longer fire residence times (Santana et al., 2011). In this way, the fuel loads and fire residence times observed in our study are higher than those of previous studies on burned shrublands and forest understories (Vadilonga et al., 2008; Santana et al., 2011; Fernández et al., 2013; Vega et al., 2005, 2014). The differences in our results compared to those of the literature reporting neutral or positive effects of prescribed burning on SOC may also be related to soil sampling. A dilution effect could be produced when too much soil thickness is sampled, since the effects of fire may be confined to the uppermost layer. Furthermore, when sampling is not carried out soon enough, ash and charred material could mix into the soil, increasing its SOC content (Badía-Villas et al., 2014, 2017).

3.2. Soil Aggregate Stability and Mean Weight Diameter

Soil aggregate stability (AS) was very high in all the studied U samples (> 76 %), and it increased concomitantly with aggregate size (**Figure 3**). No variations in AS were observed among aggregate sizes for the different soil depths, except for the 0.25-0.5 mm fraction, which showed an increase from 76.0 ± 4.6 % at 0-1 cm depth to 89.1 ± 1.4 % at 3-5 cm depth. Immediately after the prescribed burning (B0), AS only showed changes in the 0.25-0.5 mm aggregate size at 0-1 cm depth, increasing from 76.0 ± 4.6 (U) to 85.1 ± 3.8 % and diluting the previously mentioned depth gradient. Six months after burning (B6), the AS values in all aggregate sizes and depths remained virtually unchanged from those of B0. Nevertheless, one year after fire (B12), the AS of all aggregate sizes and depths showed an average reduction of 16.92 %. The mean weight diameter (MWD) in the U samples was 3.57 ± 0.43 mm at 0-1 cm depth and increased to 4.82 ± 0.41 mm at 3-5 cm depth, as represented in **Figure 4**. In B0, no significant changes in MWD were observed in any of the studied depths compared to U, although the aforementioned gradient disappeared. In B6, the MWD values increased at 1-2 and 3-5 cm depth compared to U and B0. However, those differences are not detectable in the corresponding B12 samples.

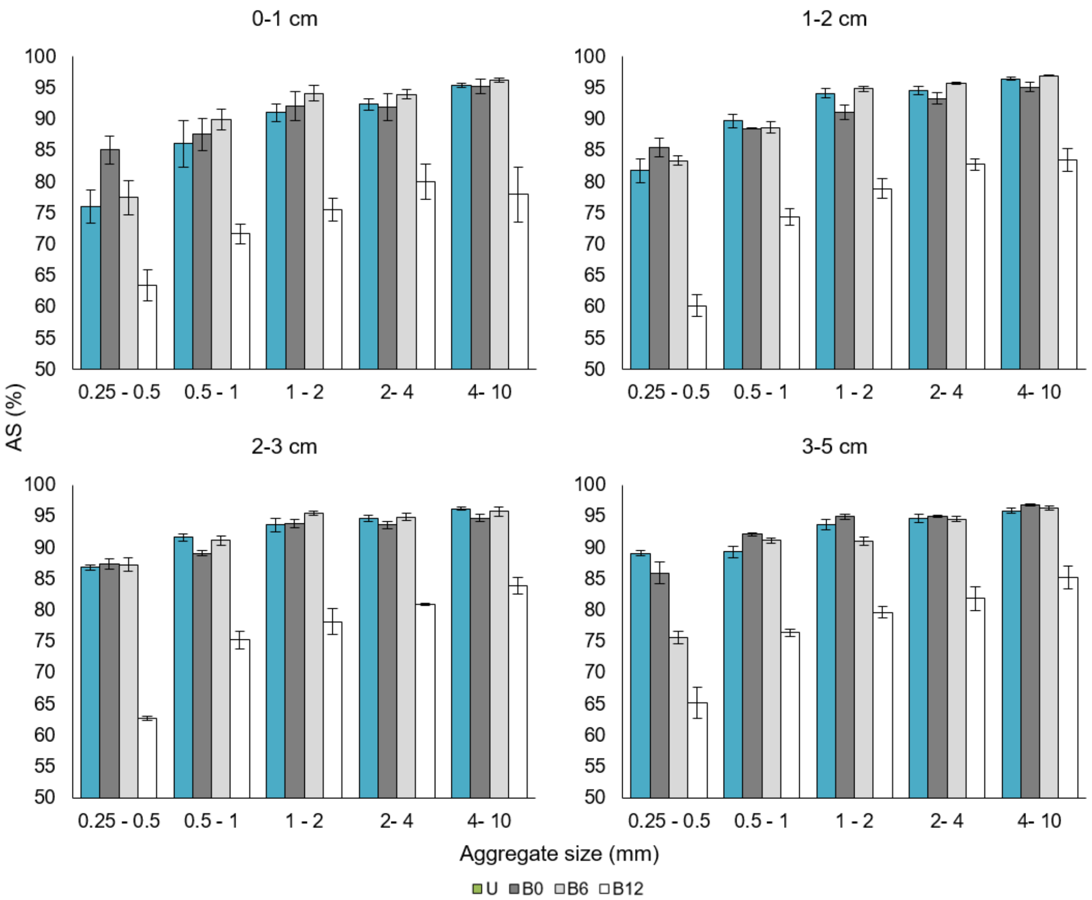

Figure 3. Aggregate Stability (AS) of unburned samples (U), immediate post-fire samples (B0), 6 months (B6) and one year (B12) post-fire samples for each aggregate size (<0.25, 0.25-0.5, 0.5-1, 1-2, 2-4 and 4-10 mm) and sampling depth (0-1, 1-2, 2-3, 3-5 cm). Mean ± SE

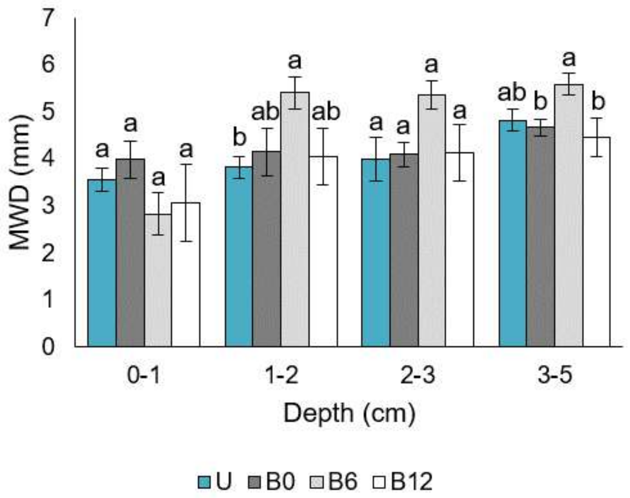

Figure 4. Mean Weight Diameter (MWD) of unburned samples (U), immediate post-fire samples (B0), 6 months (B6) and one year (B12) post-fire samples at different sampling depths. Mean ± SE. Letters indicate significant differences ($p< 0.05$) among sampling times for each soil depth

These results agree with previous studies indicating that soil aggregation can remain practically unaffected (Arcenegui et al., 2008; Jordan et al., 2011) or increase (Giovannini & Lucchesi, 1997) after fires when the temperatures in the soil remain below 220 °C. The AS of the 0.25-0.5 mm aggregate size increased after burning, despite its reduction in the SOC content, which could be explained by mineralogical modifications, as observed by Jiménez-Pinilla et al. (2016a), who detected an increase in AS by heating at 300 °C that was related to the compaction of structural units. On the other hand, Giovannini & Lucchesi (1997) reported that AS could remain unaltered after SOM combustion at temperatures of 150 °C due to transformations of the cementing iron oxides. Giovannini et al. (1990) explained the increase in soil aggregation after heating by the dehydration of soil gels at temperatures over 170 °C. Additionally, this slight increase does not seem to be related to aggregate coating by hydrophobic organic substances that increase its resistance to water slaking (Terefe et al., 2008) since SWR also decreased in B0 for this sieve fraction, as explained in section 3.3, and both properties are negatively correlated (**Table 4**). The high AS observed in both the unburned and burned samples during the study period indicate that SOM may not be the main cementing agent, since AS does not change when the SOC content is reduced by fire. This contrasts with the results of Boix-Fayos et al. (2001), who reported that the macroaggregate stability of SE-Spain soils depends on organic matter when its content is higher than 5 or 6 %. The high clay content and the presence of carbonates suggest that the aggregation in our soil might be mainly driven by inorganic binding agents that act as a permanent cement (Tisdall & Oades, 1982). Additionally, calcium carbonate is not usually affected by low-intensity fires, as it might resist temperatures up to 1000 °C (Rabenhorst, 1988). The AS reduction in B12 samples could be linked to trampling by cattle, which is known to alter soil structure (Drewry et al., 2008) and cause its compaction at ranges of 5 to 20 cm depth (Nawaz et al., 2013). This is supported by the fact that cattle grazed the plots from 2 months after B6 until B12. Despite the decrease in the B12 AS values compared to those of previous samples, the change was possibly not great enough to significantly affect the MWD, although it tended to decrease at 1-5 cm depth compared to B6.

Table 4. Regression analysis of Soil Organic Carbon (SOC), Soil Water Repellency (SWR) and Aggregate Stability (AS) among the soil sieve fractions (<0.25, 0.25-0.5, 0.5-1, 1-2, 2-4 and 4-10 mm) for each sampling time: unburned (U), immediate post-fire (B0), 6 months (B6) and one year (B12) post-fire

Time	U		B0		B6		B12	
	r	p	r	p	r	p	r	p
SOC x SWR	+0.649	**<0.0001**	+0.228	0.0566	+0.613	**<0.0001**	+0.798	**<0.0001**
SWR x AS	−0.605	**<0.0001**	−0.894	**0.0004**	−0.253	0.0607	−0.440	**0.0008**
AS x SOC	−0.568	**<0.0001**	−0.243	0.0617	−0.335	**0.0123**	−0.598	**<0.0001**

3.3. Soil Water Repellency

The occurrence of each class of SWR for each soil depth, aggregate size and sampling time is shown in **Figure 5**. The finer fractions (<0.25 to 2 mm) of the U samples at 0-1 cm depth showed high natural SWR ranging from strongly to extremely water repellent, while the coarser fractions (2 to 10 mm) were mainly wettable or only slightly water repellent. In the unburned soil, SWR showed a decreasing trend with both increasing depth and aggregate size, and at 3-5 cm depth, only a low occurrence of strong water repellency was observed in the 0.5 to 2 mm fractions, whereas slightly water repellent or wettable were the most representative classes for <0.25-0.5

mm and 0.5 to 10 mm, respectively. The SWR of the whole soil (<10 mm) showed strong water repellency that gradually decreased with depth and was wettable at 3-5 cm.

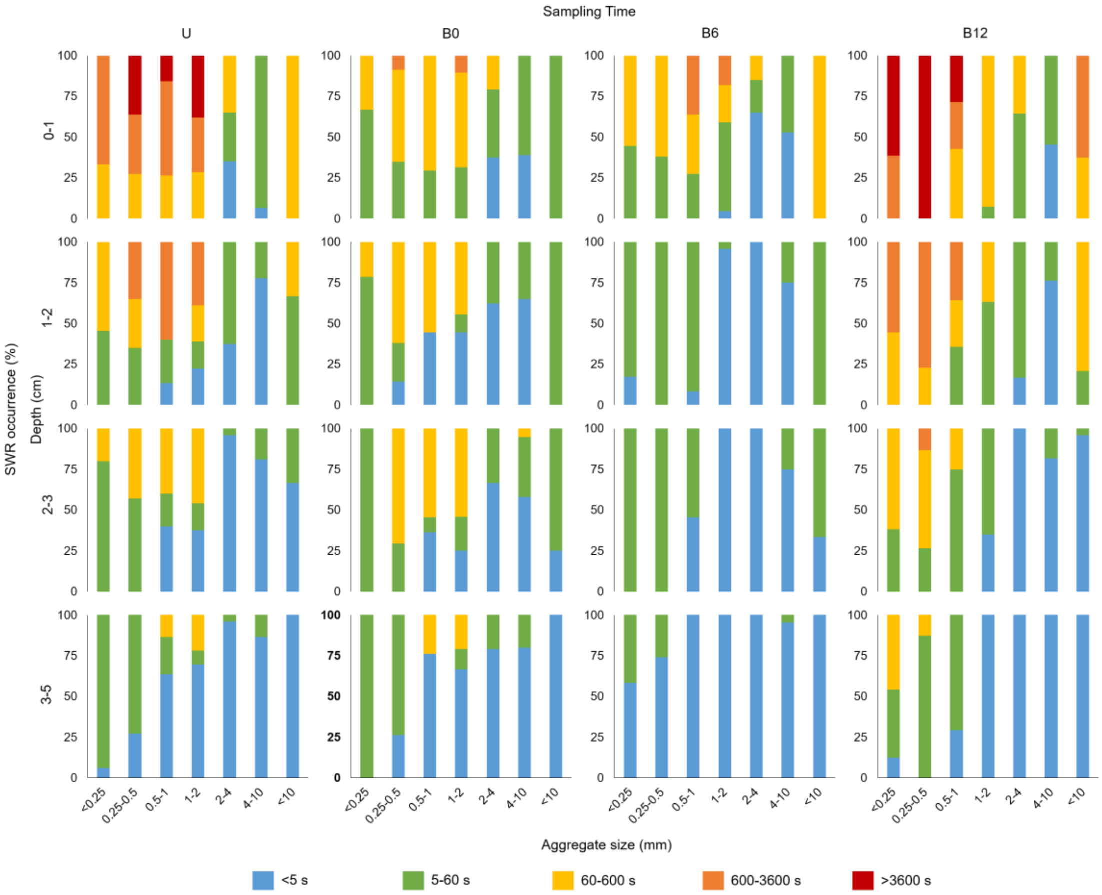

Figure 5. Occurrence (%) of Soil Water Repellency (SWR) according to the Water Drop Penetration Time (WDPT) test in the unburned (U), immediate post-fire (B0), 6 months (B6) and one year (B12) post-fire samples for each aggregate size (<0.25, 0.25-0.5, 0.5-1, 1-2, 2-4 and 4-10 mm) and whole soil (<10 mm) per sampling depth (0-1, 1-2, 2-3, 3-5 cm). SWR classes defined by Bisdom et al. 1993

Fire decreased the SWR at 0-1 and 1-2 cm depths in the finer fractions (<0.25 to 2 mm), while the coarser fractions remained virtually unchanged, as observed in the B0 samples. At 0-1 cm depth in B0, unlike in U, no extreme SWR was observed, and the occurrence of severe SWR was reduced to less than 10 % in the 0.25-0.5 and 1-2 mm aggregate sizes. In this way, the predominant classes of SWR in B0 for the finer fractions were strongly and slightly water repellent. At 0-2 cm depth, the wettability of the finer fractions also increased with burning, as the severe SWR observed in U was not observed in B0, and a higher occurrence of the wettable class samples was detected in B0 than U. The same pattern was observed for the whole soil (<10 mm), in which fire reduced

SWR to slightly water repellent at 0-1 cm depth. At B6, an opposite trend was observed between 0-1 cm and 1 to 5 cm depths. While SWR at 0-1 cm depth increased in B6 compared to B0 for the finer fractions, in the deeper soil layers, it continuously decreased to only slightly water repellent and wettable classes. One year after the prescribed burning (B12), SWR increased for all the studied soil depths in the finer fractions (<0.25 to 2 mm) compared to B6. B12 SWR increased mainly in the <1 mm fractions at 0-1 and 1-2 cm depths, showing an even higher occurrence of severe and extreme water repellency classes in these samples than the corresponding U samples. These results are reflected in the SWR occurrence of the whole soil (<10 mm), which recovered from the fire and even exceeded the pre-fire SWR occurrence, showing strong and severe water repellency classes at 0-1 cm depth. According to these results, the occurrence of SWR in the whole soil seems to be highly related to changes in the SWR of the fine fractions (<0.25 to 2 mm).

The detected SWR distribution among aggregate sizes was also found by Arcenegui et al. (2008) in Mediterranean pinewood soils, indicating that the <0.25 and 0.25-0.5 sieve fractions were the most water repellent; however, unlike the present study, SWR increased after fire. The higher occurrence of SWR in the finer sieve fractions was also observed by Jordán et al. (2011) in Mexican volcanic soils that had been unburned for a long time. Nevertheless, they detected no differences between the burned and unburned soils affected by low-severity wildfires, whereas sites affected by higher severity fires showed lower degrees of SWR. González-Pelayo et al. (2015) identified a trend to increasing SWR in the <0.25 mm sieve fraction beneath unburned shrubs (*Arbutus unedo* > *Pistacia lentiscus* ≈ *Quercus coccifera* > bare soil) in Central Portugal, although its persistence was lower than that of our findings for soil under *E. horridum*; furthermore, they observed a slight increase in SWR after prescribed burning.

We found significant differences in the SOC content among samples from different WDPT classes, showing increases in the SOC content with increasing SWR persistence (Figure 6). However, the SOC content does not fully explain the variability in SWR in all the sieve fractions of U, B0 and B6 according to the regression analysis (**Table 4**). This suggests that SOM quality and its fire-induced changes could be a possible contributing factor in SWR variations (Jiménez-Morillo et al., 2016a). Zheng et al. (2016) also indicated that, in some cases, the distribution of SOM among the different aggregate sizes might be the driving factor of SWR occurrence rather than its content. In this way, the greater SOM concentrations in the smaller aggregates may explain their strong contribution to SWR (Jiménez-Morillo 2016a) and could be a result of the presence of fine, hydrophobic interstitial SOM that accumulates in the finer sieve fractions (Mataix-Solera & Doerr, 2004). The drastic reduction in SWR observed for B0 might be related to the destruction of these hydrophobic SOM, since the SOC content decreased concomitantly. The recovery of SWR in B12 could be related to the incorporation of ashes into the soil, since they play an important role in SWR evolution following fire (Jiménez-Pinilla et al., 2016b) and a significant positive correlation between SOC and SWR was detected ($r = 0.798$; $p= <0.0001$). This SWR increase in B12 could also be attributed to the sampling being carried out after a low-rainfall season, as SWR can be more pronounced following dry periods, as reported by Doerr et al. (2000).

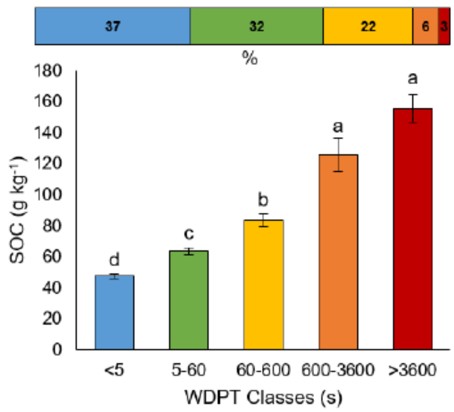

Figure 6. Soil Organic Carbon (SOC) content of all the sieve samples (n= 288) distributed in each Water Drop Penetration Time (WDPT) class. Mean ± SE. Numbers in the upper bar indicate the percentage of the total samples included in each class. Letters indicate significant differences in SOC content among WDPT classes at p <0.05

Traditionally, high SWR occurrence has been related to coarse soil textures (Giovannini & Lucchesi, 1983). González-Peñaloza et al. (2013) suggested that a limited amount of SOM may cause higher SWR in coarse-textured than fine-textured soils due to the low specific surface of larger particles. However, our studied soil showed a 22 % clay content and high natural SWR and SOC contents. This may occur if the hydrophobic organic materials causing SWR are small enough, increasing the hydrophobicity in the finer rather than the coarser fractions (de Jonge et al., 1999).

3.4. Post-fire implications of prescribed burning

Results indicate that one year after burning, the direct effects induced on SOC were still present, while SWR recovered to higher values than those of U samples. On the other hand, AS, which remained almost unaltered during the study period, was dramatically reduced in B12.

The detected SOC reduction and the biomass carbon losses have negative effects on C sequestration that could extend over the long term. Knowledge about the effects that land management practices, such as prescribed burning, can exert on mountain soils is of vital importance, since these soils store large amounts of C (Saenger et al., 2015). Therefore, further research is needed on this topic in order to assess the sustainability of burning practices in mountain environments from the point of view of C sequestration.

Prior to burning, soils showed high SWR but were covered by dense patches of *E. horridum*, which is a species that physically protects soil and improves water and nutrient retention (Montserrat et al., 1984). Burning reduced SWR, but one year after burning, it recovered to even higher levels than the pre-burning SWR in an environment where vegetation had been removed, exposing the soil to raindrop splash and increasing overland flow (DeBano, 1981). Indeed, Girona-García et al. (2018) indicated for the same study site that the recovered vegetation observed 8-12 months after fire only represented a small surface of the burned plots. However, it is difficult to isolate the effect of SWR on erosion processes since it is highly dependent on the continuity of the hydrophobic layer (Shakesby et al., 2000). Moreover, only the *E. horridum* shrubs were burned, while the pre-existing grasses remained unaltered; this, together with the occurrence of different fire severities, created a mosaic of patches (Cawson et al., 2012). Thus, further research is needed in order to detect the influence of SWR on the general overland flow, since it may vary due to the high spatial variations in infiltration (Doerr et al., 2000).

The maintenance of soil structure is of vital importance for plant growth because it determines the air and water flows. AS remained virtually unaffected by fire, although it suffered a non-fire-related significant decrease one year after the prescribed burning. The reduction in aggregate stability and therefore its destruction in extreme cases enhances soil sealing, which, combined with the development of SWR, reduces water infiltration and increases soil erodibility (Mataix-Solera et al., 2011; Cawson et al., 2016).

Fine soil fractions are of great importance in nutrient exchange processes. The occurrence of SWR in small aggregates could lead to a reduction in nutrient exchange between the soil and plants, given the reduced wettability of the soil (Mataix-Solera & Doerr, 2004). This could negatively affect the recovery of the pastures, which is the objective of the studied prescribed burning.

4. CONCLUSIONS

Fire severely decreased the SOC content in the <0.25 mm fraction at 0-1 cm depth and in the 0.25-0.5 mm at 0-2 cm depth. This translated to a 45 % SOC reduction at 0-1 cm depth in the whole soil that did not recover during the studied period. SWR was reduced by burning mainly at 0-1 cm depth for the <0.25 to 2 mm sieve fractions and the whole soil. One year after the prescribed burning, SWR recovered in these fractions, reaching even higher values than those of the unburned soil. The AS of the 0.25-0.5 mm aggregates increased after fire, but one year later, it suffered a striking decrease in all aggregate sizes and depths, probably related to cattle trampling. These findings suggest that the direct effects of prescribed burning were still present one year after burning, and further research is needed in order to assess the increased soil loss risks of the post-fire situation. Based on our results, we can also conclude that fine fractions (<2 mm) are more prone to be affected by fire, and they determine the behavior of the whole soil to a greater extent than coarser fractions.

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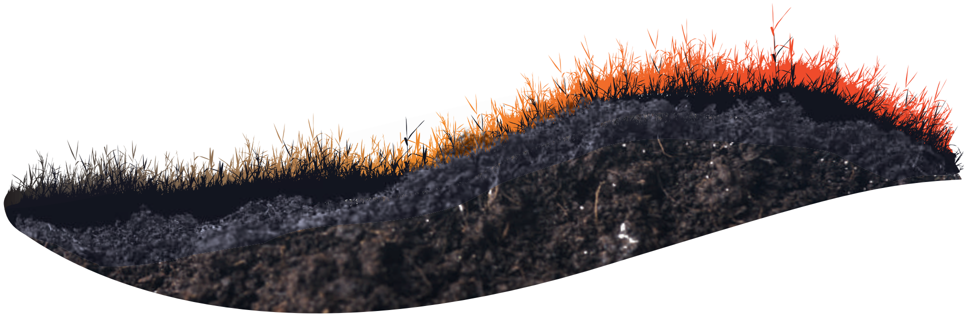

CHAPTER 5:

Effects of prescribed fire for pasture reclamation on soil chemical properties in subalpine shrublands of the Central Pyrenees (NE-Spain)

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ABSTRACT

The abandonment of the traditional pastoral activities in the subalpine grasslands of the Central Pyrenees (NE-Spain) has resulted in shrub encroachment processes that are dominated by species such as the *Echinospartum horridum*. Therefore, prescribed burning has been recently readopted in this region as a management tool to stop the spread of shrubs and recover grasslands. We aimed to assess the effect that this practice may have on soil chemical properties such as SOC, N, pH, EC, water-extractable and exchangeable cations (Ca^{2+}, Mg^{2+} and K^{+}), cation exchange capacity, inorganic N forms ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$) and available P. We studied two prescribed burnings conducted at the subalpine level of the Central Pyrenees in the municipalities of Tella-Sin (April, 2015) and Buisán (November, 2015). At each site, the topsoil was sampled in triplicate at soil depths of 0-1, 1-2 and 2-3 cm immediately before (U), immediately after (B0) and one year after (B12) burning, and litter and/or ashes were removed prior to sampling. The results indicate that in the B0 samples, burning significantly reduced the SOC and N contents as well as the exchangeable Ca^{2+} and Mg^{2+} at 0-1 cm, whereas the rest of the studied properties remained virtually unchanged. However, in the B12 samples we detected a decrease of nutrient content that was probably related to leaching and/or erosion processes.

1. INTRODUCTION

Pasturelands in the Central Pyrenees (NE-Spain) have traditionally been maintained by livestock grazing and occasional burnings (Nadal-Romero et al., 2018). However, due to rural exodus and the reduction in livestock densities, this activity has suffered from remarkable reductions over the past decades (Komac et al., 2013). The mesophytic pastures that can be found in the Pyrenees below the timberline require shrub management (i.e., grazing, burning or clearcutting) for survival (Halada et al., 2011); therefore, the reduction in grazing activity led to shrub encroachment processes that were dominated by species such as *Echinospartum horridum* (Vahl) Rothm (Komac et al., 2013; Nuche et al., 2018). The development of this species poses a threat to biodiversity and an increase in flammability risks (Caballero et al., 2010) because it forms large and dense monospecific covers (Komac et al., 2011).

A suitable procedure to reduce shrub encroachment in grazing lands can be the use of prescribed burnings (Goldammer & Montiel, 2010), which are defined as the planned use of fire to achieve precise and clearly defined objectives (Fernandes et al., 2013). Nevertheless, fire can affect most soil properties directly by burning and indirectly as a consequence of the new post-fire conditions (Santín & Doerr, 2016). The extents of the effects of fire on soils are highly influenced by environmental conditions; so, prescribed burnings are conducted when the soil and fuel moisture, temperature and topography conditions are favorable, to limit the impact of the burnings on soils and prevent fire from escaping (Vega et al., 2005; Molina, 2009). However, prescribed burnings show contrasting effects on soil properties, as has been recently reviewed in Alcañiz et al. (2018).

Previous works dealing with prescribed burnings of *Echinospartum horridum* in the Central Pyrenees have shown that this practice may severely affect soil organic matter (SOM) content (Armas-Herrera et al., 2016, 2018; Girona-García et al., 2018a, 2018b) in the first few centimeters of the topsoil. The combustion of SOM and vegetation may produce an increase in the available nutrients by either the mineralization of organic compounds or the production of ashes (González-Pérez et al., 2004; Knicker, 2007). Then, the incorporation of ashes into the soil can lead to increases in pH and electrical conductivity (EC) (Certini, 2005). The literature shows that the available concentrations of Ca^{2+}, Mg^{2+}, K^+ and Na^+ are commonly increased after prescribed burning (Arocena & Opio, 2003; Lavoie et al., 2010; Alcañiz et al., 2016). Inorganic N forms can also increase after burning from either the contribution of ashes or the mineralisation of soil organic N. For this reason, it is common to detect ammonium gains immediately after burning that will result in nitrates increases via nitrification over time (Gundale et al., 2015; San Emeterio et al., 2016). Fire may also boost the contents of available P in the soil via both the contributions of ashes as well as the mineralization of its organic forms that can occur even at relatively low temperatures (Úbeda et al., 2005; Badía-Villas et al., 2014; Larroulet et al., 2016; García-Oliva et al., 2018). This enrichment in nutrients produced by fire may promote the rapid establishment of herbaceous species. However, another consequence of SOM destruction is the loss of adsorption sites in the soil, thereby reducing the cation exchange capacity (CEC) (Badía & Martí, 2003). In this way, depending on the severity and recurrence of burning, these practices could also lead to nutrient losses (Wanthongchai et al., 2008). Nevertheless, the CEC usually remains unchanged after prescribed burning (Larroulet et al., 2016; Fonseca et al., 2017).

The main objective of our study was to detect the effects of prescribed burning of *Echinospartum horridum* for pasture reclamation on soil chemical properties, focusing on soil nutrient content and availability, at the subalpine level of the Central Pyrenees (NE-Spain). We analyzed the immediate effects of burning on total soil organic C (SOC), total N, pH, EC, water-extractable and exchangeable cations, CEC, inorganic N forms ($N\text{-}NH_4^+$ and NO_3^-) and available P, as well as their changes one year after the fire at soil depths of 0-1, 1-2 and 2-3 cm.

2. MATERIAL AND METHODS

2.1. Study sites

The study sites are located in two subalpine areas of the Central Pyrenees (NE-Spain) in the municipalities of Buisán and Tella-Sin (**Figure 1**). The Buisán plot is located in an area with a mean slope of 10 % at 1760 m.a.s.l., while the Tella plot was located on a steeper slope of 25 % at 1875 m.a.s.l., and both sites face south. The mean annual temperature in Buisán is 6 °C and 5 °C in Tella. The mean annual precipitations are 1500 mm (Buisán) and 1700 mm (Tella).

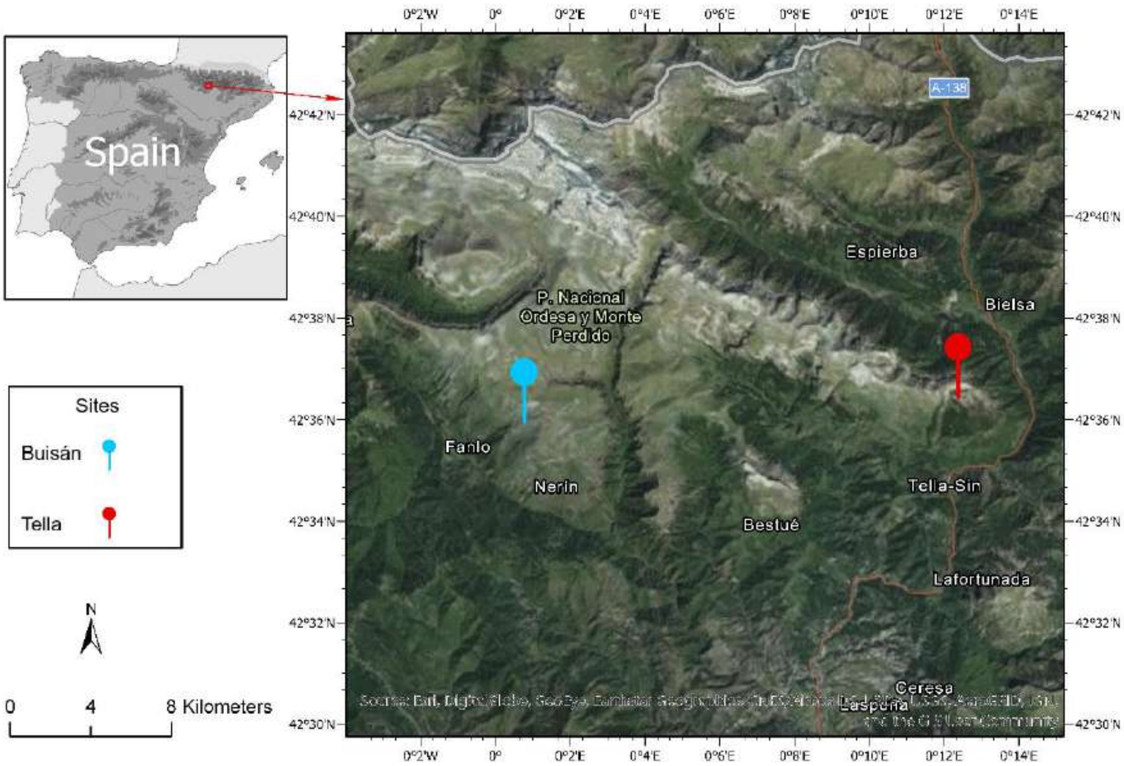

Figure 1. Location of the Buisán and Tella areas of study

The topsoil Ah horizons (0-5 cm) of both sites are characterized by high SOM contents, high CEC and fine textures; the pH in Buisán is neutral whereas it is moderately acidic in Tella. Soils in Buisán are classified as Eutric Cambisol and those in Tella as Eutric Epileptic Cambisol (IUSS Working Group WRB, 2014), and the complete soil characterization of the study sites can be found in Armas-Herrera et al. (2016) and Girona-García et al. (2018a), respectively. In Buisán and Tella, the bedrock is composed of fine detritic sediments over clayey limestones alternated with Eocene marls. As a consequence of the decreased grazing activity and the prohibition of fire after 1980, these areas have been invaded by *Echinospartum horridum,* which covered more than 75 % of the surface area before the prescribed burning was conducted. Pastures in the study sites that surround the *Echinospartum horridum* shrubs are composed of 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.

2.2. Prescribed burning characteristics

The prescribed burnings were conducted in April, 2015 (Tella) and November, 2015 (Buisán) by qualified firefighters of the EPRIF (Wildfire Prevention Team) of Huesca and BRIF (Reinforcement Brigades against Wildfires) of Daroca units. The environmental conditions met the established parameters for *Echinospartum horridum* burning: no heavy rainfall took place prior to the burning date, the temperature was between 5 and 15 °C, the relative humidity of the air was 35-70 %, and the wind speed ranged from 5 to 10 km h^{-1}. An approximation of the temperatures reached during burning at each site was obtained via type-K thermocouples placed in one sampling point at each of the different soil depths (**Table 1**). The Buisán burning was performed by applying the point source fire technique and creating a grid of spot ignitions that burned from the east to the west flanks that followed a slow progression (0.63 ha h^{-1}). In Tella, a backing fire was ignited to spread against the wind and downslope, and it was faster (2.82 ha h^{-1}) than that in Buisán. At both sites, the aerial biomass of *Echinospartum horridum* was mostly eliminated by burning, resulting in burned trunks, partially charred litter and patches of black and gray ashes.

Table 1. General characteristics of the prescribed burnings of Buisán and Tella. Temperature analysis comprises the elapsed time since a temperature increase was detected until it stabilised during the cooling stage

Study Site	**Buisán**				**Tella**			
Burning Date	November, 2015				April, 2015			
E. horridum cover (%)	75				80			
Estimated Fuel Loads (kg m^{-2}):								
Aerial biomass	9.24				9.86			
Litter (OL + OF)	1.62				1.73			
Burned surface (ha)	3.8				12.5			
Wind speed (km h^{-1})	<8				10-15			
Firing technique	Point Source Fire				Backing Fire			
Mean flame height (m)	1				0.4			
Mean flame length (m)	1.5				1.7			
Burning rate (ha h^{-1})	0.63				2.82			
Temperature analysis	Surface	1 cm	2 cm	3 cm	Surface	1 cm	2 cm	3 cm
Maximum temperature (°C)	438	31.1	18.5	18.5	n.d.	397	121	n.d
Initial temperature (°C)	13.1	9.77	9.60	8.93	n.d.	16.0	16.2	n.d
Final temperature (°C)	27.5	22.2	17.6	18.2	n.d.	25.5	25.7	n.d
Duration (min)								
< 100 °C	17.5	30.0	30.0	30.0	n.d.	33.0	42.0	n.d.
100 - 200 °C	6.00	0.00	0.00	0.00	n.d.	5.00	8.50	n.d.
200 - 300 °C	4.00	0.00	0.00	0.00	n.d.	9.50	0.00	n.d.
300 - 400 °C	2.00	0.00	0.00	0.00	n.d.	3.00	0.00	n.d.
> 400 °C	0.50	0.00	0.00	0.00	n.d.	0.00	0.00	n.d.
Pre-fire soil water content (%, w w^{-1})	n.d.	137	72.8	58.8	n.d.	100	108	84.2
Post-fire soil water content (%, w w^{-1})	n.d.	60.7	55.7	53.9	n.d.	74.5	78.6	59.0

n.d.: not determined

2.3. Soil sampling

At each burning site, we chose three representative sampling spots that were covered by *Echinospartum horridum* prior to burning. At each of these points, after removing the shrubs and organic layers from an approximate surface area of 0.25 m^2, the topsoil Ah horizon was carefully sampled at depths of 0-1, 1-2 and 2-3 cm (**Figure 2**). These samplings were carried out early in the morning immediately before the prescribed burnings were conducted, and unburned (U) samples were collected and considered the control. To detect the immediate effects of fire (B0), we sampled points adjacent to U shortly after burning (<2 h), after removing ashes and charred remains. Additionally, in both study sites, points contiguous to U and B0 were sampled one year later (B12) to assess the short-term evolution of soil properties after burning.

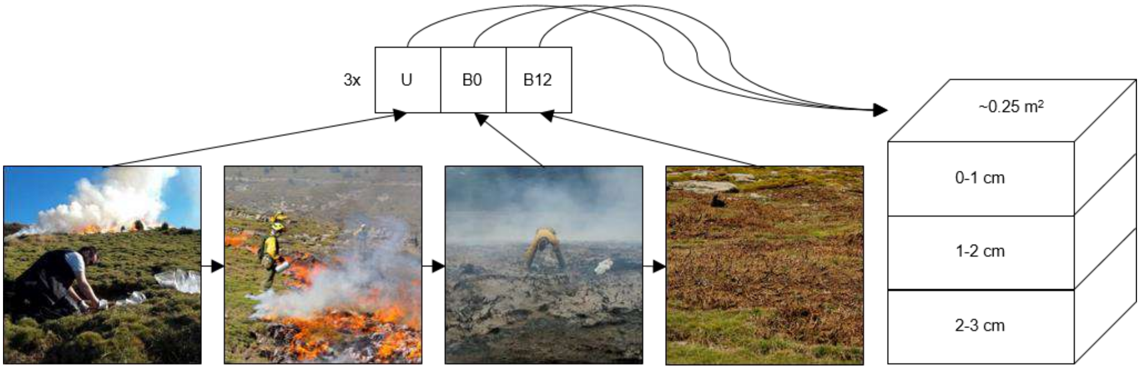

Figure 2. Sampling design followed in each study site. Unburned (U), immediately after (B0) and one year after burning (B12) samples

2.4. Sample preparation and analysis

The collected soil samples were air-dried at room temperature until constant weight and sieved through a 2 mm mesh sieve. A small portion of each sieved sample was then ground to fine powder, from which total soil organic C (SOC) and total nitrogen (N) were determined using an elemental analyzer (Vario Max CN Macro Elemental Analyser, Germany).

Soil pH was determined from potentiometric measurements of a 1:5 (w v^{-1}) suspension of soil and distilled water and the electrical conductivity (EC) was determined using an electrical conductivity meter in a 1:10 (w v^{-1}) suspension of soil and distilled water (McLean, 1982). Water-extractable (WE) cations (Ca^{2+}, Mg^{2+} and K^{+}) in the soil samples were determined by atomic absorption (Mg^{2+}) and emission (Ca^{2+} and K^{+}) spectrometry (AAS/AAE Spectrometer Varian SpectrAA 110) in 1:10 (w/v) filtered extracts of soil and distilled water after 2 hours of shaking (Sharpley & Kampath, 1988). Ammoniacal (N-NH_4^+) and nitric (N-NO_3^-) forms of nitrogen were determined according to the methods in Bremner & Keeney (1965) in 1:5 (w v^{-1}) filtered extracts of soil and 1M KCl after 30 minutes of shaking. The ammonia was separated by steam distillation from an aliquote of the extract and collected in a boric acid solution; then, it was determined by titration using 0.005N H_2SO_4. Then, in the same extract, Devarda alloy was added to reduce the remaining nitrate to ammonium and the same procedure was followed for its distillation and titration. Available P was determined following the method of Olsen & Sommers (1982). P was extracted using 0.5M $NaHCO_3$ buffered at pH 8.5 (1:20 w $v^{-1}$of soil and extractant). Then, an aliquot of each sample was taken and its P content was determined colorimetrically by measuring the concentration of the complex formed by the reaction of phosphate with acid ammonium molybdate, using a UV/visible spectrophotometer (Cole-Parmer, Jenway 6300 Spectrophotometer, United Kingdom). To determine the exchangeable Ca^{2+}, Mg^{2+} and K^{+} as well as the cation exchange capacity (CEC), a sequential extraction procedure was followed. Exchangeable cations were determined by atomic spectroscopy in the leachate obtained after three consecutive extractions (total shaking time of 15 minutes) with 1M CH_3COONa buffered at pH 8.2 (ratio 1:20, w v^{-1}). After that, samples were washed three times with ethanol (ratio 1:20, w v^{-1}) to remove the excess of the displacing solution without disturbing the adsorbed Na^{+}. Then, the adsorbed Na^{+} was displaced after three consecutive extractions (total shaking time of 15 minutes) with 1M CH_3COONH_4 buffered at pH 7 (ratio 1:20, w v^{-1}), and it was determined by atomic emission spectrometry, considering its value equal to that of the CEC (Bower et al., 1952; Rhoades, 1982).

2.5. Statistical analysis

To identify the differences in the studied soil properties related to the treatments (burning and time), as well as their variations within soil depths, one-way ANOVA tests were used because the interaction between time and depth was significant in most cases. Sampling time (U, B0, B12) was considered a fixed factor, and the data were split by soil depth (0-1, 1-2 and 2-3 cm) to detect the effects of fire and time at each of the studied soil depths. Furthermore, changes in soil properties among soil depths were tested using soil depth (0-1, 1-2 and 2-3) as a fixed factor, for which the data were split by sampling time (U, B0, B12). These tests were performed using StatView for Windows version 5.0.1 (SAS Institute Inc. Cary, North Carolina, USA). We also conducted a principal component analysis (PCA) to identify further relationships between soil properties, using a Pearson correlation, with XLSTAT software (XLSTAT 2017: Data Analysis and Statistical Solution for Microsoft Excel. Addinsoft, Paris, France).

3. RESULTS AND DISCUSSION

3.1. Prescribed burning intensity and severity

The fire severity of both prescribed burnings was estimated as low-moderate based on the indicators defined by Parsons et al. (2010). After burning, part of the litter was charred, and a thin layer of black to gray ash could be found with recognizable litter beneath it. The soil structure remained unchanged (Girona-García et al., 2018b), and aggregates were not weakened by the consumption of soil organic matter. The *Echinospartum horridum* shrubs were mostly consumed, and only their main trunks remained. The partial consumption of litter allowed for the transfer of heat into the soil, especially at the Tella site, as can be observed in the temperature analysis shown in **Table 1**. It is noteworthy that these measurements can only be considered observations, and the fire intensity was approximated because the temperatures were measured only at one point in each site. In the Buisán site, a maximum temperature of 438 °C was recorded on the soil surface and the temperature remained over 400 °C for 4.8 minutes. However, little heat transfer into the soil was detected as the temperatures at a depth of 1 cm depth raised to only 31.1 °C and very slight increases were observed in deeper soil layers. On the other hand, at the Tella burning, temperatures at a depth of 1 cm reached a maximum of 397 °C and stayed in a range of 300-400 °C for 3 minutes, whereas at 2 cm, temperatures increased to 121 °C and stayed at 100-200 °C for 8.5 minutes. Apart from the fire intensity and soil thermal inertia, the contrasted heat transfer into the soil that was observed during burning could be related to the water content of the soil (**Table 1**). The high pre-fire soil water content in Buisán (137 ± 3 %) and Tella (100 ± 32 %) could have limited the heating of the soil as heating is normally slowed until after complete water vaporization (Campbell et al., 1995; Badía et al., 2017). According to that, the soil water content in Tella tended to decrease after burning at the three studied soil depths, while in Buisán the water content decreased at only the 0-2 cm depth. From all the gathered data, we can conclude that the Tella burning was characterized by a fast (2.82 ha h^{-1}) and intense fire, whereas the Buisán burning was less intense but the fire residence time was longer (0.63 ha h^{-1}).

3.2. Effects of fire on soil organic matter

The soil organic C (SOC) and total N (N) contents were very high in the unburned (U) soils of both the Tella and Buisán sites (**Figure 3**). At the Buisán site, the SOC concentration was 243 ± 10 g kg^{-1} at 0-1 cm and decreased to 78.8 ± 14.1 g kg^{-1} at 2-3 cm soil depth; and the N content was of 14.6 ± 0.7 g kg^{-1} at 0-1 cm and decreased to 6.15 ± 0.91 g kg^{-1} at 2-3 cm. On the other hand, a higher SOC content was detected at the Tella site, which was 338 ± 59 g kg^{-1} at 0-1 cm and decreased to 216 ± 77 g kg^{-1} at 2-3 cm. The N content at this site was also higher than that in Buisán, which was 20.9 ± 2.9 g kg^{-1} at 0-1 cm and decreased to 15.2 ± 4.6 g kg^{-1} at 2-3 cm.

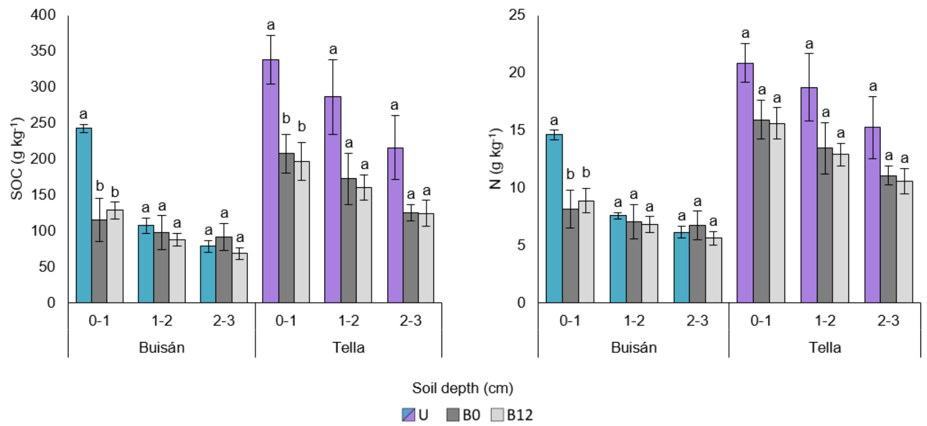

Figure 3. Soil organic C (SOC) and total N (N) in unburned (U), immediate post-fire samples (B0) and one year after burning samples (B12) for each soil depth and site (mean value ± SE of three field replicates). For same sampling depth, lowercase letters indicate significant differences among sampling times ($p < 0.05$)

At the Tella site, prescribed burning (B0) significantly reduced the SOC at 0-1 cm (-38 %) compared to U and a decreasing trend, that was close to statistical significance was also detected at soil depths of 1-2 cm (p = 0.0770) and 2-3 cm (p = 0.0633) cm soil depth. The N content also showed a decreasing trend (-24 %) at 0-1 cm that was close to significance (p = 0.0716). At the Buisán site, only the first cm of soil was significantly affected, where burning decreased the SOC and N contents in B0 by -52 % and -44 %, respectively. This severe disturbance could be explained by the temperatures reached during prescribed burning, as explained in the previous section, since the combustion of SOM begins when temperatures in the range of 200-250 °C are reached (Certini, 2005; Santín & Doerr, 2016). Furthermore, the slow spread of fire at the Buisán site indicates a higher fire residence time compared to that of the Tella site, which could explain the greater SOC and N reductions. Fire effects were still detectable at both sites one year after burning (B12) and recovery signs in SOC and N contents were not observed when compared to the contents of the U samples. The lack of short-term changes in SOC and N at the Tella site could be related to the removal of ash and charred material by wind and/or rain after burning. On the other hand, at the Buisán site, ashes mixed with partially charred litter were still observed at B12, suggesting limited incorporation of ash into the soil. Extensive discussions of the effects of prescribed burning on SOC and N at the Buisán and Tella sites can be found in Armas-Herrera et al. (2016, 2018) and Girona-García et al. (2018a).

3.3. Fire effects on soil pH, electrical conductivity and nutrients

Unburned (U) soils at the Buisán site showed pH values between 7.19 ± 0.10 and 7.55 ± 0.12 at 0-1 cm and 2-3 cm, respectively (**Figure 4**). In B0, an increase in pH was observed at 0-1 cm (7.59 ± 0.10), and this effect was still present at B12 (7.68 ± 0.07). On the other hand, at the Tella site, soils presented more acidic pH values (average of 4.5 at all studied depths) in U soils than those at the Buisán site, and these values remained unchanged in the B0 samplings indicating that this property was not affected by the fire. However, at the Tella site, the pH of the B12 samples dramatically increased at all studied soil depths to values between 6.26 and 6.70. These pH increases in acidic topsoils could be related to a series of factors such as the: 1) accumulation of K and Na hydroxides, 2) formation of Mg and Ca carbonates and/or 3) elimination of organic matter acidic groups (Knicker, 2007 and references therein).

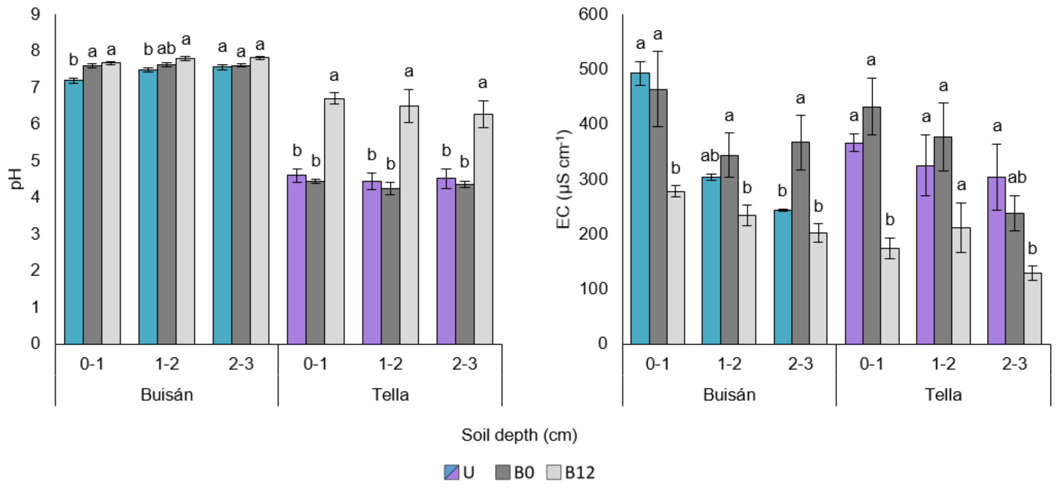

Figure 4. pH and electrical conductivity (EC) in unburned (U), immediate post-fire samples (B0) and one year after burning samples (B12) for each soil depth and site (mean value ± SE of three field replicates). For same sampling depth, lowercase letters indicate significant differences among sampling times ($p < 0.05$)

A decreasing gradient in electrical conductivity (EC) with depth was detected in the Buisán U samples while in the Tella U samples, no differences were observed among soil depths (**Figure 4**). The fire induced no direct changes in EC at either the Buisán or Tella sites although, in B12, the EC significantly decreased in all the studied soil depths in both sites.

In the U soils of Buisán, the content of water-extractable cations (WE-Ca^{2+}, WE-Mg^{2+} and WE-K^{+}) was higher at 0-1 cm than that in the underlying layers (**Figure 5**). After burning (B0), no changes were detected in WE-Ca^{2+} and WE-K^{+}, although WE-Mg^{2+} was significantly decreased at 0-1 cm. In B12, significant reductions were detected in WE-Ca^{2+} (0 to 3 cm) and WE-Mg^{2+} (0-1 cm) compared to U and B0 and WE-K^{+} remained unchanged. However, at the Tella site, the WE-cations showed no differences in B0, but their contents also decreased at all studied soil depths in B12, indicating losses by soil erosion and/or leaching (Francos et al., 2018).

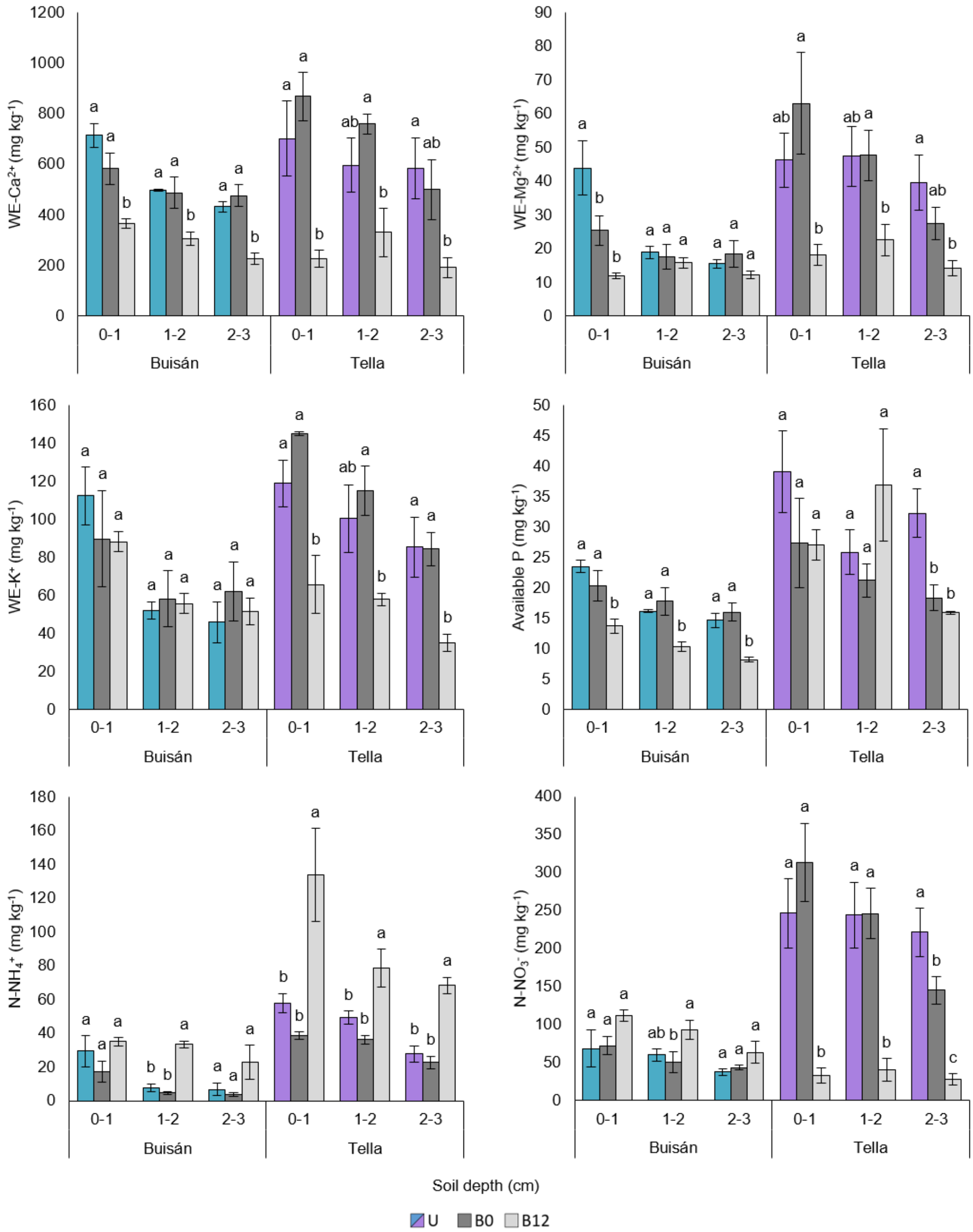

Figure 5. Water-extractable cations (WE-Ca^{2+}, WE-Mg^{2+} and WE-K^{+}), available P and inorganic N forms (N-NH_4^+ and N-NO_3^-) in unburned (U), immediate post-fire samples (B0) and one year after burning samples (B12) for each soil depth and site (mean value ± SE of three field replicates). For same sampling depth, lowercase letters indicate significant differences among sampling times ($p < 0.05$)

Our results contrast those traditionally reported in the literature after fire as it is common to find increases in pH, EC and WE-cations related to the release of cations by the combustion of SOM, as well as the incorporation of ashes into the soil (Badía & Martí, 2003; Pereira et al., 2011; Badía et al., 2014; Bodí et al., 2014). Nevertheless, in our study, these effects could not be observed because soils were sampled immediately after burning, and ashes were meticulously removed prior to sampling; however, these effects could have probably occurred within the first year after burning. Furthermore, the results obtained in B12 indicate that ashes were either redistributed at the soil surface or leached downwards into the soil, as previously observed by Bodí et al. (2014), since WE cations, and thus EC, decreased at all studied soil depths. On the other hand, the differences observed in our study compared to those conducted in Mediterranean environments could be related to the high mean annual precipitation of our study sites.

Inorganic N species (N-NH_4^+ and N-NO_3^-) at both the Buisán and Tella sites showed no differences between U and B0 at the studied soil depths (**Figure 5**). At both sampling times and sites, the nitrate content was higher than the ammonium content, indicating the occurrence of active nitrification processes. Despite the reduction in N in B0 at 0-1 cm, no changes were observed in ammonium or nitrate contents, which is unexpected because they are by-products of organic N combustion (Certini, 2005). Furthermore, apart from organic N mineralization, increases in inorganic N forms are usually found after prescribed burning due to the incorporation of ashes (Alcañiz et al., 2018 and references therein). Thus, the removal of ashes prior to sampling explains the neutral effects of prescribed burning on soil inorganic N forms that were observed in our study immediately after the fire. In B12, no changes were detected in ammonium or nitrate contents at the Buisán site. Nevertheless, at the Tella site, an increase in the ammonium content and a decrease in the nitrate content were detected at all studied soil depths in B12. This finding contrasts the inorganic N dynamics after fires that are commonly reported in the literature, in which an immediate pulse in ammonium content is followed by increases in nitrate content related to nitrification processes up to one year later (Gundale et al., 2005; Badía et al., 2014; San Emeterio et al., 2016). This could be a consequence of the reduction in soil biological activity after burning that is evidenced by a drastic reduction in microbial biomass (Armas-Herrera et al., 2016, 2018) and thus, nitrification rates because ammonium could be adsorbed in the soil and nitrates could be leached when they are not rapidly taken up by soil biota or plants (Mroz et al., 1980).

These N losses could have a negative impact on vegetation succession if there is no prompt plant regrowth (Knicker, 2007).

The available P contents at both the Buisán and Tella sites remained virtually unaffected by fire (**Figure 5**), which is in accordance with the results of previous studies conducted after prescribed and experimental burnings (Niemeyer et al., 2005; Marcos et al., 2009). Many studies have also indicated that available P increases after burning (Úbeda et al., 2005; Badía-Villas et al., 2014; Larroulet et al., 2016), and these increases are mainly related to the incorporation of ashes into the soil. In our case, ashes were removed prior to sampling, so this effect could not be detected in B0. On the other hand, the lack of changes in available P is unexpected given the temperatures that were reached in the topsoil, as organic P mineralization occurs at temperatures over ~200 °C (García-Oliva et al., 2018), which would have led to increases in available P (Fontúrbel et al., 2016). However, the absence of differences might also be related to the fact that P losses by volatilization do not occur until temperatures of ~775 °C are reached (Bodí et al., 2014). Santín et al. (2018) also observed that available P did not significantly change after a moderate/high-severity prescribed eucalypt forest burning, and this result was related to the oligotrophic characteristics of that forest system. One year after burning, the available P values at the Tella site were heterogeneous, and no significant differences were found when these values were compared to those of U and B0. Nevertheless, the available P significantly decreased at the Buisán site at all studied soil depths. The losses of available P after burning may be due to leaching (Pereira et al., 2012), and similar results were also observed by Alcañiz et al. (2016) one year after prescribed understory burning in a Mediterranean forest.

3.4. Fire effects on soil cation exchange complex

The cation exchange capacity (CEC) in the U samples of both study sites showed high values, that ranged from 31.7 to 41.6 $cmol_{(+)}\ kg^{-1}$. Burning had no significant effects on CEC, as seen in its B0 values (**Figure 6**), although a decreasing trend was detected at the Buisán site. Similar results were found by Larroulet et al. (2016) and Fonseca et al. (2017), who also detected no significant changes in CEC after prescribed shrub burning in semi-arid regions.

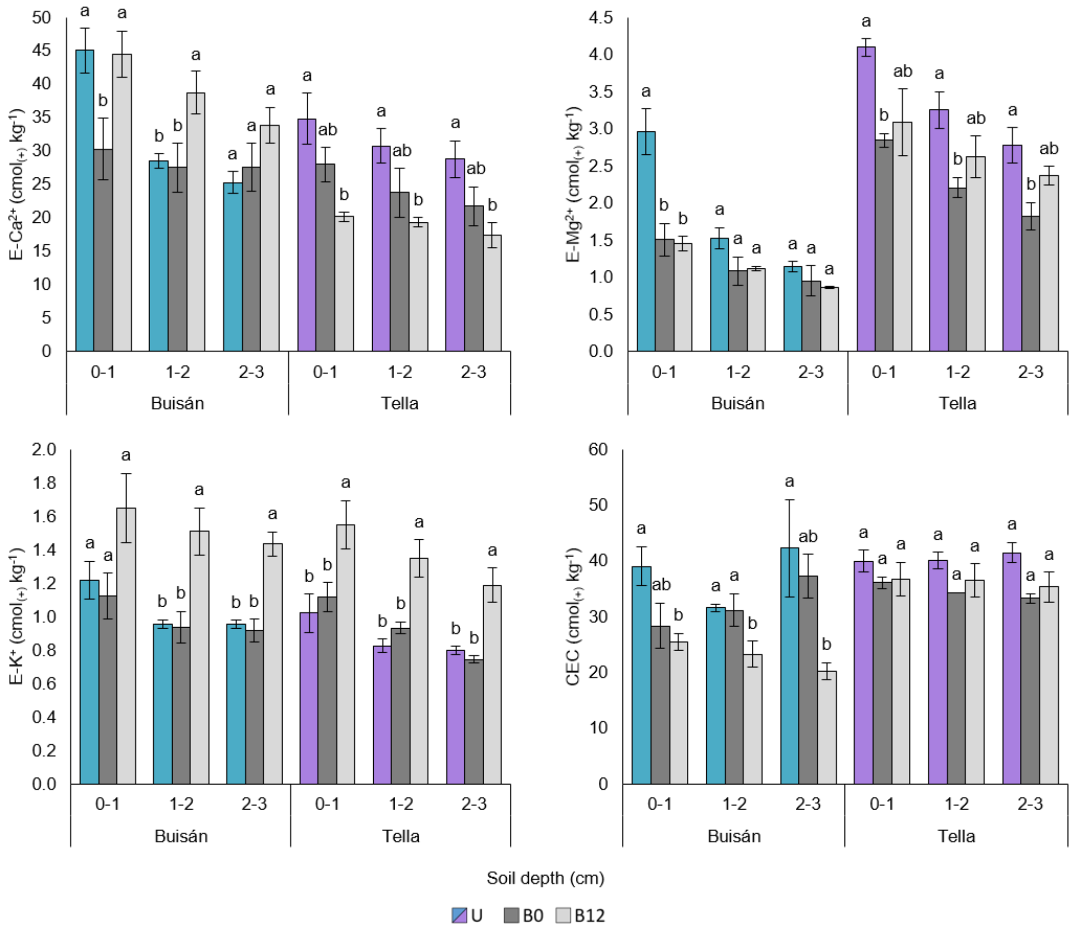

Figure 6. Exchangeable cations (E-Ca^{2+}, E-Mg^{2+} and E-K^{+}) and cation exchange capacity (CEC) in unburned (U), immediate post-fire samples (B0) and one year after burning samples (B12) for each soil depth and site (mean value ± SE of three field replicates). For same sampling depth, lowercase letters indicate significant differences among sampling times ($p < 0.05$)

The CEC in soils is tightly related to SOM, so the greater impacts on SOC and N that were observed at Buisán site could explain the decreasing trend exhibited by this property. This suggests that although SOM was reduced by burning, this reduction had not reached a threshold in which CEC was significantly affected because SOM content was still high after the fire. Additionally, experimental studies that addressed the effects of heat on CEC indicated that this property could be affected when temperatures exceed 250 °C (Badía & Martí, 2003), 300 °C (Inbar et al., 2014) or 350 °C (Thomaz, 2017) for a certain period of time. In the B12 samples, the CEC values at the Tella site showed no differences when compared to the U and B0 samples; nevertheless, in Buisán, the CEC values decreased significantly at depths of 0 to 3 cm. As SOM undergoes mineralization and/or stabilization processes, CEC increases concomitantly (Stevenson, 1982). Then, the detected decrease in Buisán in the B12 samples could be related to the incorporation of new SOM that is less transformed and therefore has lower CEC values.

The exchangeable cation contents (E-Ca^{2+}, E-Mg^{2+} and E-K^{+}) were similar in the U soils of both study sites, with Ca^{2+} being the predominant cation (**Figure 6**). At the Buisán site, burning decreased E-Ca^{2+} at 0 to 2 cm and Mg^{2+} at 0 to 1 cm, whereas E-K^{+} remained unchanged. On the other hand, at the Tella site, a significant reduction in E-Mg^{2+} and a decreasing trend in E-Ca^{2+} were observed at all studied soil depths in the B0 samples. In the same way as at the Buisán site, K^{+} showed no changes after burning in the Tella site. In this way, the results show the loss of divalent exchangeable cations after burning at both sites, which is probably a consequence of the destruction of organic functional groups (González-Pérez et al., 2004). Consequently, the exchange sites would have been occupied by K^{+}, therefore showing no differences in its content in the B0 samples.

Our findings contrast the results found in the literature that show that increases (Arocena & Opio, 2003; Lavoie et al., 2010) or neutral effects (Wang et al., 2013; Fontúrbel et al., 2016; Larroulet et al., 2016; Fonseca et al., 2017) on exchangeable cations occur after prescribed burning. Apart from the differences in burning intensity and vegetation type, the contrasting effects detected in our study compared to the literature could be related to the removal of ash prior to sampling and the detailed sampling scale since the studies mentioned above sampled greater soil thicknesses, which could dilute the effects of burning (Badía-Villas et al., 2014). One year after burning at the Buisán site, E-Ca^{2+} recovered to U values and E-K^{+} showed an increasing trend, although E-Mg^{2+} still showed values similar to B0 at the Buisán site. An opposite trend was detected at the Tella site, where E-Ca^{2+} significantly decreased, K^+ significantly increased, and E-Mg^{2+} showed a recovering trend in all the studied soil depths. The different evolutions of these properties observed at both sites could be attributed to surface processes and topographical characteristics. The Buisán site is characterized by low slopes, and no signs of erosion were observed during the study period. Furthermore, one year after burning, charred remains and ashes were still present in the plots. This could have been caused by the snowfall that followed the burning, which stabilized the ash and remaining litter, allowing a slower release of cations over time (Hamman et al., 2008). On the other hand, the burning at the Tella site was performed in April on a steep slope, and was followed by spring rains and summer drought, which could have resulted in leaching and erosion processes. In this way the probable short-term increase in cations after the fire was reversed by erosion and/or leaching, explaining the loss of exchangeable cations (Francos et al., 2016).

3.5. General discussion

SOM is of vital importance for nutrient cycling and cation exchange because nutrients can be volatilized or transformed into available forms via the combustion of SOM (Knoepp et al., 2005). Moreover, SOM, as well as the clay type and content determine the CEC (Ulery et al., 2017). Prescribed burnings are characterized by low intensities; therefore, the temperatures that are reached could be sufficient to produce the combustion of a part of the SOM but lower than the temperatures necessary to induce mineral alterations (Bodí et al., 2014). Despite the effects of fire on SOM, the CEC did not decrease accordingly, suggesting that the SOM content threshold that would have reduced the CEC had not been reached. However, the elimination of organic functional groups can lead to the loss of divalent cations, resulting in a decrease in the CEC immediately after burning. The displacement of exchangeable cations after burning could have led to an increase in water-extractable cations. However, this effect was not observed and could be related to a reduction in cation extractability in water that is probably related to cation precipitation (Badía & Martí, 2003). Divalent cations are released after combustion of organic materials as water-soluble oxides that can be rapidly transformed to less soluble carbonates and chlorides (Thiffault et al., 2008). For this reason, no changes were observed in EC at either the Tella or Buisán sites and only a minor increase in pH was detected at the Buisán site, although transient changes could have also been produced between the sampling times. In a similar way, burning had no effects on inorganic N forms and available P. The increases in pH, EC and nutrients usually reported in the literature after prescribed burning (Alcañiz et al., 2018) are related to the incorporation of ashes into the sampled soil, which we tried to avoid by all means. Although fire exerted few direct changes on the studied soil nutrients, some differences compared to the unburned soil could be observed one year after burning. Apart from the different seasons when each prescribed burning was performed, the slope also played an important role in the post-fire evolution. As explained in the previous section, the Buisán burning was conducted in a plain area and was followed by snowfall that allowed the ashes and partially charred litter layers to stabilize so leaching of soluble ions only occurred in the B12 samples. On the other hand, nutrient losses in the soil after the Tella burning could be explained by: 1) the soil losses as the prescribed fire was conducted in April on a south-facing steep slope, making it more prone to erosion, 2) leaching during the spring rainy season that is favored by the acidic soils. These effects are favored by the slow vegetation recovery at both study sites as reported in Armas-Herrera et al. (2018) and Girona-García et al. (2018a). At the Buisán site, one year after burning, vegetation represented only a small surface of the burned plots, which were mainly covered by partially charred litter and ashes. In the B12 samples from the Tella site, herbaceous plant coverage was of only 14 %, whereas bare soil represented 42 % of the ground surface.

These results were well summarized in the PCA analysis (**Figure 7**), in which samples were clearly separated by site and treatment.

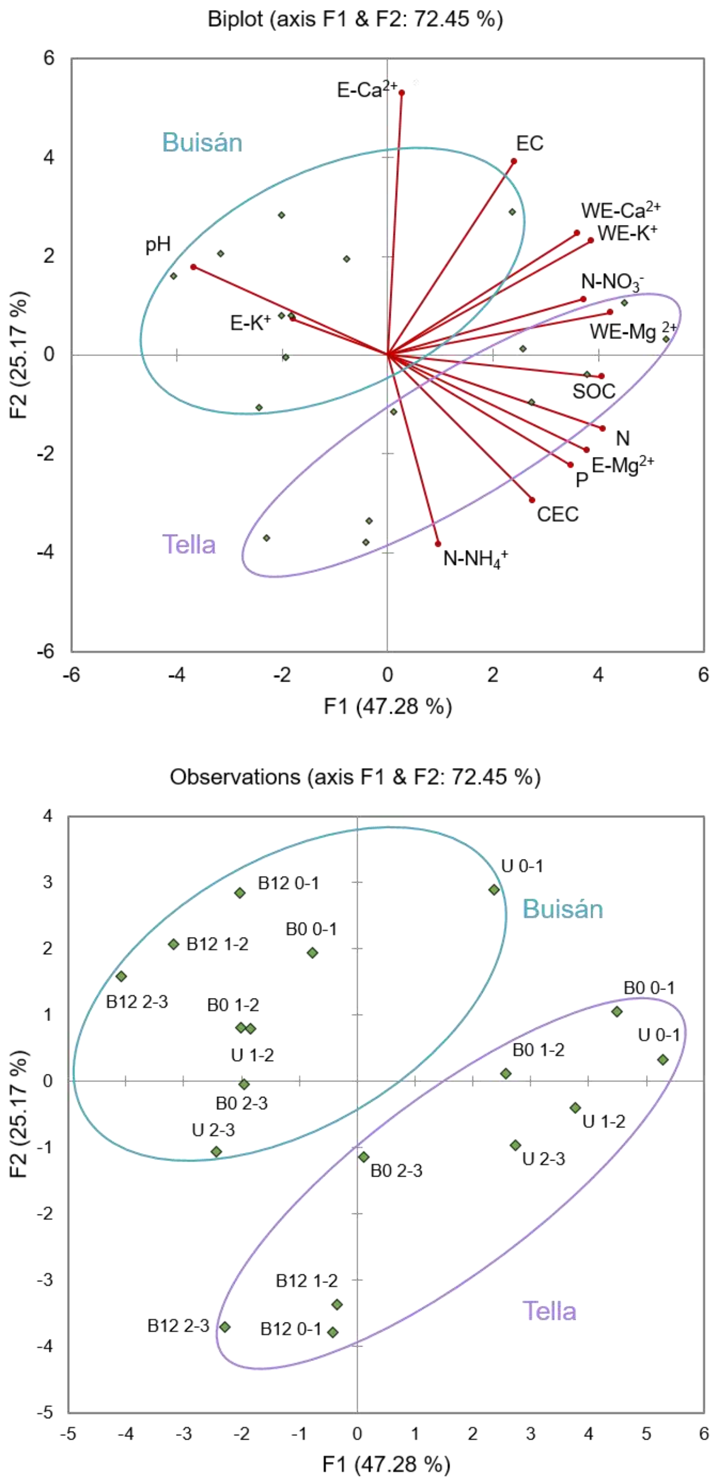

Figure 7. Results of the Principal Component Analysis (PCA). Variables: Soil organic C (SOC), total N (N), pH, electrical conductivity (EC), water-extractable cations (WE-Ca^{2+}, WE-Mg^{2+} and WE-K^{+}), inorganic N forms (N-NH_4^+ and N-NO_3^-), available P (P), exchangeable cations (E-Ca^{2+}, E-Mg^{2+} and E-K^{+}) and cation exchange capacity (CEC). Observations: unburned (U), immediate post-fire samples (B0) and one year after burning samples (B12) for each soil depth and site

Axis 2 (25.17 %) distributed samples by study site, showing that the Buisán site is characterized by higher pH values and therefore higher cation contents. On the other hand, the Tella site showed higher SOC, N, P and inorganic N contents. Axis 1 (47.28 %), however, separated the samples by treatment according to the previous discussion. The U samples at 0-1 cm from the Buisán site showed higher positive loadings compared to the equivalent B0 samples. The U and B0 samples from the deeper layers at the Buisán site formed a large cluster that indicated the limited depth in which burning exerted direct changes. Additionally, the B12 samples showed higher negative loads, which is in accordance with the decreases detected at this sampling time for the studied properties. At the Tella site, burning did not have the same effects on the studied properties as those at the Buisán site; therefore, U and B0 are not clearly separated by axis 1. However, in the same way as at the Buisán site, the B12 samples from the Tella site also showed higher negative loadings.

4. CONCLUSIONS

Despite the spatial and temporal variations expected from sampling such a thin topsoil layer (0-1, 1-2, and 2-3 cm depth), we showed the importance of how samplings are performed (i.e., sampled soil depth, time since burning and ash removal) to isolate the direct effects of fire on soils. Our results indicate that the SOM content was severely affected in the first centimeter of the topsoil, although it had few repercussions on soil nutrient content and availability. However, as a consequence of site characteristics (i.e., burning season, slope and precipitation), high nutrient losses were detected one year after burning that were probably related to leaching and/or erosion. Therefore, the long-term impact of prescribed fire on soils may differ depending on the burning season and topography, and these changes could negatively impact the recovery of vegetation over time. The results highlight the need to further monitor the evolution of the studied properties to assess the sustainability of this practice from the perspective of soil and plant recovery.

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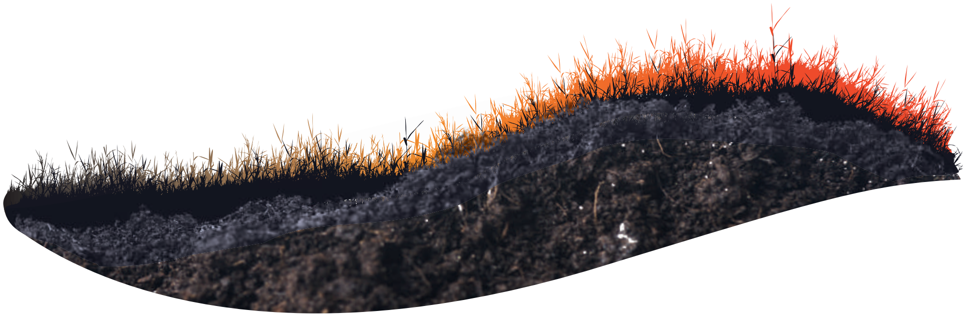

CHAPTER 6:

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

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ABSTRACT

Prescribed burning has been readopted in the last decade in the Central Pyrenees to stop the regression of subalpine grasslands in favor 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 analyze 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 analyzed the soil total organic C (SOC), total nitrogen (TN), microbial biomass C (MBC), 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 SOC (-52 %), TN (-44 %), MBC (-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 centimeters.

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 favor 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 favorable 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 characterizes 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 analyze 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 m.a.s.l. 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 sheep 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.

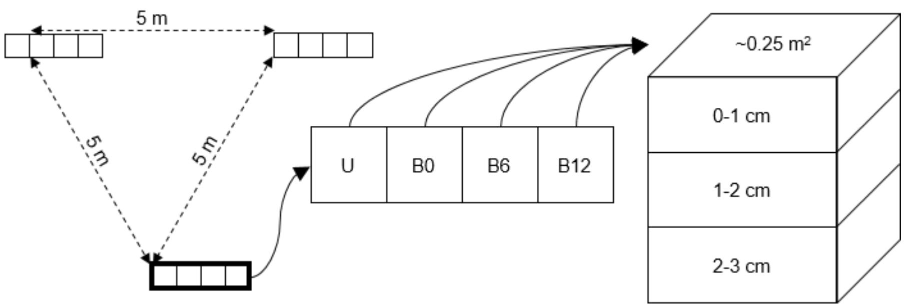


Figure 1. Design of the sampling plots. Unburned (U), immediately after (B0), 6 months (B6) and one year (B12) after burning sampling

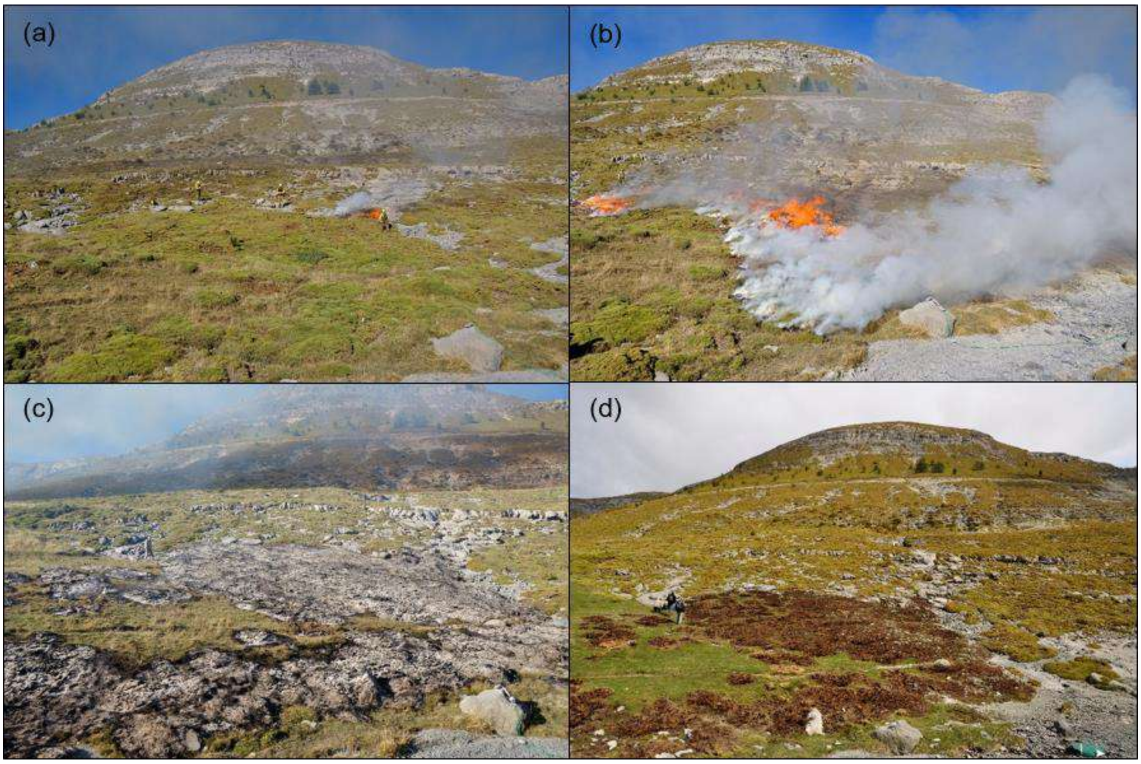


Figure 2. View of the study site before (a), during (b), immediately after (c) and one year after (d) prescribed burning

2.4. Sample preparation and laboratory methods

Samples were fresh sieved through a 2 mm mesh and kept in a refrigerator at 4 °C 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 Analyzer, 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 (SOC). Microbial biomass C (MBC) was determined following the chloroform fumigation-extraction method (Vance et al., 1987) using a calibration factor of K_c = 0.38. The MBC/SOC 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 mineralization 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 mineralization coefficient (CMC) as SR per oxidizable C unit and time; and the microbial metabolic quotient (qCO_2) as SR per MBC 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.

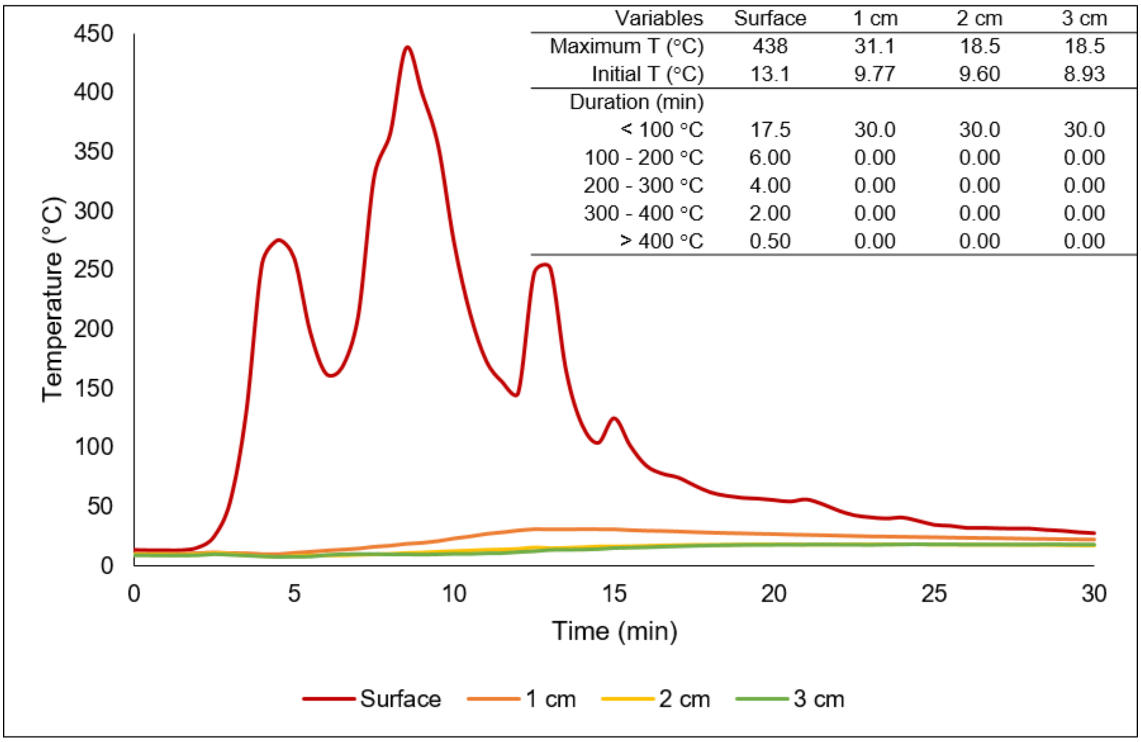
2.5. Statistical analysis

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 analyze 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 vaporization (Campbell et al. 1995; Badía et al. 2017). This affirmation is supported by the fact that water contained in the soil was not totally vaporized 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

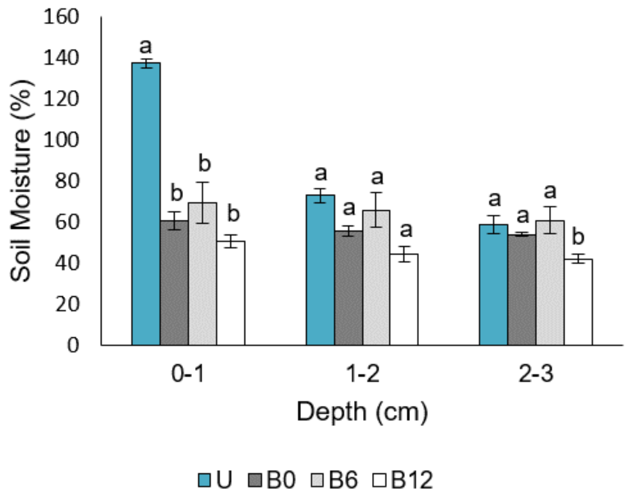


Figure 4. Soil water content in unburned (U), immediate post-fire samples (B0) and samples taken six months (B6) and one year (B12) after burning for each studied soil depth (mean value ± SE of three field replicates). For same sampling depth different letters indicate significant differences among sampling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

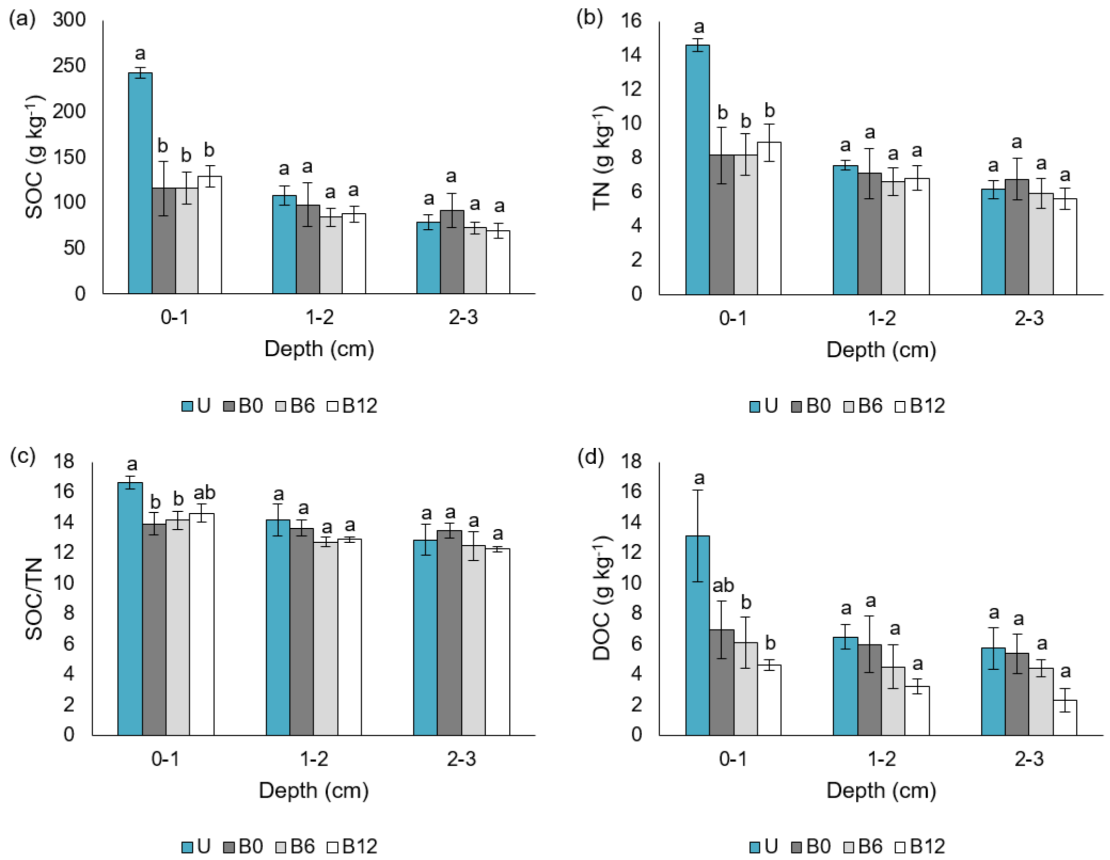


Figure 5. Fire effects on: a) soil total organic C (SOC); b) soil total N (TN); c) SOC/TN ratio and d) K_2SO_4-extractable C (DOC) in unburned (U), immediate post-fire samples (B0) and samples taken six months (B6) and one year (B12) after burning for each studied soil depth (mean value ± SE of three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

At B6, soil SOC 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 mineralization and soil biological activity in the topsoil (Yi et al., 2015). A slight, not statistically significant, increase in SOC 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).

Fire also affected organic matter turnover and quality by reducing soil SOC/TN 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 stabilization or higher C losses (Badía & Martí, 2003; González-Pérez et al., 2004). At B6, SOC/TN values presented no significant differences as compared to B0; nevertheless, B12 SOC/TN 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 (MBC) 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).

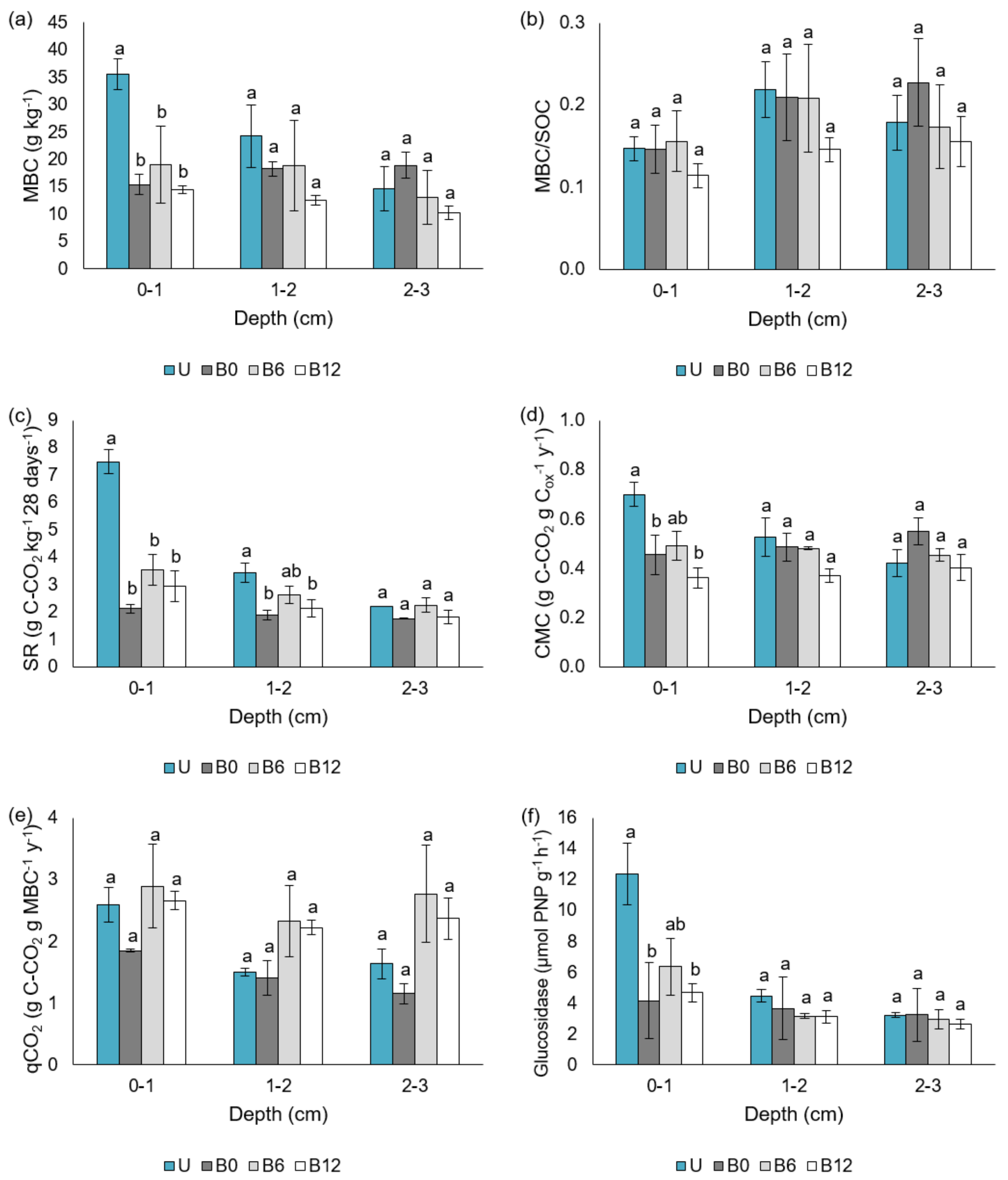


Figure 6. Fire effects on: a) microbial biomass C (MBC) b) MBC/SOC ratio; c) soil respiration (SR); d) C mineralization coefficient (CMC); e) microbial metabolic quotient (qCO_2) and f) β-D-glucosidase activity (Glucosidase) in unburned (U), immediate post-fire samples (B0) and samples taken six months (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 ($P < 0.05$)

Immediately after burning, MBC 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 MBC 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 MBC after burning although the effect was not as severe as in the present study. On the other hand, authors report an increase in MBC 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 MBC 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 MBC values recover (Bárcenas-Moreno et al., 2011).

No significant changes were observed in the MBC/SOC ratio with neither fire, time nor depth because both parameters evolved following the same trends (Figure 6b). Soil MBC/SOC ratio can be used as a soil quality indicator since in soils under degradative processes, such as fires, MBC 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 MBC/SOC increases 1-3 months after fire due to higher nutrient availability (Fontúrbel et al., 2016). g^{-1} h^{-1}

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.

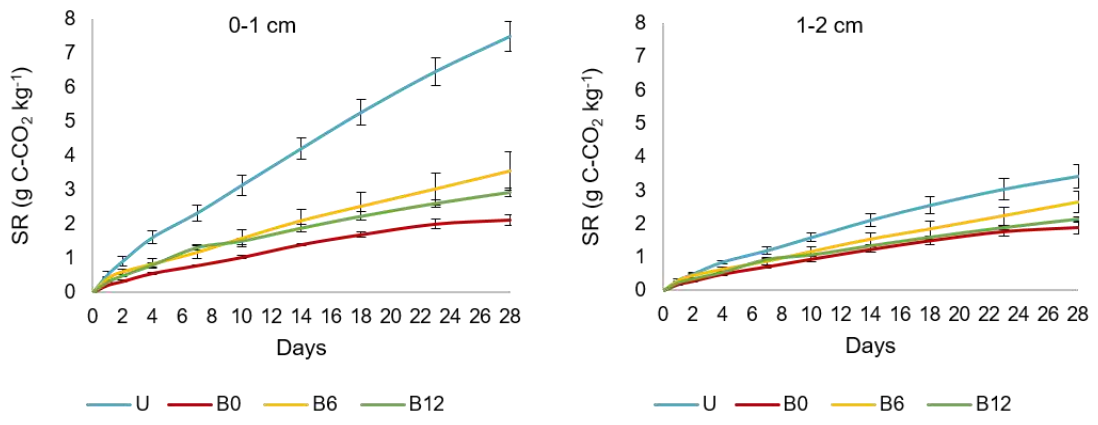


Figure 7. Soil respiration (SR) expressed as cumulative C-CO_2 emitted during the incubation assays (28 days) in unburned (U), immediate post-fire samples (B0) and samples taken six months (B6) and one year (B12) after burning for the fire-affected soil depths (0-1 and 1-2 cm)

A statistically significant reduction in the C mineralization 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 behavior 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 mineralization 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 behavior 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 colonized 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 colonization 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 mineralization 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 mineralization 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-dependent.

4. CONCLUSIONS

All the studied soil properties (SOC, TN, SOC/TN, MBC, 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.

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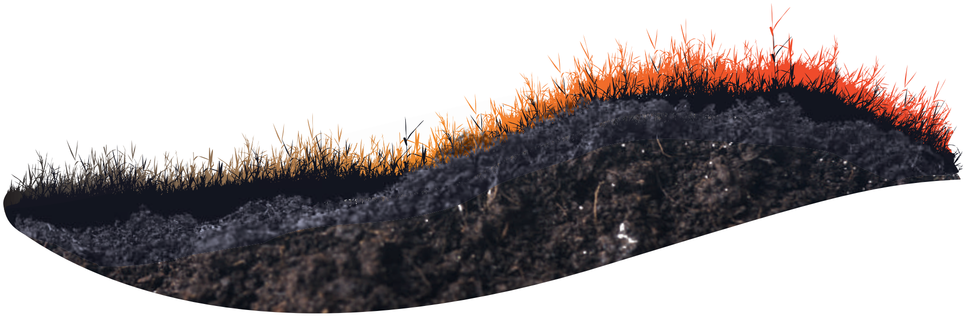

CHAPTER 7:

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

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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 °C (Buisán), 768 °C (Asín) and 595 °C (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.

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; Badía et al., 2017a; 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, **Figure 1**); these sites are referred to as Buisán, Asín and Yebra in the remainder of the text.

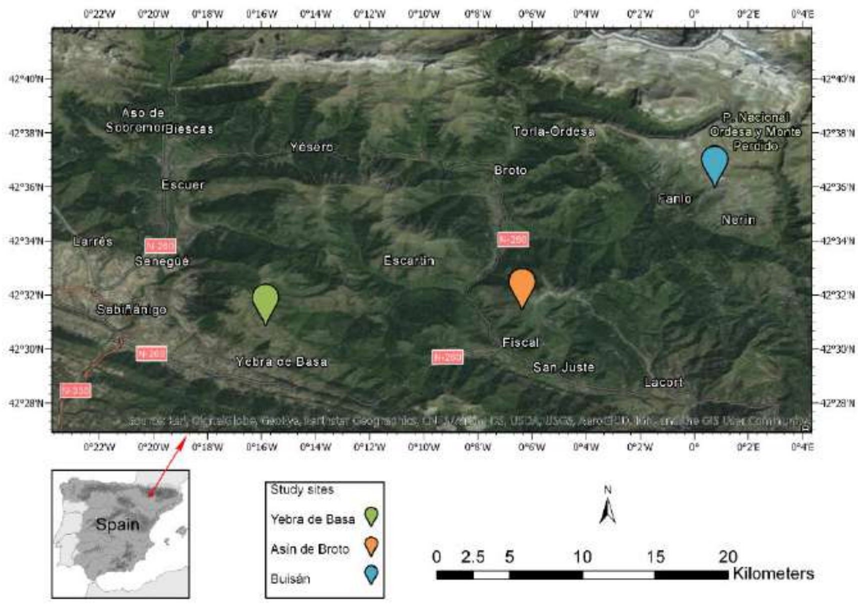

Figure 1. Locations of the study sites

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 °C for Yebra; 1650 m a.s.l., 1120 mm and 8.8 °C for Asín; and 1760 m a.s.l., 1500 mm, 6 °C for Buisán.

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 (°C)	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 (°C)	438	768	595
Initial temperature (°C)	13.1	7.5	4.9
Final temperature (°C)	27.5	24.3	10.2
Temperature residence time (min):			
< 60 °C	2.50	15.0	24.9
60-100 °C	15.0	5.75	1.33
100 - 200 °C	6.00	2.50	1.50
200 - 300 °C	4.00	1.42	0.83
300 - 400 °C	2.00	0.58	1.25
> 400 °C	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.

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.

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 °C, 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**.

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) (**Figure 2**).

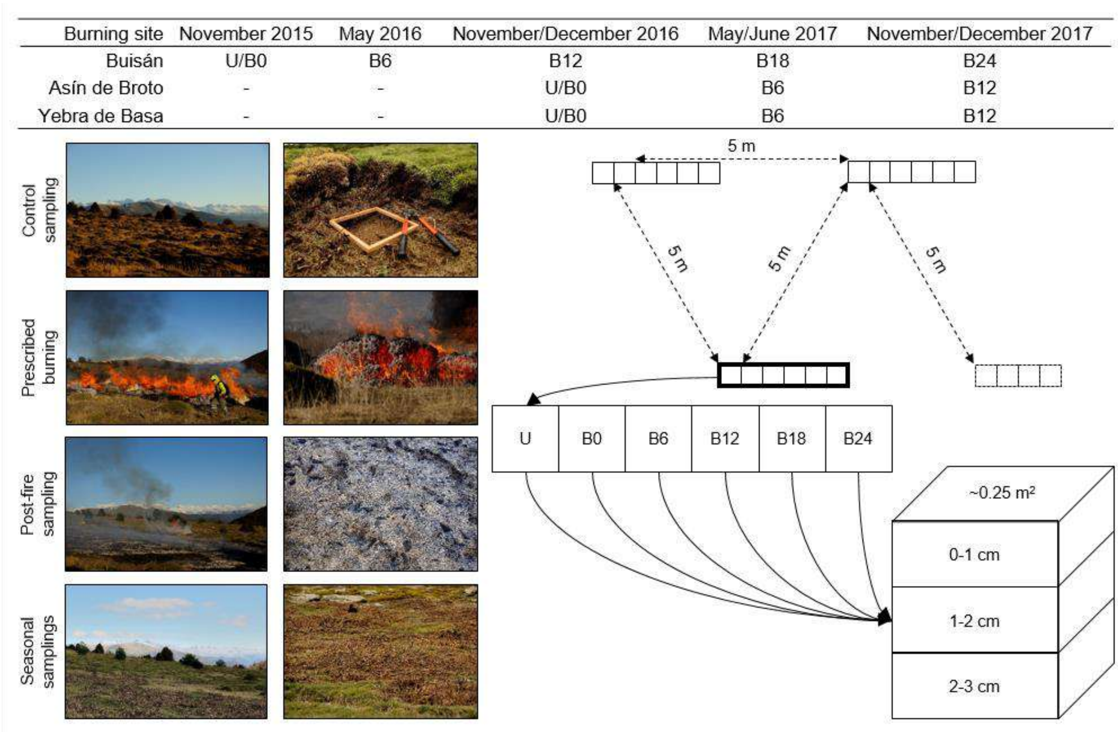

Figure 2. Sampling design followed throughout the study period for each study site. U: unburned samples; B0: immediate postfire samples; B6, B12, B18, B24: seasonal samplings every 6 months after burning

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., 2017b). All samples were collected in plastic bags to avoid desiccation and were rapidly transported and stored at 4 °C 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.

2.4 Sample preparation and analysis

The fresh (not-dry) samples were sieved to fine earth (2 mm) and kept at 4 °C 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 °C dried soil. Each of the first 3 centimeters of the undisturbed soil samples obtained with the steel cylinders was carefully separated, dried at 105 °C 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 in 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:

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

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 °C 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 °C (Buisán), 768 °C (Asín) and 595 °C (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°C 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 (**Figure 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}.

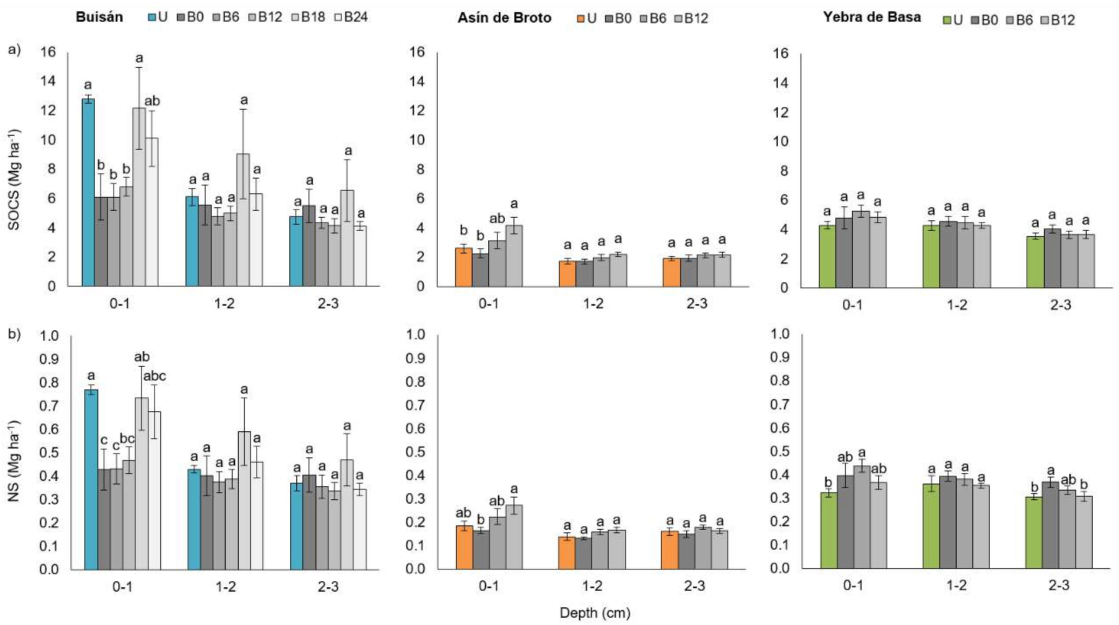

Figure 3. Soil total organic carbon stocks (SOCS) and total nitrogen stocks (NS) for each study site, sampling time and soil depth (mean ± standard error). U: unburned samples; B0: immediate post fire samples; B6, B12, B18, B24: seasonal samplings every 6 months after burning. Lowercase letters indicate significant differences at p<0.05 among sampling times for each studied soil depth and site

The soil N stocks (NS) showed the same trends observed in the SOCS for U soils (**Figure 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.

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.

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

		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

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 (**Figure 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.

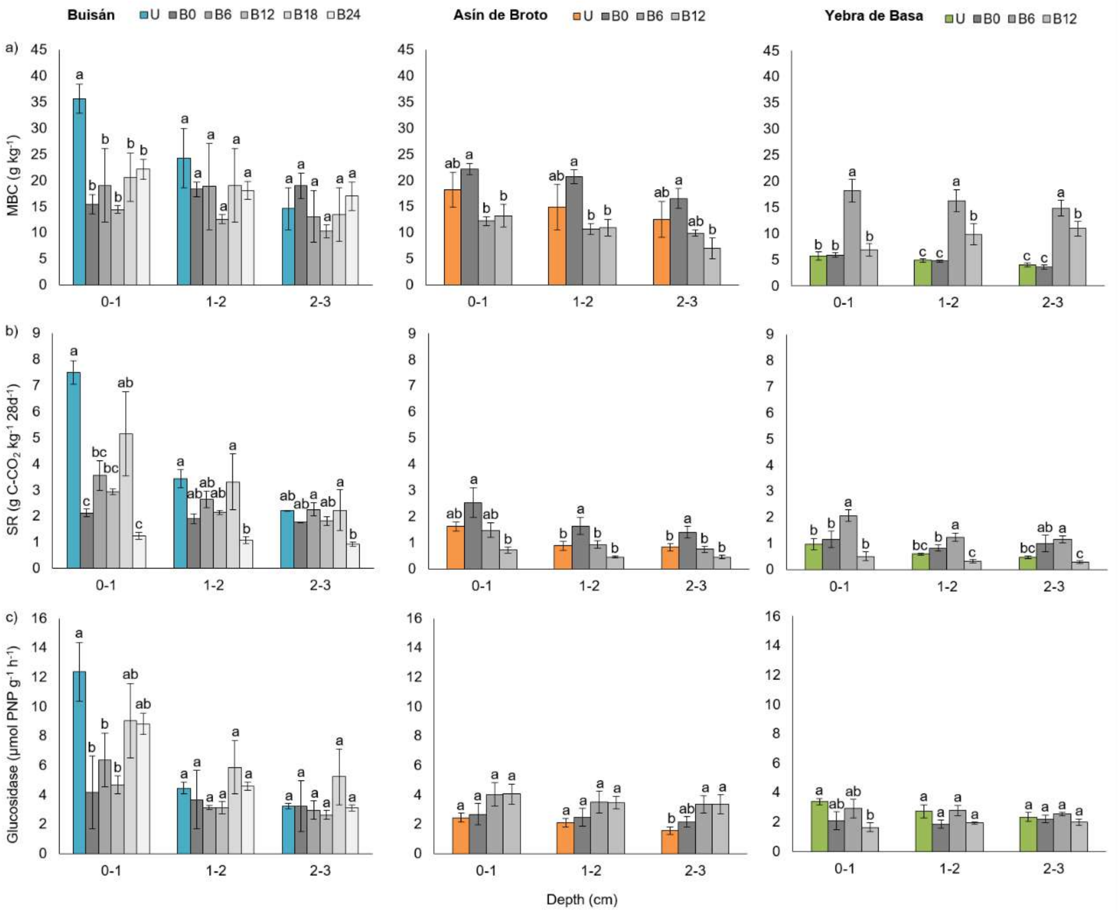

Figure 4. Microbial biomass carbon (MBC), soil basal respiration (SR) and β-D-glucosidase activity (Glucosidase) for each study site, sampling time and soil depth (mean ± standard error). U: unburned samples; B0: immediate post fire samples; B6, B12, B18, B24: seasonal samplings every 6 months after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

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.

The soil basal respiration (SR) in the U samples from the Buisán site was significantly higher than those from the Asín and Yebra sites (**Figure 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.

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

		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

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 (**Figure 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 (**Figure 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.

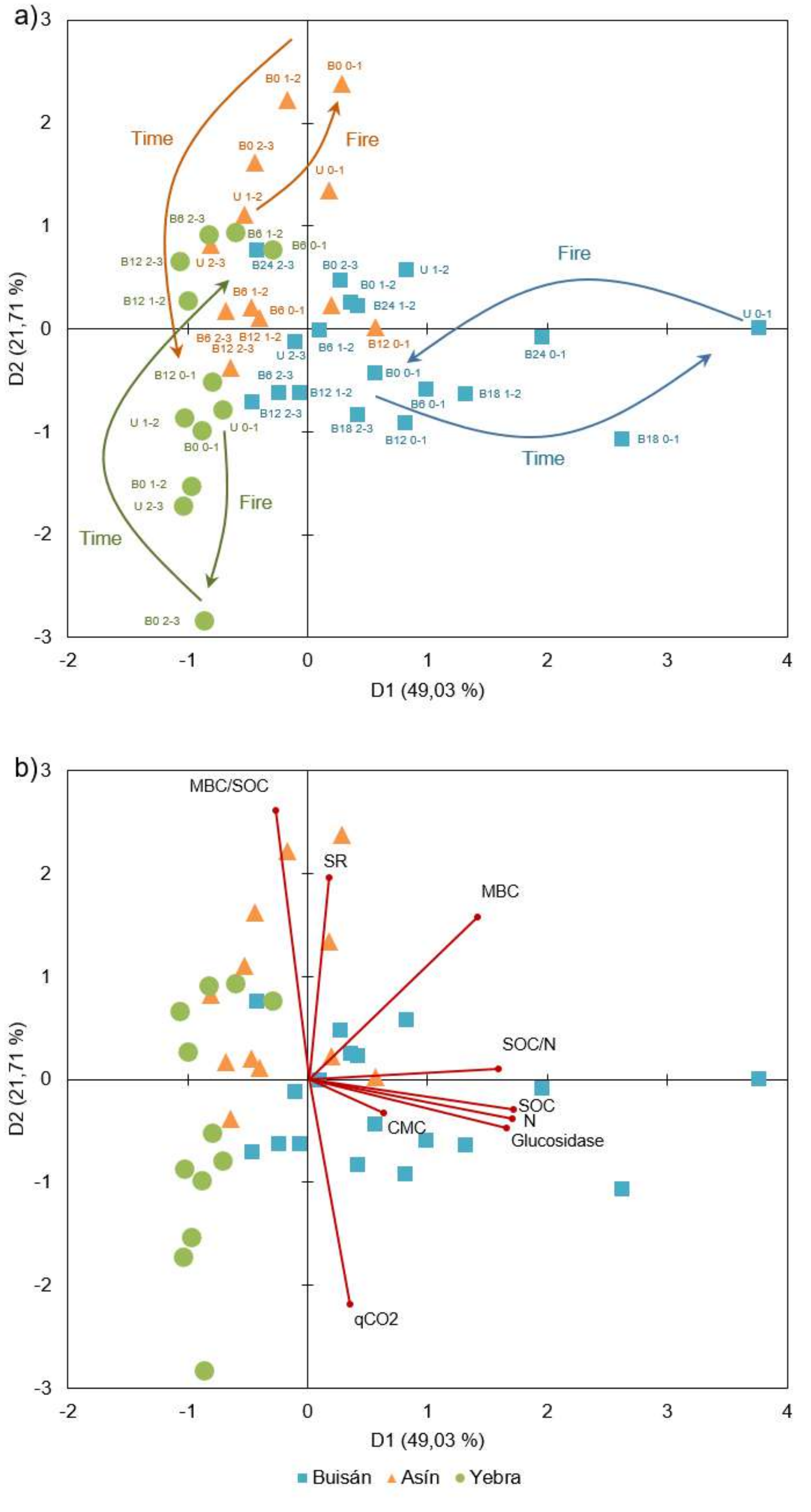

Figure 5. Principal component analysis (PCA) after varimax rotation for the observations (a) and the variables (b). In figure a) abbreviations refer to unburned samples (U), immediate post fire samples (B0) and seasonal samplings every 6 months after burning (B6, B12, B18, B24) for each soil depth. Abbreviations 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 °C 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 °C 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 °C (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 °C (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 post fire 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 C sequestration and pasture development could balance the losses produced by the burning of biomass over the long term.

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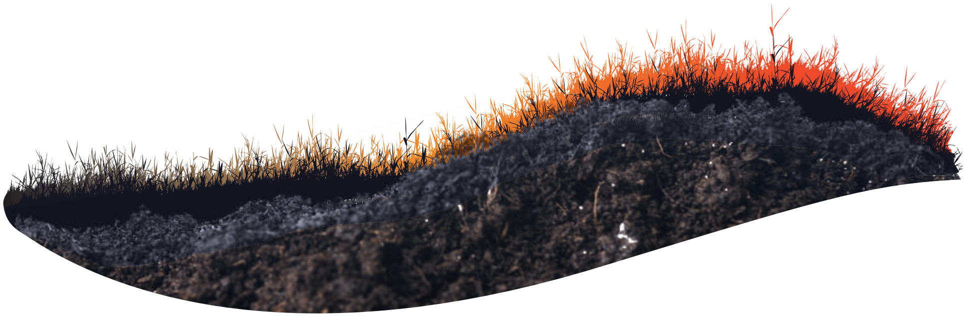


CHAPTER 8:

Conclusions and future research directions.

CONCLUSIONS

Effects of prescribed burning on soil organic carbon, aggregate stability and water repellency

1. Fire severely decreased the SOC content in the <0.25 mm fraction at 0-1 cm depth and in the 0.25-0.5 mm at 0-2 cm depth. This translated to a 45 % SOC reduction at 0-1 cm depth in the whole soil that did not recover during the studied period.

2. The SWR was significantly reduced by burning mainly at 0-1 cm depth for the <0.25 to 2 mm sieve fractions and the whole soil. One year after the prescribed burning, SWR recovered in these fractions, reaching even higher values than those of the unburned soil.

3. The AS of the 0.25-0.5 mm aggregates increased after fire, but one year later, it suffered a striking decrease in all aggregate sizes and depths, probably related to cattle trampling.

4. The fine aggregate fractions (<2 mm) are more prone to be affected by fire, and they determine the behavior of the whole soil to a greater extent than coarser fractions (2-4 and 4-10 mm).

Effects of prescribed burning on soil nutrient content

5. Despite the severe affection of fire on SOM at 0-1 cm depth, it had few direct repercussions on soil nutrient content and availability.

6. As a consequence of site characteristics (i.e., burning season, slope and precipitation), high nutrient losses were detected one year after burning that were probably related to leaching and/or erosion.

Effects of prescribed burning on soil organic carbon stocks and dynamics

7. The prescribed burning of shrubs had contrasting direct effects on SOCS and related biological properties among sites.

7.1. At the Buisán site, all the studied soil properties (SOCS, NS, MBC, SR and 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.

7.2. At the Asín and Yebra sites, fire had no direct effects on SOCS, NS, SOC/TN, MBC, SR and β-D-glucosidase.

8. At the Buisán site, the SOCS recovered to values similar to those of the unburned soils 2 years after burning. One year after burning, the SOCS at Asín were 60 % higher than those of the unburned soils at 0-1 cm depth.

9. The results obtained at the three sites for the short- and mid-term showed a decrease in carbon mineralization, which was probably due to the changes in the quality of new SOM inputs and the variations in cycling rates.

10. Burning may allow for higher C sequestration in the soil in the long term, which is related to the reduced biological activity and the incorporation of partially charred vegetal remains

General conclusions

11. It is important how samplings are conducted (i.e., sampled soil depth, time since burning and ash removal) to isolate the direct effects of fire on soils.

12. The direct effects of burning on soil are highly dependent on the environmental conditions and how fire is applied.

12.1. When the rapid spread of fire was favored, the soil properties remained virtually unchanged.

12.2. Burning in autumn may be advisable against burning in spring. The snowfall that followed the burnings may have stabilized the ashes and remaining litter, favoring its incorporation into the soil and preventing erosion.

FUTURE RESEARCH DIRECTIONS

The present dissertation, because it consists on a general characterization of the effects of shrubs prescribed burnings on soil properties in humid mountain environments, raises numerous unanswered questions and thus, opportunities and directions for future research. Based on the findings stated above and the issues that arose during the development of the project, there are a number of gaps in knowledge that would benefit from further research, as:

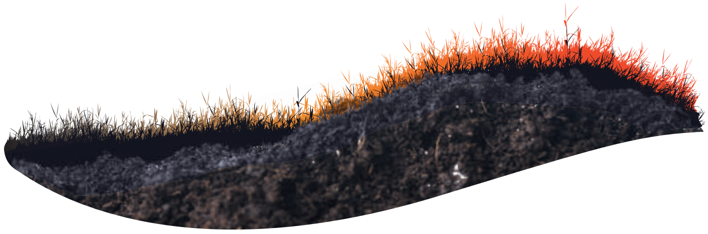
Further work is needed regarding how the different environmental conditions as well as the ignition techniques applied influence the effect of fire on soils in order to determine the optimal conditions for burning.

The *Echinospartum horridum* prescribed burnings are conducted to recover pasturelands. For this reason, it is of interest the study of the plant-soil relationship to detect whether the changes in soil properties after burning affect the recovery of the herbaceous vegetation.

It is essential to study whether the apparent improved carbon sequestration in the soil and the pasture development could balance the losses produced by the burning of biomass over the long term.

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. Because either clearing and prescribed burning have not always had satisfactory outcomes, further research based on a comparative approach is needed to assess the suitability and sustainability of both practices.

It would also be of interest to study the soil loss by erosion after prescribed burning, especially in areas such as the Pyrenees, where the rainfall is high and the slopes are usually steep.



TESIS DOCTORAL

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