



## Long-term evolution of shrub prescribed burning effects on topsoil organic matter and biological activity in the Central Pyrenees (NE-Spain)



Andoni Alfaro-Leranz <sup>a,\*</sup>, David Badia-Villas <sup>a</sup>, Clara Marti-Dalmau <sup>a</sup>, Mohamed Emran <sup>b</sup>, Ana Paula Conte-Dominguez <sup>c</sup>, Oriol Ortiz-Perpiña <sup>a</sup>

<sup>a</sup> GEOFOREST, Departamento de Ciencias Agrarias y del Medio Natural, Escuela Politécnica Superior de Huesca, Instituto de Investigación en Ciencias Ambientales (IUCA), Universidad de Zaragoza, Ctra. Cuarte s/n, 22071 Huesca, Spain

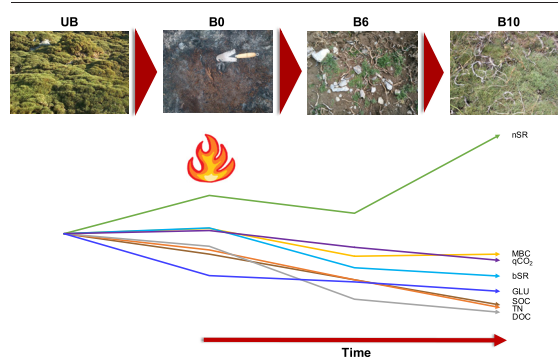
<sup>b</sup> Land and Water Technologies Department, Arid Lands Cultivation Research Institute (ALCRI), City of Scientific Research and Technological Applications (SRTA-City), 21934 New Borg El-Arab City, Alexandria, Egypt

<sup>c</sup> Escuela Politécnica Superior de Huesca, Universidad de Zaragoza, Ctra. Cuarte s/n, 22071 Huesca, Spain

### HIGHLIGHTS

- Prescribed burning is used as a tool to reduce shrub biomass and its continuity.
- The effects of prescribed burnings on topsoil were analyzed up to 10 years.
- The  $\beta$ -D-glucosidase activity was directly affected by the fire.
- Several mid- and long-term effects were observed.
- The reduction in microbial activity was related to the loss of organic matter.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Since the last half of the 20th Century, scrubs have been invading subclimatic grasslands in the montane and subalpine stages of Spain due to the decrease of the grazing activity. This shrub encroachment reduces biodiversity and the ecopastoral value of the region and leads to the accumulation of woody fuel, which represents a high fire risk. To control the encroachment, prescribed burnings are performed but their effects on soils over the years are still undetermined. This study aims to research about the long-term effects of a prescribed burn of *Echinopartum horridum* (Vahl) Roth. on topsoil organic matter and biological activity. Soil sampling was carried out in Tella-Sin (Central Pyrenees, Aragón, Spain) and four treatments were selected: unburned (UB), immediately burned (B0), burned 6 years before (B6, mid-term) and burned 10 years before (B10, long-term). Among the obtained results, an immediately after burning decrease on  $\beta$ -D-glucosidase activity (GLU) was found, which did not recover over time. Other properties did not have an immediate reduction but did so over time: total soil organic carbon (SOC), labile carbon (DOC), total nitrogen (TN), basal soil respiration (bSR). And others were not affected at all: microbial biomass carbon (MBC), and the microbial metabolic quotient (qCO<sub>2</sub>). Moreover, the normalized soil respiration (nSR) increased with the time, which implies an acceleration of the potential mineralization of soil organic carbon. In short, although the elimination of the dense shrubs by fire has not entailed major immediate soil modifications, which would be typical of a low severity prescribed burn, several mid- and long-term effects in the C cycle have been observed. Future studies will have to discern what is the main cause of these modifications (soil microbial composition, edaphoclimatic changes, lack of soil cover and soil loss, soil fertility, etc.).

\* Corresponding author.

E-mail address: [a.alfaroler@unizar.es](mailto:a.alfaroler@unizar.es) (A. Alfaro-Leranz).

## 1. Introduction

Mountain landscapes and their plant cover have been changing throughout history, driven by both abiotic and biotic factors (Gómez, 2008). There are mainly two types of grasslands in the Central Southern Pyrenees: the permanent ones (known as alpine grasslands), which are above the tree line and mainly maintained by abiotic factors, and the secondary communities (subalpine and montane grasslands), which were created and maintained by the destruction or modification of forests, mostly by human action. From the late Middle Ages to the beginning of the Contemporary Age, forests were cut or burned to supply villages and to expand summer grasslands for livestock (García-Ruiz et al., 2020; Gómez, 2008). These secondary grasslands below the alpine stage are protected by Natura Net 2000 for their natural and cultural value, have a high species diversity and their low fuel load makes them represent a lower fire risk as opposed to a dense thicket (Vélez, 2012). After the beginning of the Contemporary Age, and particularly throughout the second half of the 20th Century, the reduction of livestock and the abandonment of fire as a tool have led to a progressive recuperation of forests and to the shrub re-colonization, giving greater homogeneity to the landscape (Castellnou et al., 2010; Gómez, 2008). Gartzia et al. (2014) reported that 21 % of croplands and 20 % of pasture lands were colonized by different shrub species during the last quarter of the past century in the Central Southern Pyrenees. One of the most invasive species is *Echinopartum horridum* (Vahl) Roth., Family *Fabaceae*, a native species from the French Massif Central and the Central Pyrenees. It has a cushion shape, with densely thorny branches, armed with rigid spines, and may reach 50 cm in height and 1 m in diameter. Its natural habitat comprehends ridges and eroded hillslopes, over thin and stony soils over calcareous bedrocks, but, due to secondary succession, it can also be found on old pasture lands and croplands, and under forests borders (Instituto Pirenaico de Ecología and Gobierno de Aragón, 2005). This shrub represents a high fire risk. It forms barely monospecific communities in dense patches with a high dry and senescent biomass content (Komac et al., 2013; Palacio et al., 2006). In turn, the germination of *E. horridum* is stimulated by fire, making it capable of quickly regenerating after fires (Alados et al., 2019). The accumulation and continuity of woody fuel in the Southern Pyrenees, together with the current climate change scenario, could lead to the occurrence of large wildfires (Resco de Dios et al., 2021).

To avoid this, the reintroduction of the use of the fire as a fuel management tool is proposed, but instead of being carried out by shepherds, it is carried out by professional technicians. These technical fires, called “prescribed burnings” (PBs), are performed under certain atmospheric and edaphic conditions (window of prescription) to control the fire (low-intensity fires) and minimize its damage to the soil properties. PBs are considered a good management tool to reduce the fuel load and the incidence of wildfires, with a good cost/effectiveness ratio (Valkó and Deák, 2021). PBs are not only useful for a temporary reduction of shrub biomass, but also to train the wildfire extinction crews before the fire season, as they are performed between late autumn and early spring.

The fire effects on soil properties are diverse and depend on both the temperatures reached and their residence time, the fire intensity and the fire regime (Alcañiz et al., 2018; Lombao et al., 2020). Furthermore, these effects vary according to other factors, such as soil sampling conditions (time after the fire, soil sampled thickness), soil type and soil moisture content (Badía et al., 2017b), and also the measured attributes, depending on their sensitivity to heat (Pereira et al., 2023). In the case of PBs effects on soil properties are also diverse, and can either be negative, positive or neutral, in most cases not as severe as wildfires (Alcañiz et al., 2018). But, even so, the most sensitive properties, like the microorganisms or the organic matter (Santín and Doerr, 2016), could be severely affected especially under dense shrubs. In fact, some studies performed in controlled burned areas with *E. horridum* show immediate and short- to mid-term effects on topsoil organic matter and microbial activity (Armas-Herrera et al., 2018; Girona-García et al., 2019, 2018a). In this sense, in the controlled burning of a calcareous forest topsoil, the activity of soil enzymes responsible for participating in the C-cycle ( $\beta$ -D-glucosidase) and certain fractions of soil

organic matter have been shown to be particularly sensitive to heat (Pereira et al., 2023). Concretely,  $\beta$ -D-glucosidase (GLU) is considered a soil quality indicator and is directly related to the quantity and quality of soil organic matter (Turner et al., 2002).

The PBs effects on immediate and short-term soil properties are partially studied, but very few studies investigate about the long-term effects on soil microbial community and numerous studies are not long enough to observe the microbial response to fire during the whole ecosystem recovery process (Dove et al., 2022). Therefore, the objective of this study was to evaluate the immediate, mid- and long-term effects of a prescribed burn, of a thicket of *E. horridum* that invaded subalpine pastures, on various soil microbial activity-related properties. In particular, the changes at the centimetric scale of the topsoil (0–3 cm) were evaluated in soil biochemical properties, as basal soil respiration (BSR), microbial biomass carbon (MBC),  $\beta$ -D-glucosidase activity (GLU), total soil organic carbon (SOC), total nitrogen (TN) and labile organic carbon (DOC), as well as other related soil properties, comparing effects right after the fire event, 6 years after and 10 years after the event, with the non-burned shrubland areas. Previous studies performed in the Central Pyrenees show the immediate to mid-term effects of different prescribed fires on topsoil properties, like in Armas-Herrera et al. (2018), where the effects were studied until 5 years after the PB. This study aims to provide further information about the long-term (10 years) response of the soil microbial community to prescribed burnings in the subalpine stage, giving an idea of the ecosystem resilience to fire-induced perturbations and how soil organic matter (quantity and quality) and soil biological activity are related.

## 2. Material and methods

### 2.1. Study area

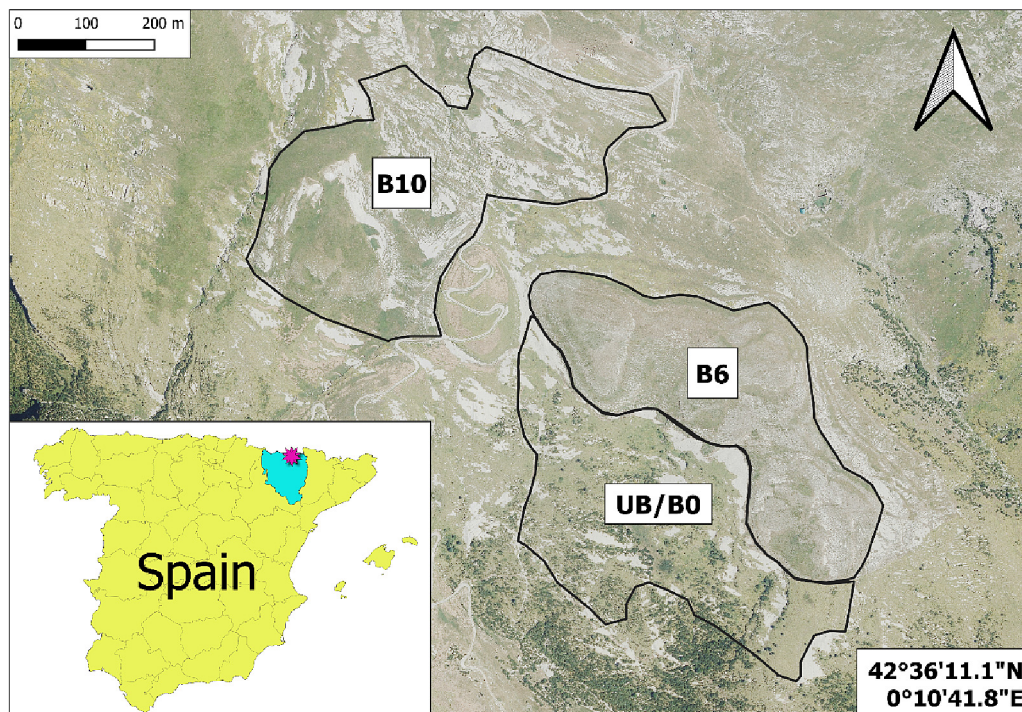
The study area is located in Tella-Sin municipality, Central Southern Pyrenees, Huesca (Aragón), NE Spain (Fig. 1).

Limestone outcrops alternate with areas of sufficient soil thickness to support high quality pastures, rich in species ( $n = 25.2 \pm 4.1$ ) and in biodiversity (Shannon index of  $3.88 \pm 0.87$ ), dominated by *Festuca nigrescens* Lam. accompanied (among many other species) by *Nardus stricta* L. or *Bromus erectus* Huds. Many pasture areas in this location have been invaded by a dense canopy of *E. horridum*, a plant community with very few species ( $n = 6 \pm 3$ ) and, therefore, very low diversity (Shannon index of  $0.86 \pm 0.47$ ), low pastoral value, and occasionally by *Buxus sempervirens* L. (Armas-Herrera et al., 2016; Mora et al., 2022). Soils are shallow (<50 cm soil depth) and are characterized by high organic matter content, high aggregate stability and slightly acid reaction; the texture class is silty clay loam in the whole profile. Based on its properties, the soil is classified as Hypereutric Epileptic Cambisol (Loamic, Humic) (IUSS Working Group WRB, 2014). The area is located around 1800 m above the sea level, the mean annual precipitation is around 1700 mm and mean annual temperature is around 5 °C (Armas-Herrera et al., 2016). Currently, this area is widely grazed from early June till late September, mainly by sheep and goats. However, the actual amount of livestock is not enough to stop by itself the re-encroachment after the fire (Mora et al., 2022).

### 2.2. Fire characteristics

Prescribed burns were performed 0, 6 and 10 years before the sampling time (Table 1) by firefighters of the EPRIF (Wildfire Prevention Team) of Aragón and the 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 *E. 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<sup>-1</sup> (Girona-García et al., 2019).

Temperature measurements were taken in a single spot in March 2021 using type-K thermocouples at the soil surface, 1 and 2 cm-deep ( $n = 3$ ). A wide thermal range could be found, from the 812 °C at the soil surface to



**Fig. 1.** Location of the study area (Tella-Sin) in the Central Pyrenees (NE-Spain) and experimental plots: Unburned (UB), burned and sampled on the day (B0), sampled at 6 (B6) and 10 (B10) years after burning. In each plot, 4 soil replicates (n) for 3 soil depths (0–1, 1–2 and 2–3 cm) have been sampled.

ambient temperature below 2 cm (Table 2). Even though, the litter layer was not completely consumed by the fire.

### 2.3. Sampling

The sampling was executed in March 2021. Four representative sampling points ( $0.5 \times 0.5$  m area per point) were chosen within each control and burned plots (UB, B0, B6, B10) to assess the immediate, mid-term, and long-term fire effects, respectively, on soil properties. Each of the four points were randomly selected with a five-meter separation from each other. Samples from B0 treatment were taken beside the UB ones, a few minutes after the PB was performed, following a similar design to Girona-García et al. (2019).

Prior to soil sampling, aerial vegetation, ashes and litter were carefully removed from the mineral soil surface within each sampling point to facilitate the work. Aerial vegetation samples were kept for shrub biomass analysis, and litter and ash were discarded. Soil was sampled at three depths (0–1, 1–2 and 2–3 cm) in each sampling. Previous studies show that there are no changes in soil properties at greater depths in prescribed fires performed at similar locations (Girona et al., 2019). Subsamples for biological determinations were separated and stored at 4 °C, and the rest of the soil was air-dried and sieved at 2 mm. Plant roots and other plant residues were hand-removed from soil samples.

**Table 1**

Dates of prescribed burning and shrub dry biomass of each plot at the sampling time.

Code	Prescribed burning time	Date	Shrub dry biomass (t/ha)
UB	Control (unburned)	–	$57.7 \pm 11.4$
B0	Immediate (0 years)	March 2021	$<1^a$
B6	Mid-term (6 years)	April 2015	$7.7 \pm 1.6$
B10	Long-term (10 years)	February 2011	$14.3 \pm 3.6$

<sup>a</sup> Charred residues.

### 2.4. Laboratory analysis

The moisture content of the soil samples was determined gravimetrically after oven drying at 105 °C until a constant weight was reached, and all results were calculated on a 105 °C dry soil basis. The dry shrub biomass of each sampling point was calculated gravimetrically by oven drying the vegetation samples at 60 °C until a constant weight was reached and expressed as tones of dry fuel per hectare for each sampling plot.

The total soil organic carbon (SOC) and nitrogen (TN) concentrations were determined using a CN Vario Max elemental analyzer (Elementar, Hanau, Germany). The labile carbon (DOC) was calculated as the  $K_2SO_4$  extractable carbon and then measured by chromic oxidation (Vance et al., 1987).

**Table 2**

Characteristics of 2021 prescribed burning: soil temperature (single point) and moisture in the soil samples before the fire (UB) and after the fire (B0).

Maximum soil temperature	(°C)
At surface	812
At 1 cm	39
At 2 cm	21
Temperature residence time at surface	(minutes)
<60 °C	123.53
60–100 °C	6.15
100–200 °C	6.32
200–300 °C	2.02
300–400 °C	1.53
>400 °C	5.97
Soil moisture	(%)
Before the fire	
0–1 cm	$112.9 \pm 30.0$
1–2 cm	$94.1 \pm 26.5$
2–3 cm	$81.7 \pm 23.8$
After the fire	
0–1 cm	$90.2 \pm 21.6$
1–2 cm	$87.8 \pm 27.6$
2–3 cm	$85.1 \pm 36.9$



Microbial biomass carbon (MBC), expressed as g MBC per kg soil, was determined by the fumigation-extraction method (Vance et al., 1987), using an extraction factor of  $K_{ec} = 0.38$  (Girona-García et al., 2019); this method consists of soil fumigation with chloroform followed by an extraction with 0.5 M  $K_2SO_4$  (1:5 w/v). Soil respiration or  $CO_2$  efflux was measured in an incubation assay of soil samples under optimal temperature (25 °C) and moisture (50 % water-holding capacity) conditions. The released  $CO_2$  was captured with NaOH traps (Anderson, 1982) and determined at intervals of 7 days during an incubation period of 28 days. Soil microbial respiration processes were distinguished as i) basal soil respiration (BSR) expressed as mg  $CO_2$  per kg soil per day, ii) mineralization coefficient or normalized soil respiration (nSR) expressed as mg  $CO_2$  per g SOC per day, and iii) metabolic quotient ( $qCO_2$ ) expressed as mg  $CO_2$  per g microbial biomass carbon (MBC) per day (Armas-Herrera et al., 2016; Girona-García et al., 2018a). The enzymatic  $\beta$ -D-glucosidase activity (GLU) was determined in soil incubated with 4-nitro-phenyl- $\beta$ -D-glucopyranoside enzyme substrate (Eivazi and Tabatabai, 1988) for 1 h at 37 °C. The  $p$ -nitrophenol (PNP) released as a product of GLU was extracted by filtration after adding  $CaCl_2$  and tris hydroxymethyl aminomethane buffer at a pH of 12. Finally, the PNP concentration was determined spectrophotometrically at 400 nm and expressed as mg PNP per g soil per h.

Soil pH and electrical conductivity (EC) were measured in 1:2.5 w/v and 1:5 w/v soil aqueous suspensions, respectively. Soil exchangeable  $K^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$  were extracted with 1 N  $NH_4OOCCH_3$  ( $AcONH_4$ ) buffered at pH 7.0 (1:20 w/v), after three consecutive extractions, 5 min of shaking and 5 min of centrifuging per extraction (Bower et al., 1952). Exchangeable  $Ca^{2+}$  and  $Mg^{2+}$  were determined by atomic absorption spectrometry and exchangeable  $K^+$  was determined by flame photometry.

Soil water repellency (SWR) was calculated with water drop penetration test (WDPT), proposed by Bisdom et al. (1993) and using the classes of Doerr (1998), on air-dried and sieved soil.

### 2.5. Statistical analysis

Statistical analysis was performed using the RStudio open-source software. The values reported in the text are expressed as the mean  $\pm$  standard deviation unless otherwise noted. A two factorial ANOVA was run to analyze the significant variance of soil response to the prescribed burning time (treatment) and soil depths, and the interaction among the two factors (variable treatment \* depth). The pairwise comparison of Tukey's HSD test ( $p < 0.05$ ) was also used to evaluate the statistical significance of the differences in the response variables. In order to satisfy the assumptions of the ANOVA tests, the variables were subjected to normality and homoscedasticity tests and were transformed whenever necessary, using the boxcox function ("MASS" library). A Pearson correlation was performed too to identify relationships between the studied soil properties. In addition, a Principal Component Analysis (PCA) was also conducted to obtain a synthetic view of the variation of soil variables, at the three soil depths, along the studied time sequence.

## 3. Results and discussion

Most of the studied parameters reveal significant negative impacts in the mid- and long-term, although no significant immediate changes were observed for many of them (Fig. 2). ANOVA test showed no significant interaction between the two factors (treatment, soil depth) for all the measured parameters, denoting that no differences were found in the changes associated with the different treatments between the three studied soil depths. The lack of differences between the response to fire of the three soil thicknesses characterized is already a first indicator of low severity of the burning, unlike severe laboratory burning that fundamentally affects the first and second cm (Badía et al., 2017b; Pereira et al., 2023). The low temperatures recorded 1 cm-deep compared to the surface (Table 2) also indicate a low severity burn.

### 3.1. Organic matter and biological activity

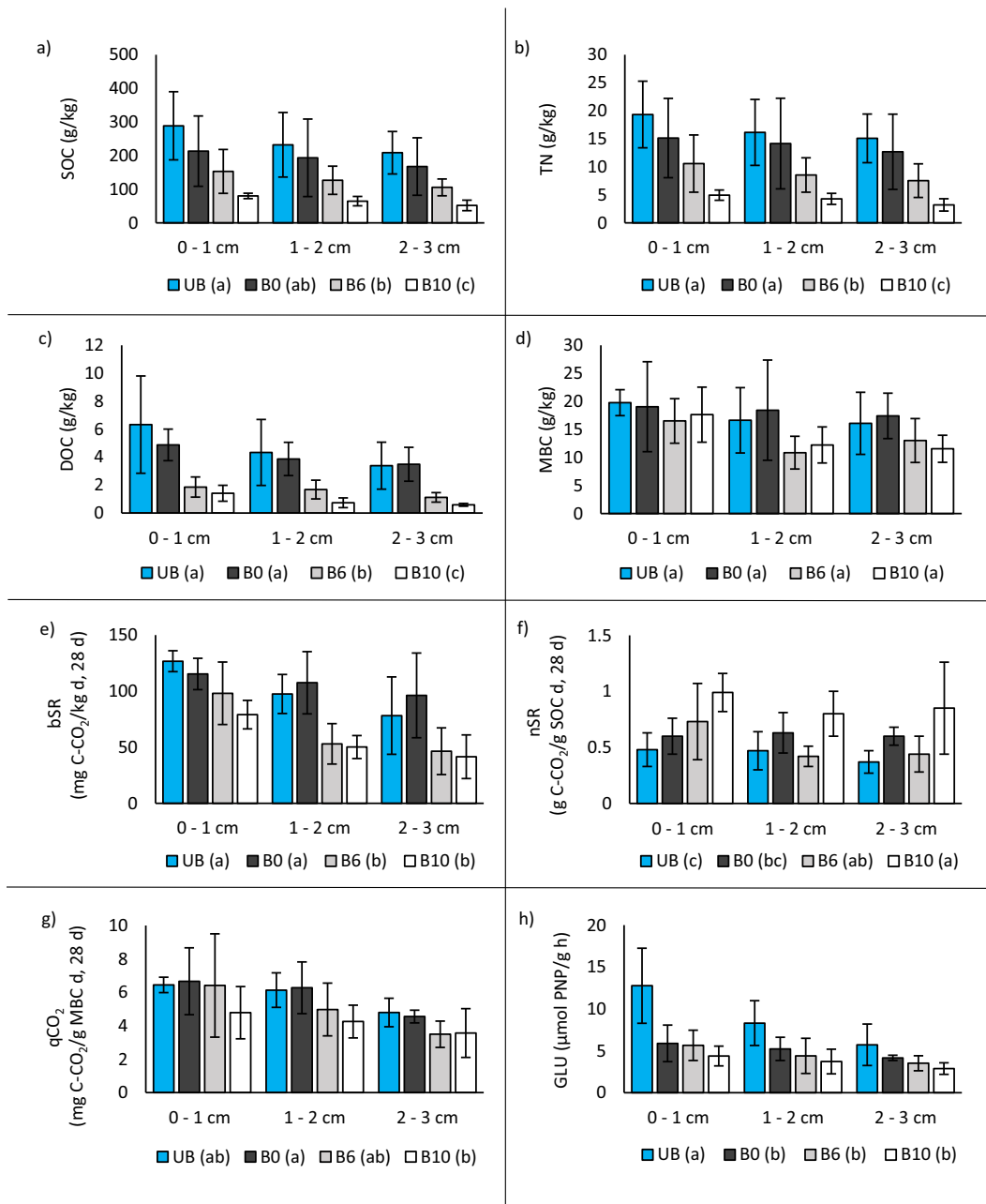
A constant decreasing tendency of SOC and TN was observed over time, although none of them was immediately (BO) affected by the fire in a significant way, but the difference became significant from the mid-term (B6) (Fig. 2a, b). Other low severity PBs also showed hardly any immediate reduction in the soil organic matter (Fontúrbel et al., 2016; Girona-García et al., 2019; San Emeterio et al., 2016, 2013), because its combustion is produced under temperatures above 300 °C (Santín and Doerr, 2016) and TN volatilization temperature is 200 °C (Francos et al., 2019; Mataix-Solera and Guerrero, 2007). Only in intense burns these parameters decrease immediately due to their combustion in the shallowest topsoil centimeters (Armas-Herrera et al., 2016; Francos et al., 2019; Girona-García et al., 2018b, 2018a; Granged et al., 2011), while, in other cases, when the fire facilitates the incorporation of partially charred organic matter to the soil, its content immediately increase (Alcañiz et al., 2016).

The short to long-term effects found in other studies are also diverse. Similar results to this study were found by Armas-Herrera et al. (2018) in the same location: 5 years after the fire, SOC and TN were lower than in the UB plot, which was related to erosion processes, as did other authors (Campo et al., 2008; Hatten et al., 2005; Khouri and Prendes, 2006). In the current study, all plots are located on steep slopes (25–35 % in average), the mean annual precipitation (MAP) is high (1700 mm/yr) and a considerable recover of vegetation can also be observed 6 years after the fire (Table 1), but the original plant cover, close to 100 %, is normally reached 15 years after burning (Mora et al., 2022). Badía et al. (2017a) reported a considerable proportion of bare soil within the first year after a *E. horridum* PB, which could favor erosion the first years after the fire. Francos et al. (2019) also had similar results, but they related it to leaching and plant absorption. In addition, other authors blamed the mineralization of organic matter for the decrease of SOC (Alcañiz et al., 2016; De Marco et al., 2023; Memoli et al., 2021), which could also be the case of the current study, as it is explained in more detail below.

On the other hand, Girona-García et al. (2019), who studied three similar locations of the Central Pyrenees, found that in two of them soil organic matter increased in the topsoil centimeter 1–2 years after the fire due to the late incorporation of ash and charred materials. In the case of the present study, we observed that the litter layer was not totally consumed by the fire, due to its low severity. This layer kept ashes and partially charred materials away from the mineral soil and therefore no immediate SOC increases were found despite the low fire severity, as found by Fontúrbel et al. (2016). Other authors reported no significant variability from short to long-term changes on those parameters (Campo et al., 2022; López-Poma and Bautista, 2014; Muñoz-Rojas et al., 2016). The above-mentioned variability of results is specially linked to variations in the duration of the fire as well as plant and soil moisture content (Alcañiz et al., 2016). Thus, fires of long duration, with dry fuels and soils produce greater organic matter loss (Alcañiz et al., 2016; Girona-García et al., 2019).

DOC barely decreased right after the fire but suffered a significant and permanent decrease (>50 %) 6 years after (Fig. 2c). These results match the findings of (Girona-García et al., 2018a), who found a significant permanent decrease of DOC 6 months after a similar prescribed fire, in a 1-year study, in another location of the Central Pyrenees, which was attributed to its mineralization by the remaining active microorganisms, to the decrease of soil organic matter inputs from the organic layers and to leaching and runoff. That hypothesis could also explain the changes in this particular case, as previously explained. On the contrary, San Emeterio et al. (2016) did not find any variation in DOC between UB and burned soils along the 2-year period of study, but the effects of the fire could be diluted in the sampling depth (0–10 cm).

MBC did not suffer any significant variations among the studied time, but a slight downward trend ( $p$ -value = 0.053) over time can be observed (Fig. 2d). The other studied carbon fractions are significantly correlated with it (Table 3). Girona-García et al. (2019) found an immediate permanent decrease of MBC in the topsoil centimeter in one location of the Central Pyrenees, while no changes were found in the other two study sites.



**Fig. 2.** Evolution of biochemical properties for the different treatments (UB, B0, B6 and B10) within the sampled soil depths (0–1, 1–2 and 2–3 cm). Lowercase letters in the legend indicate significant differences between treatments ( $p < 0.05$ ) for the whole sampled soil depth (0–3 cm). In each histogram bar, the mean ( $n = 4$ ) and the standard deviation are represented.

**Table 3**

Pearson correlation coefficients, showing the degree of linear association (negative and positive) between soil properties, for all treatments and all sampled depths ( $n = 48$ ).

Variable	pH	EC	Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>	SOC	TN	DOC	bSR	MBC
pH										
EC	0.26									
Ca <sup>2+</sup>	0.38**	0.87**								
Mg <sup>2+</sup>	-0.39**	0.44**	0.41**							
K <sup>+</sup>	-0.44**	0.07	0.01	0.75**						
SOC	-0.35*	0.65**	0.65**	0.76**	0.43**					
TN	-0.28	0.68**	0.71**	0.76**	0.40**	0.99**				
DOC	-0.23	0.75**	0.68**	0.64**	0.30*	0.91**	0.89**			
bSR	0.11	0.75**	0.72**	0.60**	0.38**	0.74**	0.74**	0.77**		
MBC	-0.17	0.44**	0.42**	0.64**	0.55**	0.69**	0.67**	0.61**	0.69**	
GLU	-0.25	0.59**	0.49**	0.50**	0.29	0.76**	0.73**	0.82**	0.68**	0.50**

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

They affirm that this parameter is a sensitive soil property, and its direct impacts depend on the temperatures reached and the soil water content. Other authors have also found, like in the present study, the absence of direct effects on this parameter (Girona-García et al., 2019; Memoli et al., 2021), while others observed immediate negative impacts (Armas-Herrera et al., 2016; Fontúrbel et al., 2016; Girona-García et al., 2018a). The non-significant reduction of this parameter in the mid-term is in accordance with San Emeterio et al. (2016), who found a non-significant difference ( $p < 0.1$ ) between the unburned and burned plots by the end of their study (19 months). Differences in soil water content between plots were blamed for that variation. Girona-García et al. (2019) also found effects on MBC over the time and up to one year, which were positive in one of the studied locations and negative in another one.

The bSR, unchanged right after burning, suffered a significant permanent decrease 6 years after the fire which was maintained at 10 years (Fig. 2e). This drop coincides with that of the DOC content, which is believed to be one of the main responsible of changes in this parameter (Castro-Díez et al., 2012), both being highly and significantly correlated ( $r = 0.77$ ), as shown in Table 3. The effects of fire on bSR are diverse. Like in this study, Girona-García et al. (2019) did not find any direct or short-term effects of the PBs on 2 Pyrenean locations. However, they found a decrease in the topsoil centimeters in another one, like other authors (Armas-Herrera et al., 2018, 2016; Girona-García et al., 2018a; Memoli et al., 2021). The nSR was significantly higher 6 and 10 years after the fire (Fig. 2f). Given that MBC did not significantly vary, and that the SOC suffered a progressive decrease along the years, there are more microorganisms per SOC unit and, therefore, the emitted C-CO<sub>2</sub> per SOC unit is also higher. The soil microbial activity could have also had an important role on the organic matter loss, as previously mentioned. De Marco et al. (2023) and Panico et al. (2020), who also found increases in nSR 1 year after a wildfire, suggest that the fire could stimulate the mineralization and provoke a SOC loss over time. They hypothesize that nSR is dependent on the quality of organic matter, more specifically, a higher C/N ratio could imply a more recalcitrant organic matter and a higher nSR. In the case of our study, C/N ratio also increased significantly (0–3 cm) between UB (14.25 ± 0.85), B6 (14.86 ± 1.57) and B10 (16.08 ± 1.97), probably due to the mineralization of the most labile organic matter fractions before B10. Other study in the Central Pyrenees also found no immediate variations in nSR after a PB (Armas-Herrera et al., 2016). On the contrary, Girona-García et al. (2018a) found an immediate decrease due to the reduction of labile carbon. The qCO<sub>2</sub> did not significantly vary between UB and B0 (Fig. 2g), matching the results of similar studies performed in the Central Pyrenees (Armas-Herrera et al., 2016; Girona-García et al., 2018a). It was, in fact, lower in B10 than in B0. Higher qCO<sub>2</sub> levels are often related with the microbial response to stress conditions and with C/N ratio (Akburak et al., 2018). No changes in MBC mean that the microbial community was not affected by the fire and that changes in its activity (bSR, nSR and qCO<sub>2</sub>) are related with changes in organic matter quantity and structure.

GLU suffered an immediate significant reduction provoked by the fire, which did not recover even 10 years after the fire (Fig. 2h). The direct effect of the fire might have been produced by the enzyme denaturation due to the heat (Knicker, 2007). The fact that GLU is one of the few parameters that, in this study, undergoes a significant decrease right after the fire would be a symptom of its high sensitivity to fire (Pereira et al., 2023). This immediate negative effect has been reported before by many authors (Armas-Herrera et al., 2016; Girona-García et al., 2018a; López-Poma and Bautista, 2014; Wang et al., 2013). The persistence of the negative effects could be caused by a decrease in labile C content (Girona-García et al., 2019; Wang et al., 2013), due to the temporary loss of *E. horridum* canopy cover and its resulting exposure to rainfall, which is confirmed by the significant permanent decrease of DOC 6 years after the fire and their high correlation (Table 3). GLU is also very correlated with SOC (Table 3), as it reflects soil quality and is a primary source of enzyme substrates and their physical stabilization (López-Poma and Bautista, 2014). Changes on the soil cover can also be the cause of lower GLU. López-Poma and Bautista (2014) found higher GLU values in soils with shrub cover, compared to grass or bare soil. As opposed to this study, most authors found a recovery tendency on GLU in the short- and mid-term (Armas-Herrera et al., 2018; Girona-García et al., 2019; López-Poma and Bautista, 2014; Wang et al., 2013), while a few did not find any significant effects (Fontúrbel et al., 2016; San Emeterio et al., 2016), but, in those cases, the low soil temperature during the PB and the shallow soil sampling depth respectively could be the cause.

Changes in the microbial community could be driven by the changes on the plant cover, which regulates its stability (Picariello et al., 2021). Right after the fire, the plant cover was almost totally consumed, leaving soil exposed to abiotic processes, that could cause erosion, cooling, heating and drying. Ten years after the PB, the shrub vegetation biomass has not yet reached the initial values (Table 1) and, just as with the vegetation, the values of the biological activity prior to burning have not yet recovered.

### 3.2. Other soil parameters

The initial pH, slightly acidic, became neutral after burning, without being significantly different either by fire or over time (Table 4). Neither was EC directly affected by fire, although it decreased significantly with time (B6), as did most basifying cations (Table 4). The correlation between pH and EC is not significant, as shown in Table 3 ( $r = 0.26$ ). Otherwise, EC shows a significant correlation with the main exchangeable cations (Ca<sup>2+</sup> and Mg<sup>2+</sup>) and organic matter. Many authors have also found that pH and EC sometimes are not affected the same way after a fire (Girona-García et al., 2018c; Granged et al., 2011; Mills and Fey, 2004), while others like Muñoz-Rojas et al. (2016) have found, in fact, a high correlation between them. The decrease in EC and the main exchangeable cations over time is not surprising in an environment with about 1700 mm/yr of MAP (Úbeda et al., 2005; Ulery et al., 1993) and with a recovering vegetation

**Table 4**

Evolution of properties (0–1, 1–2 and 2–3 cm depth) for unburned and burned soils (at 0, 6 and 10 years after burning): pH, Electrical conductivity (EC) and Exchangeable cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>). Lowercase letters indicate significant differences between treatments ( $p < 0.05$ ). The P-value indicates whether there are significant differences between treatments.

		pH	EC (μS/cm)	Ca <sup>2+</sup> (cmol <sub>(+)</sub> /kg)	Mg <sup>2+</sup> (cmol <sub>(+)</sub> /kg)	K <sup>+</sup> (cmol <sub>(+)</sub> /kg)
0–1 cm	UB	6.3 ± 1.1a	283.5 ± 32.7a	39.76 ± 7.76a	1.53 ± 0.48a	0.68 ± 0.26a
	B0	7.2 ± 0.8a	307.3 ± 38.4a	45.36 ± 11.42a	1.89 ± 0.61a	0.83 ± 0.39a
	B6	6.6 ± 0.2a	129.0 ± 37.2b	28.14 ± 7.14b	1.54 ± 0.54a	0.82 ± 0.07a
	B10	6.7 ± 0.2a	114.5 ± 23.1b	18.06 ± 3.66c	1.01 ± 0.15b	0.65 ± 0.14a
1–2 cm	UB	6.3 ± 1.2a	249.1 ± 25.8a	39.44 ± 11.55a	1.29 ± 0.33a	0.68 ± 0.20a
	B0	6.9 ± 0.9a	243.2 ± 56.0a	36.95 ± 5.23a	1.32 ± 0.70a	0.71 ± 0.44a
	B6	6.5 ± 0.4a	112.6 ± 23.5b	25.24 ± 7.06b	1.22 ± 0.32a	0.72 ± 0.07a
	B10	6.6 ± 0.1a	108.9 ± 31.6b	16.45 ± 2.59c	0.83 ± 0.16b	0.62 ± 0.14a
2–3 cm	UB	6.5 ± 0.9a	238.2 ± 53.3a	39.62 ± 10.91a	1.30 ± 0.44a	0.63 ± 0.23a
	B0	6.9 ± 0.7a	239.8 ± 62.7a	39.35 ± 7.34a	1.55 ± 0.91a	0.78 ± 0.55a
	B6	6.5 ± 0.5a	122.3 ± 42.2b	23.36 ± 7.57b	1.01 ± 0.25a	0.61 ± 0.14a
	B10	6.5 ± 0.1a	103.8 ± 27.4b	15.17 ± 2.42c	0.68 ± 0.13b	0.53 ± 0.15a

that absorbs essential nutrients (Alcañiz et al., 2016). The incorporation of basic ash, rich in soluble ions and salts (Muñoz-Rojas et al., 2016), into the soil is likely to happen and causes these parameters to increase in the short term (Alcañiz et al., 2018; Gil et al., 2010), sometimes right after the PB

(Francos et al., 2019; Girona-García et al., 2018c; Granged et al., 2011) and sometimes within the next few years (Alcañiz et al., 2016; Fontúrbel et al., 2016; Girona-García et al., 2018c). Those effects are expected to disappear in the mid- and long-term. In the current study, ashes did not have the chance to incorporate to the mineral soil before the B0 sampling, because the litter layer was not completely consumed by the fire and kept most ashes away from the mineral soil. The same thing happened to Fontúrbel et al. (2016), who found changes on pH in the organic horizon, but found no changes in the mineral soil.

A decrease of SWR with depth was found in UB samples (Fig. 3). In B0, one of the samples showed an increase in SWR in 1–2 cm depth and the rest showed the same trend as in UB. A possible explanation is that fire severity was high enough in this point to provoke the migration of some hydrophobic compounds from 0 to 1 cm to 1–2 cm (De Bano, 1981). SWR decreased over the time and completely disappeared after 6 years, following the same tendency as SOC and related parameters. The combustion of organic matter and the subsequent destruction of hydrophobic substances can cause the decrease of SWR (Granged et al., 2011), and the post-fire erosion and reduction of organic matter could even produce its complete disappearance. Other authors reported a decrease of SWR next to the surface after PBs (Girona-García et al., 2019; Granged et al., 2011) and an increase in the lower layers of soil (Girona-García et al., 2018b), but most of the times SWR recovers UB values with the time (Girona-García et al., 2019; Granged et al., 2011), unlike in the present study.

### 3.3. Overall changes in soil properties: PCA

In the principal component analysis (PCA), the first two dimensions accounted for 82.2 % of the total variance in the dataset (Fig. 4). Dimension 1 (Dim1), which explains 66.1 % of the variance, is related to indirect fire effects, mainly associated with carbon and nitrogen, and Dimension 2 (Dim2) explains the remaining 16.1 % of variance. Dim1 distributes the samples by organic matter content and composition and the biological parameters related to it, on the left side, and nSR and SAS on the right side. UB and B0 samples showed the highest carbon and nitrogen content and biological activity, while B6 and B10 showed the lowest. C/N and nSR showed higher values in B6 and B10 than in UB and B0. On Dim2, B0 samples showed higher positive loadings due to their higher pH values, while UB showed the lowest and B6 and B10 showed medium values, although these differences are not significant. To summarize, this PCA highlights, on one hand, how the immediate perturbation of the fire did not alter most of the studied soil properties. And, on the other hand, it shows how most of the studied properties were affected 6 and 10 years after the fire. That is to say, it shows how the indirect effect of the fire affected the soil, which did not recover even 10 years after the perturbation.

### 4. Conclusions

The prescribed bush burning in Tella's steep slopes have barely generated any direct, immediate effects in the topsoil centimeters (0–3 cm) on most of the studied parameters, which is related to its high thermal inertia, as shown by the fact that, below the 2 cm depth, the soil temperature had not increased due to the passage of the fire (it was, in consequence, of low severity). Only the sensitive enzymatic activity related to cellulose degradation, GLU, showed a strong reduction after fire (B0), especially in the first centimeter (where it was reduced up to 2/3 of the original value). This reduction was maintained over time (up to 10 years), strongly related with the reduction of DOC. However, there were negative effects over time, both at 6 and 10 years after burning, related with the organic matter loss and changes in its quality, i.e.: the reduction of SOC, DOC, TN and bSR. We interpret that the reduction and changes observed 6 and 10 years after burning in the organic matter were produced by a combination of the loss of litter and surface soil (laminar erosion on a centimeter scale), and the organic matter mineralization by microbial activity. The steep slopes of the hillsides and the abundant rainfall in this area of the subalpine stage would favor the erosion, at least during the first years after burning,

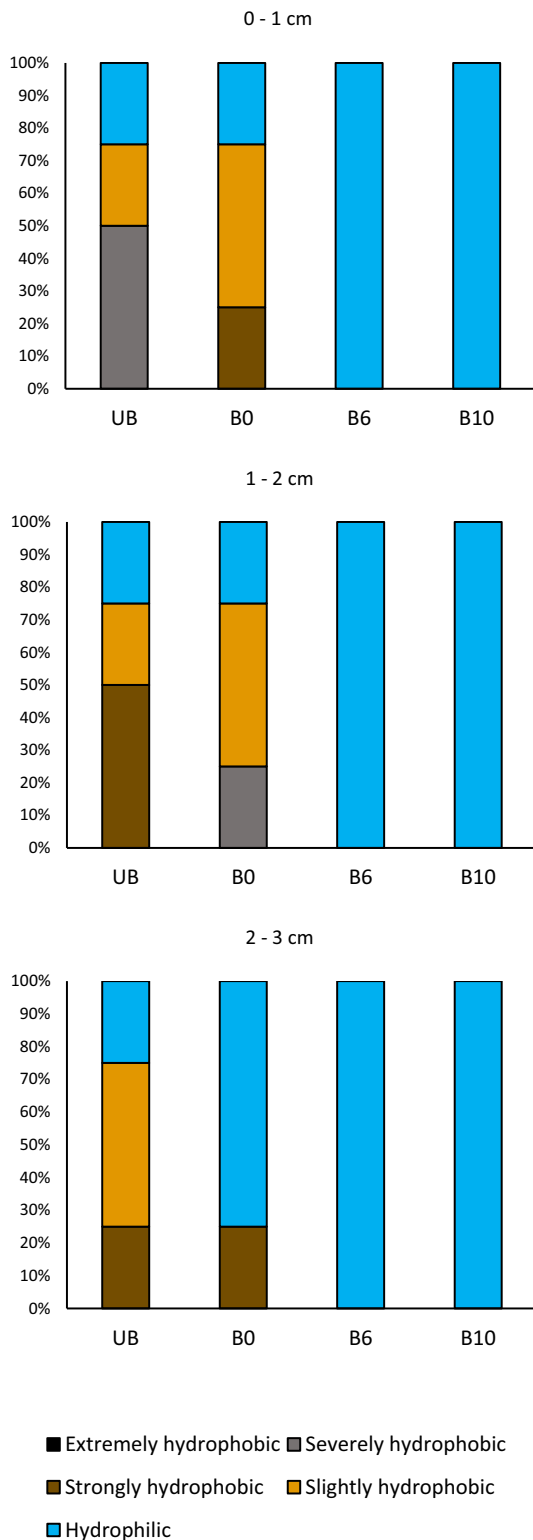
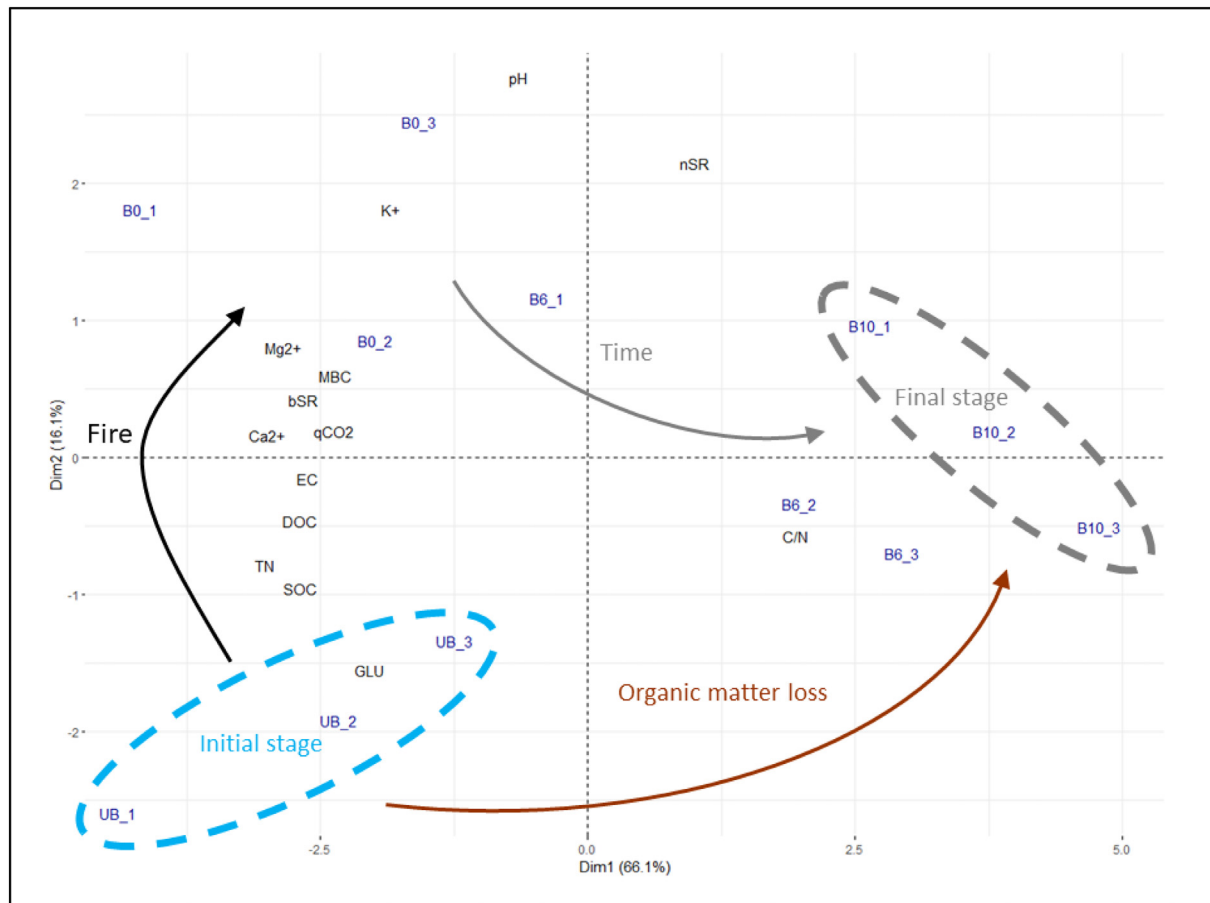


Fig. 3. Occurrence (%) of soil water repellency (SWR) according to the Water Drop Penetration Test (WDPT) for the different soil depths (0–1, 1–2 and 2–3 cm) in the unburned (UB), immediately burned (B0), 6 years after the fire (B6) and 10 years after the fire (B10) plots. SWR classes defined by Doerr (1998).



**Fig. 4.** Principal Component Analysis (PCA): diagram of the factorial loads in the first two axes of the soil properties (in red) and the soil samples (in blue). Underscore symbol + number in the soil samples indicates the different sampled depths: 1 (0–1 cm), 2 (1–2 cm) and 3 (2–3 cm). Abbreviations of soil properties have already been indicated in the text.

while a protective vegetation cover was still being reinstalled after its burning. The activity of the unaltered microbial community, together with the reduction of organic matter inputs from the litter layer contributed to the organic matter loss over time.

Further research is needed to ascertain whether changes in soil organic matter dynamics are linked to changes in the functionality of the microbial population and how it changes over longer time. This study proved that prescribed fires, even when properly performed, causing barely no direct effects on soil, can sometimes negatively affect soil organic matter over long time periods. In order to help land managers to choose where to perform prescribed fires, it would be interesting to determine which site characteristics were decisive in the organic matter loss.

#### CRediT authorship contribution statement

**Andoni Alfaro-Leranz:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization. **David Badia-Villas:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Clara Marti-Dalmau:** Formal analysis, Resources, Data curation, Writing – original draft, Writing – review & editing. **Mohamed Emran:** Formal analysis, Writing – original draft, Writing – review & editing. **Ana Paula Conte-Dominguez:** Formal analysis, Investigation, Resources. **Oriol Ortiz-Perpiña:** Methodology, Formal analysis, Resources.

#### Data availability

Data will be made available on request.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.163994>.



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