SHORT- AND MID-TERM EVOLUTION OF TOPSOIL ORGANIC MATTER AND BIOLOGICAL PROPERTIES AFTER PRESCRIBED BURNING FOR PASTURE RECOVERY (TELLA, CENTRAL PYRENEES, SPAIN)

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SHORT TITLE: EVOLUTION OF SOIL PROPERTIES AFTER PRESCRIBED BURNING IN THE PYRENEES

HIGHLIGHTS

Prescribed fire is used in the Pyrenees as a tool for managing grazing lands

SOM and biological properties we examined at short- and mid-term after burning

Fire effects remained at mid-term except microbial C that returned to pre-fire values

Warming due to cover loss and nutrients release have stimulated SOM mineralization

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ldr.2937

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Abstract

We determined the short and medium-term effects of prescribed burns on soil organic matter content and biological activity in grazing areas invaded by the shrub *Echinospartum horridum* (Vahl) Rothm. in the Pyrenees of Huesca (Spain). Soil samples were collected at 0-1, 1-2 and 2-3 cm depths in triplicate just before the burn, immediately after the burn (T0), and one and five years later (T1, T5). We analysed the contents of total soil organic C (SOC) and N, soil respiration, microbial biomass C, and β-D-glucosidase and acid phosphatase activities. Fire provoked an immediate high decrease in the contents of SOC (-40.2 %) and N (-26.3 %) in the first 3 cm, which were even lower at T5 (-50.3 % and -46.5 %, respectively). This can be explained as follows: (i) low incorporation of burned organic matter due to removal by wind and runoff; (ii) changes in microclimate increasing soil temperature and enhancing mineralization; and (iii) a stimulating effect on decomposition due to the release of nutrients. Soil biological activity was affected at T0 in the first 3 cm (-49.3 % glucosidase, -48.2 % phosphatase and -54.5 % respiration rate). Microbial biomass C content was also affected by fire at T0 (-32.3 %) but was close to its initial value at T5. The results suggest that these soils are particularly sensitive to fire. Adjusting the frequency and intensity of the burns is necessary to minimize their impact on the soil and to ensure the suitability of this management practice.

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

INTRODUCTION

Grazing lands are important to the global carbon (C) cycle because they store between 20 and 35 % of the world's soil organic matter (SOM) (Janzen, 2005) and account for about 25 % of the global potential for soil C sequestration (Follett & Reed, 2010). Grazing lands in Europe are currently largely restricted to mountain areas, but they contain ecosystems that have the richest biodiversity of the continent (Tälle *et al.*, 2016). C storage in the soils of the mountain areas is one of the largest on a global scale (Jobbágy & Jackson, 2000). This is due to low temperatures, which slow down the decomposition and loss of organic matter (Körner 2003). Because of this, mountain soils contain a large proportion of labile SOM, which makes them more sensitive to climate changes (Budge *et al.*, 2011).

Most of the grazing land in European mountain areas depends on its maintenance by regular disturbance by grazing and/or fire, which prevents succession to woody vegetation. However, the cessation of fire use due to wildfire risk and a decrease in livestock pressure over the past several decades have allowed encroachment of shrubs into the grazing lands throughout the European mountain ranges (Cernusca *et al*., 1996; Galvánek & Lepš, 2008). Shrub encroachment can have significant impacts on ecosystems, including alterations of the dynamics of water and nutrients in soil, increased fire and avalanche hazards, and a loss of biodiversity and pasture resources (Montané *et al*., 2010). In the Pyrenees, woody species have increased their biomass by 47 % between 1975 and 2005 (Komac *et al.*, 2013). One of the most important encroaching species in this area is the thorny cushion dwarf *Echinospartum horridum* (Vahl) Rothm. *E. horridum* grows naturally in rocky habitats, but spreads rapidly onto undergrazed pastures, where it behaves as an invasive species, forming dense, almost monospecific stands (Komac *et al*., 2011b).

In recent years, prescribed burning has gained renewed attention as a substitutive source of disturbance and, thus, as a tool for fighting shrub encroachment (Fernandes *et al*. 2013). However, it has some drawbacks, such as increased erosion rates during the months following fire, the loss of nutrients (Gómez-Rey *et al.*, 2013) and the enhanced spread of pyrophytic species (Girard *et al.*, 2008). Prescribed fire is less expensive than the mechanical removal of shrubs (Goldammer & Montiel, 2010) and may be the only realistic way to stop shrub encroachment in certain mountain areas (Lyet *et al.*, 2009), coupled with moderate grazing (Komac *et al.*, 2011a). However, few studies exist on the environmental effects of prescribed burning of shrubland in subalpine environments (San Emeterio *et al.*, 2016; Girona-García *et al*., 2017).

The response of soils to fire is complex and depends on fire severity, as well as on soil and vegetation characteristics (Certini, 2005). Fire impacts on soils include the loss of SOM and nutrients by combustion and increased post-fire runoff and erosion rates, which are related to decreased aggregate stability (Badía-Villas *et al.*, 2014; Mataix-Solera *et al*., 2011). On the other hand, fire causes some organic matter to become “pyromorphic" humus, which includes refractory and oxidation resistant organic molecules (Knicker *et al*., 2005). Fire also affects soil microbiota, which is responsible for the cycling of SOM, and fungi are much more sensitive than bacteria (Boerner *et al.*, 2000), which is reflected in the changes of the composition of the microbial community (Barreiro *et al*. 2016). Soil enzyme activities are also affected by both the denaturation and deactivation of enzymes at high temperatures (Knicker, 2007) and by changes in the availability of microbial substrates as a result of fire (Boerner *et al*. 2000).

The objective of this study was to investigate the short to medium-term impacts of prescribed burns on the soils of grazed land in mountain areas to improve this management tool in terms of the frequency and intensity of the burns. To this aim, we analysed soils one year and five years following a controlled burn of *E. horridum* scrub in the Central Pyrenees (Huesca, Spain). The immediate effects of this experimental burning on the SOM and soil biological properties were reported in a previous work (Armas-Herrera *et al.*, 2016). In that work, larger-than-expected immediate effects of the prescribed burning on these properties were found, which were more in line with the typical effects of summer wildfires than with the low impacts generally attributed to prescribed fire. Examining the changes in the short and mid-term were beyond the scope of that study. Based on previous results, our preliminary hypotheses were that (a) the soils would start to recover at the short term (one year or less) and (b) that a complete recovery would occur in the medium term (five years or less). The following specific objectives were pursued: (a) to determine the depth and degree to which the soils remained affected over the first years following a burn in terms of SOM content and soil biological properties, and (b) to study the resistance and resilience of the soil microbial biomass and activity to fire disturbance, as inferred from soil respiration and enzyme activity values.

MATERIAL AND METHODS

Study area and field work

The study site is located in an extensive grazing area in the municipal district of Tella-Sin (Huesca) at 1875 m.a.s.l. The average annual precipitation is 1700 mm and the average annual temperature is 5 °C. The soils, classified as Leptic Eutric Cambisols (Loamic, Humic), are shallow and irregular in depth, with limestone outcrops alternating with soils thick enough to support high quality pastures of *Bromion erecti* Koch 1926 and *Nardion strictae* Braun-Blanquet 1926, which are grazed by sheep, goat, horse, and cattle herds. The livestock population is barely one third of what was supported 50 years ago. As a result, a large area is now occupied by dense shrub thickets of *E. horridum* with canopy cover close to 100 % (**Figure 1A**).

Prescribed burning of *E. horridum* shrublands is performed in the Central Pyrenees by the Forest Fire Prevention Team (EPRIF) at the request of local sheperds, with the aimed of promoting the growth of grass species that can be grazed by the livestock. In April 2015, a prescribed burn was performed in a 12.5 ha plot with slopes ranging from 10 to 40 %. Temperatures during the fire were measured with thermocouples located in the mineral soil at 1 cm and 2 cm depths. The burn was initiated from the top and from the foot of the slope at the same time, with both flame fronts eventually merging in the middle area, resulting in very high fire intensity (**Figure 1B**). The highest temperatures measured were 397 °C at a 1 cm depth and 121 °C at a 2 cm depth. Further information on the experimental burn, the study area, and the soil properties can be found in Armas-Herrera *et al.* (2016). Near the plot burned in 2015 (approximately 100 m), a plot with similar area (12.6 ha) was burned in February 2011. The two plots had a similar slope aspect, gradient and length, elevation, parent material and soil morphology. We do not have data on the temperatures reached in the 2011 burn, but EPRIF team reported to us that both burns were very similar in terms of intensity and severity.

The immediate and short-term effects of the 2015 burn were studied at three sampling points located equidistant along an NE-SW transect from the top to the bottom part of the burned plot. In each point, samples were taken from the top soil layer at three depth intervals (0-1, 1-2 and 2-3 cm). We focused on the very top layer of soil on the basis of our previous work (Badía et al., 2014; Aznar et al., 2016) which shows that high temperatures during fire often only reach the first one or two cm of soil depth. The samples were collected immediately before (unburned control) and a few hours after the burn (burned T0) in April 2015 and one year after the burn in April 2016 (burned T1). In addition, a similar transect was conducted in the burned plot in 2011, and soil samples were collected in April 2016, five years after the burn (burned T5). All samplings were carried out at the end of the winter (with the precise date depending on the weather of the year) to avoid confounding with seasonal trends.

Plant cover was calculated at T1 and T5 by interpreting orthophotographs of the soil surface taken at 1.7 m height, with an Olympus digital camera (OM-D EM-10). Photographs of the soil surface covered an area of 3.26 m^2 (2.08 x 1.57). For each image, we calculated the proportion of bare soil, stones or rocks, burned plant material, herbaceous vegetation and organic debris (**Table 1**). One year after burning (T1), the plot burned in 2015 was abundantly recolonized by *E. horridum* saplings, with few thorns and at a 1-6 cm height, as well as by bulb and rhizome sprouts from *Carex flacca* Schreb., *C. humilis* Leyss., *Euphorbia cyparissias* L., *Iris latifolia* (Mill.) Voss, *Teucrium chamaedrys* L. and *Carduus carlinifolius* Lam (average plant cover of 14 %) (**Figure 1C**). In turn, the plot burned in 2011 (T5) had 43 % cover of very thorny *E. horridum* at approximately 10-15 cm in height in 2016 (**Figure 1D**).

Laboratory analysis

All soil samples were sieved at 2 mm and kept at 4 °C until analysis. The water content was determined at 105 °C, and the results of all analyses were calculated on a dry weight basis. Total soil organic C (SOC) and nitrogen (N) were measured on a Vario Max CN macro elemental analyser (Elementar Analysensysteme GmbH, Hanau). Microbial biomass C was obtained by the chloroform fumigation-extraction method (Vance *et al.*, 1987). Potential soil C mineralization rates were determined in a 28 day-incubation of soil samples at optimum moisture (50 % field capacity) and temperature (25 °C). The emitted CO_2 was captured with alkali traps (Anderson, 1982) and was determined on days 1, 2, 4, 7, 10, 14, 18, 22, and 28 following the beginning of the incubation. The accumulated C-CO_2 values were expressed per dry weight and time (C-CO_2 efflux). β-D-glucosidase and acid phosphatase enzyme activities were analysed according to the methods of Eivazi & Tabatabai (1988) and Saá *et al*. (1993), respectively.

Data analysis

Soil properties were analysed using a repeated measures ANOVA with the time elapsed since the burn (Time) as the fixed factor and soil depth (Depth) as the repeated measure. Although the samples were obtained from two different plots (unburned control, burned T0, and burned T1 from the burned plot in 2015 and burned T5 from the burned plot in 2011), they were considered representations of the different stages in a single post-fire sequence and were all included in the analysis. The variables were checked for normality and sphericity, and when necessary, they were inverse-, log- or root-transformed before ANOVA. Differences between single groups within a statically significant factor were assessed using Fisher's Least Significant Difference (LSD) test. A significance level of $P < 0.05$ was used for all tests. All analyses were carried out using SPSS for Windows (version 20, SPSS Inc., Chicago, IL, USA).

Although the recovery of soil properties to their pre-fire levels is not necessarily desirable, their maintenance and reversion over time are meaningful for assessing the sustainability of burning and the suitable burning frequency. Thus, we assessed the fire resistance, recovery and resilience of SOC, N, microbial biomass C, soil respiration rates and acid phosphatase and β-D-glucosidase activities with the following formulas, given by López-Poma & Bautista (2014):

Resistance (RS): $-100\ [(UB-B_0)/UB]$
Recovery (RC): $-100\ [(UB-B_x)/UB]$
Resilience (RL): $-(RS-RC)$ at $t = 1$ year and $t = 5$ years

where UB is the value of the variable in the unburned control soil, B_0 represents the value at T0, and B_x corresponds to the value at T1 or T5. RS and RC values of zero indicate maximum resistance and full recovery at a given time after fire, respectively, whereas negative values are indicators of lower RS and RC. RS can be positive when the value of the target variable in burned soils is higher than in unburned soils. A value of zero RL means no resilience, a positive value indicates resilience and a negative value represents a further decrease in the values of the response variable compared to the initial impact of fire.

RESULTS

The contents of total SOC and N decreased with depth, being significantly different at 0-1 cm, 1-2 cm and 2-3 cm, and the prescribed burn led to a significant decrease of their contents (**Tables 2,3, Figures 2A, 2B**). The interaction *Time* x *Depth* was not significant, i.e., no differences were found in the changes associated with time after the burn between the three soil depths. The amount of SOC and N tended to decrease with time after the burn. Thus, one year later (T1), SOC and N contents were slightly (not significantly) lower compared to the values just after the burn (T0) and were even lower in the soils burned five years earlier (T5), where the N content was significantly lower compared to the soils just after fire (T0). These results indicate a low resilience for both parameters, being lower for N. The different behaviour of both elements is reflected in the C/N ratio (**Figure 2C**), which decreased in the short term (T0 and T1) due to the prescribed burn, but at T5 it was higher although still significantly different to those of soils analysed before burning.

The amount of microbial biomass C did not show significant variations with depth, but it was significantly affected by fire (**Figure 2D**). The interaction *Time* x *Depth* was not significant, but a lower immediate impact of the burn was observed at a greater soil depth, as reflected in the resistance indexes (**Tables 2,3**). The burn effect was similar at T0 and T1. The microbial C contents at T5 did not show significant differences with respect to the control soils, thus revealed the highest values of resilience among all variables studied.

The activities of enzymes β-D-glucosidase and acid phosphatase showed smaller or no significant differences with soil depth, whereas they decreased significantly just after the burn (T0) (**Tables 2,3, Figure 3A, 3B**). The enzyme activities continued to decline at the short term (T1), although more slowly with soil depth for the β-D-glucosidase, as mirrored by the significant interaction between *Time* and *Depth*. Both enzymes showed higher activities at T5, but were still much lower than before burning. The resilience of enzymatic activity was low for both enzymes, especially in the case of phosphatase, and resilience was somewhat higher for β-D-glucosidase.

The C-CO_2 efflux was highest in the uppermost cm of soil (0-1 cm) and was lowest at the 2-3 cm depth. It decreased significantly at T0 (**Tables 2,3, Figure 3C**), but there was no significant interaction of *Time* x *Depth*, indicating that this decrease was similar for the upper 3 cm of soil. The C-CO_2 efflux decreased further at T1 and showed comparatively higher values at T5, although they were lower than that observed in the soils analysed before the burn.

DISCUSSION

Impacts of prescribed burning on soil organic matter

The decrease of SOM within the first 3 cm contrasts with the results found in other studies with similar time scales in areas subjected to prescribed fires. Most studies on this topic only describe small decreases (not significant) as a result of the lower intensity and severity fires (Alexis *et al.*, 2012; San Emeterio *et al.*, 2016) or increases due to the inputs of ashes and charred residues derived from burning (Alcañiz *et al*., 2016; Larroulet *et al*., 2016; Rutigliano *et al*., 2007). In these studies, the initial SOM values generally recovered in the short or medium term. More in line with our results, Badía-Villas *et al*. (2014) and Aznar *et al*. (2016) reported, under laboratory controlled conditions, significant C losses ranging from -50 % to -75 % in the first 2 cm soil depth, where temperature reached about 300 °C at 1 cm soil depth, and Girona-García *et al*. (2017) recently reported C losses of -52% after burning of a *E. horridum* stand in a nearby area in the Pyrenees. The C/ N ratio decrease found in the short term after the burn is well known in the literature for soils after a fire, and can result from the release of C from certain thermally-unstable plant constituents (e.g. cellulose), as well as from the preferential immobilization of N over C into pyrogenic heteroaromatic compounds during charring (Knicker, 2007; Knicker, 2011). The mid-term trend to return to a C/ N value close to that of the soil prior to the burn indicates a certain degree of recovery of the quality of SOM, which contrasts with the significant loss of the total SOM content.

In view of our results, it seems evident that the ashes derived from the burn were hardly incorporated into the soil. This suggests a loss of the ash material, which can be rapidly removed from the soil surface by wind and post-fire rain (Bodí *et al.*, 2014). SOM losses persisted for some time after fire, likely due to high decomposition rates, enhanced by the release of nutrients by fire, and accelerated by the soil warming resulting from the loss of vegetation cover and partially the litter.

Temporal evolution of soil biological activity

In general, the results obtained in this work are consistent with data reported in the literature regarding the fire effects on the composition, biomass and activity of microbial communities suggesting that they depend mainly on the severity of the fire (Knicker 2007). Our results in the short and mid-term (T1 and T5) coincide with those of Fontúrbel *et al*. (2016), who observed a decrease in β-D-glucosidase and acid phosphatase activities (0-2 cm) one year after a prescribed scrub burn at low intensity (58 °C at the soil surface), which remained unrecovered four years later; however, contrary to our results, the soil microbial C totally recovered one year later. Barreiro *et al*. (2010) found an initial decrease of β-D-glucosidase activity ranging from -73 to -86 % in the first 2 cm of soil after a prescribed burning. Similar to our results, these values were even lower one year after the burn than immediately after the burn, although five years later, β-D-glucosidase activity had been fully recovered, which contrasts with our results. Choromanska & DeLuca (2001) found that soil microbial C was not recovered until two years after a prescribed burning (0-10 cm); and Fritze *et al*. (1993) detected similar values in soil microbial C between 5 and 9 years after burning. The apparent recovery of microbial biomass C detected at T5 does not necessarily imply the recovery of the original microbial community structure prior to the burn, since it may have been affected by changes in soil conditions, e.g. in the availability of substrates for microorganisms (Fontúrbel *et al.*, 2012). On the other hand, our findings coincide with those generally reported regarding the greater sensitivity of phosphatase than β-D-glucosidase to fire. López-Poma & Bautista (2014) observed that β-D-glucosidase and phosphatase activities were similarly affected by a controlled burn (492 ± 32 °C, on the soil surface). However, whereas β-D-glucosidase recovered fully after 3 years in the first 5 cm of the soil, acid phosphatase did not recover during the same period. Some authors have suggested that fire intensity is related to an intense mineralizing effect of organic P and that the release of inorganic P could reduce the activity of acid phosphatase (Saá *et al*., 1993, 1998).

On the other hand, the low respiration rates detected in burned soils (T0, T1, and T5) compared to unburned soils can be attributed to the high intensity and severity of the burn, which were higher than expected for a prescribed burning (Armas-Herrera *et al.*, 2016). Many studies on this subject observed decreases in the soil respiration rates in the short term after a prescribed burning (Choromanska & DeLuca, 2001; Fritze *et al.*, 1993; Pietikaeinen & Fritze, 1995), which is consistent with our results. However, in none of these studies, the decrease was associated with a decrease in SOC; moreover, increases of SOC were detected. Bonamomi et al. (2017) provided evidence that, in burned soils, the greater the fire impact, the lower the respiration rates, which may be due to a greater accumulation of recalcitrant C and a greater loss of labile C.

Implications for the management of the pastoral ecosystem

The results of this work provide valuable conclusions regarding the sustainability of prescribed burns in mountain ecosystems. On the one hand, it is particularly important to control fire intensity so that temperatures in the top soil centimetres do not exceed the threshold of the temperature at which SOM consumption becomes significant (≈200-250 °C) (Certini, 2005). In low intensity burns, the impacts on soil are low, and recovery is fast. Thus, the impacts are often considered neutral or even positive due to an enrichment in bioavailable nutrients, the protection from erosion by partially burned litter remaining after fire, and even an enrichment in SOM by the incorporation of partially burnt material (Knicker 2007; Fernandes *et al*. 2013).

On the other hand, it is of great importance to adjust the burn frequency to guarantee the sustainability of this practice (Williams *et al.*, 2012). Burning at a frequency lower than the recovery time will not have long-term impact on soils, whereas burning at a shorter frequency will eventually result in cumulative effects, until a new stable state is reached. In any case, low recovery rates limit the optimum frequency of burning. According to our results, the recovery of soil conditions to that prior to a burn appears to be still far at current time, five years after the prescribed burn.

Much likely, prescribed burning at a sustainable frequency will not be able to prevent the *E. horridum* shrub from dominating the area. Furthermore, the invasive *E. horridum* has a great capacity for regrowth after a fire, which results from massive germination of its seeds and allows this plant to regenerate quickly and recolonize the burned areas (Marinas *et al*. 2004). For this reason, it is not possible to rely solely on regular burns, and it is essential to maintain a sufficient livestock density that limits the regeneration of the shrubland (Fuhlendorf *et al.*, 2009).

Future research should examine the long-term consequences of prescribed burns in the soils of the Central Pyrenees, especially in regards to its effects on SOM cycling and soil biological activity, soil loss by erosion, the interaction of prescribed burns and livestock density, and the monitoring of the evolution of vegetation cover. This knowledge will help in adopting sustainable management practices that consider both the suitable frequency of application of prescribed burns that allows the functional recovery of the soil, and the type and degree of grazing that reduces shrub encroachment in mountain pastures, guaranteeing the conservation of these ecosystems.

CONCLUSIONS

All soil properties studied were modified by fire, with variations in the extent of the effects, the soil depths affected, and the recovery and resilience in the short (one year) and medium term (five years). Fire seems to have a significant impact on the dynamics and turnover of organic matter in the first 3 cm of the soil, which remained even after five years. The magnitude of this impact is consistent with the high temperatures recorded in soil during the prescribed burn. The loss of vegetation cover and partially the litter probably increased the soil temperature, thereby stimulating the mineralization of SOM at short and mid-term, while the release of nutrients sequestered in the SOM as a result of the fire could also stimulate its decomposition. Only the content of microbial C seems to have recovered in the medium term to its initial values prior to the burn. Our results contrasted with those obtained in most studies carried out on soils after prescribed burning, where the effects on soil were of a smaller magnitude, and the recovery to values prior to the burn was relatively fast.

Given this information, it is necessary to continue the study of the evolution of SOM content and soil biological activity in the mid- and long term, as well as to complement it with measures of the magnitude of soil loss, while monitoring the evolution of vegetation cover and the consumption of *E. horridum* by livestock; this will allow an assessment of the appropriateness of this practice and an adjustment to the frequency and intensity of burns to minimize their impacts on the soil.

ACKNOWLEDGEMENTS

We are grateful to Rafael de Partearroyo and Raúl Vicente (EPRIF, Huesca) for allowing us to participate in the prescribed burning program, and to Janielle S. Pereira and Daniel Gómez García for their help during the field work. This study was supported by the Spanish Government Project CGL2013-43440-R and a grant from the Instituto de Estudios Altoaragoneses (Provincial Council of Huesca). It is a contribution by the PaleoQ group (Aragón Regional Government).

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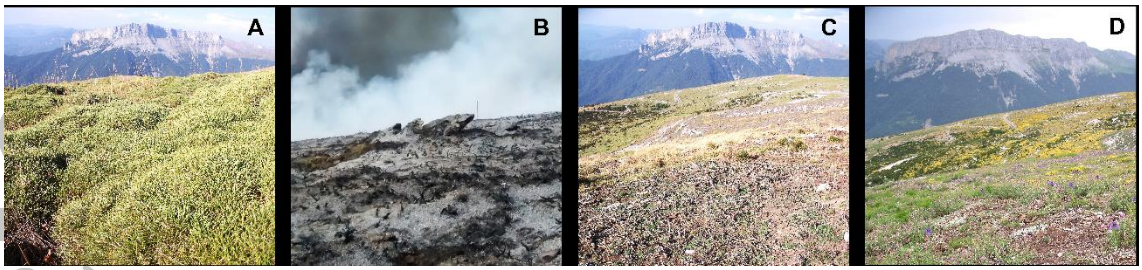
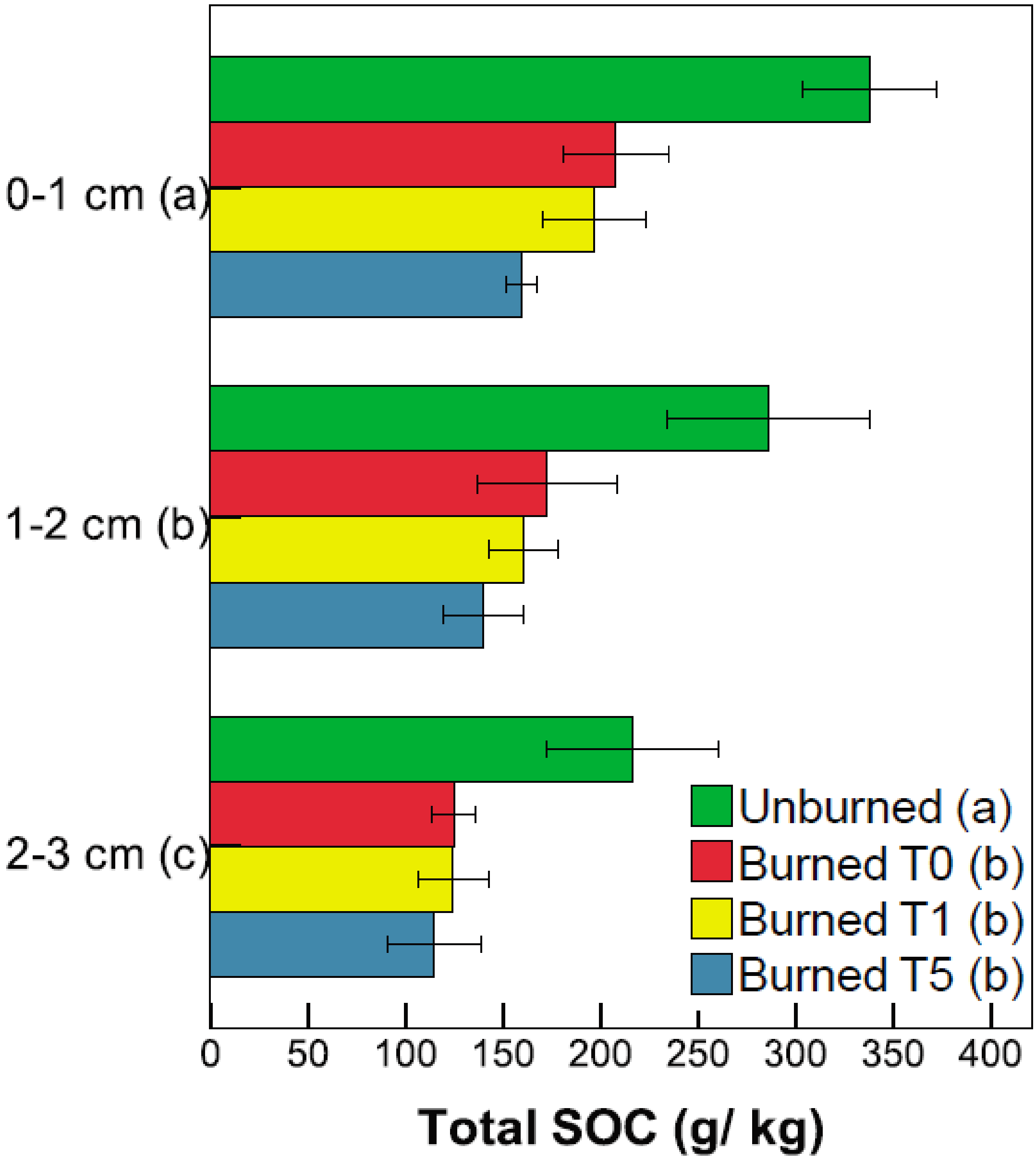
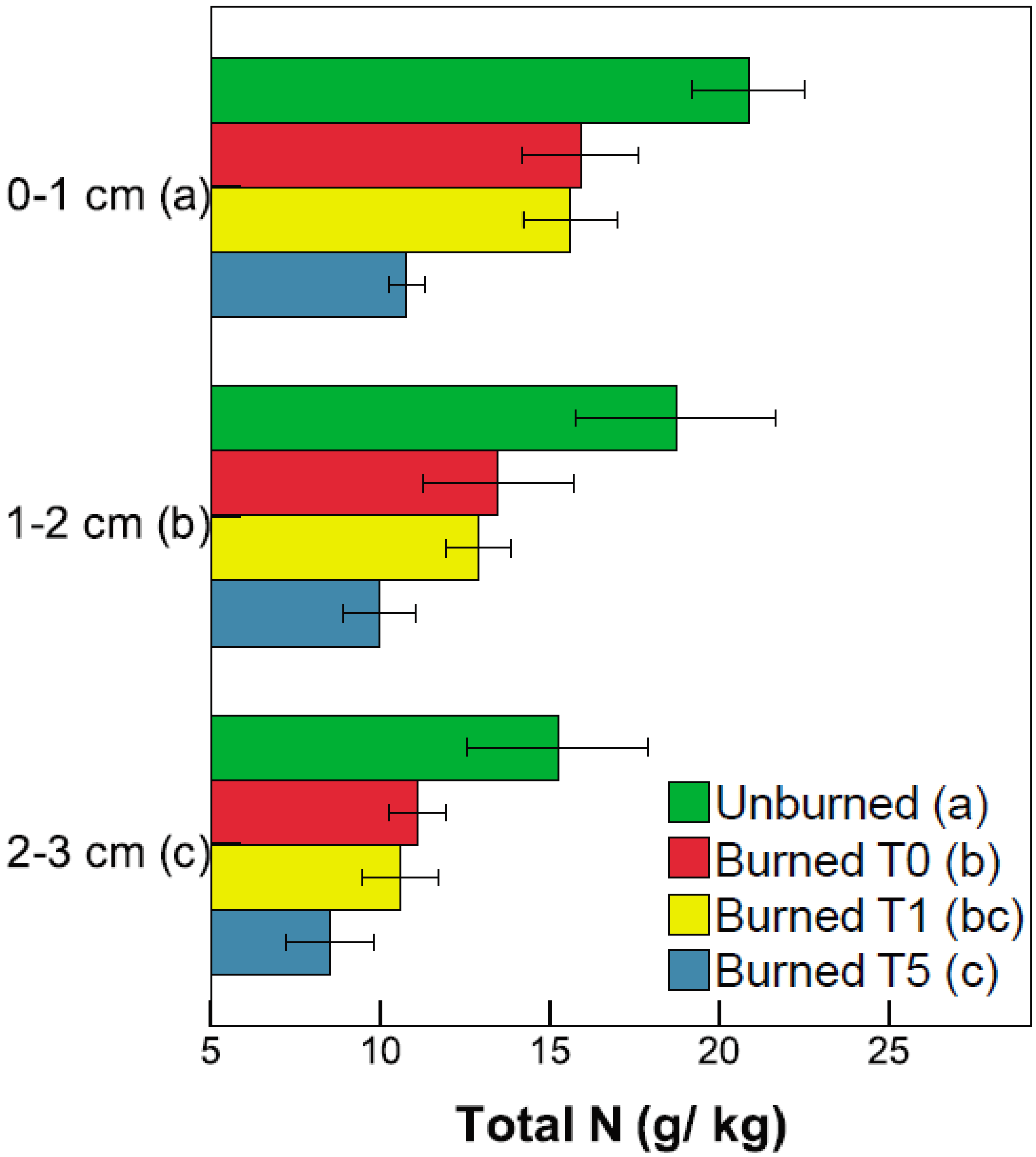


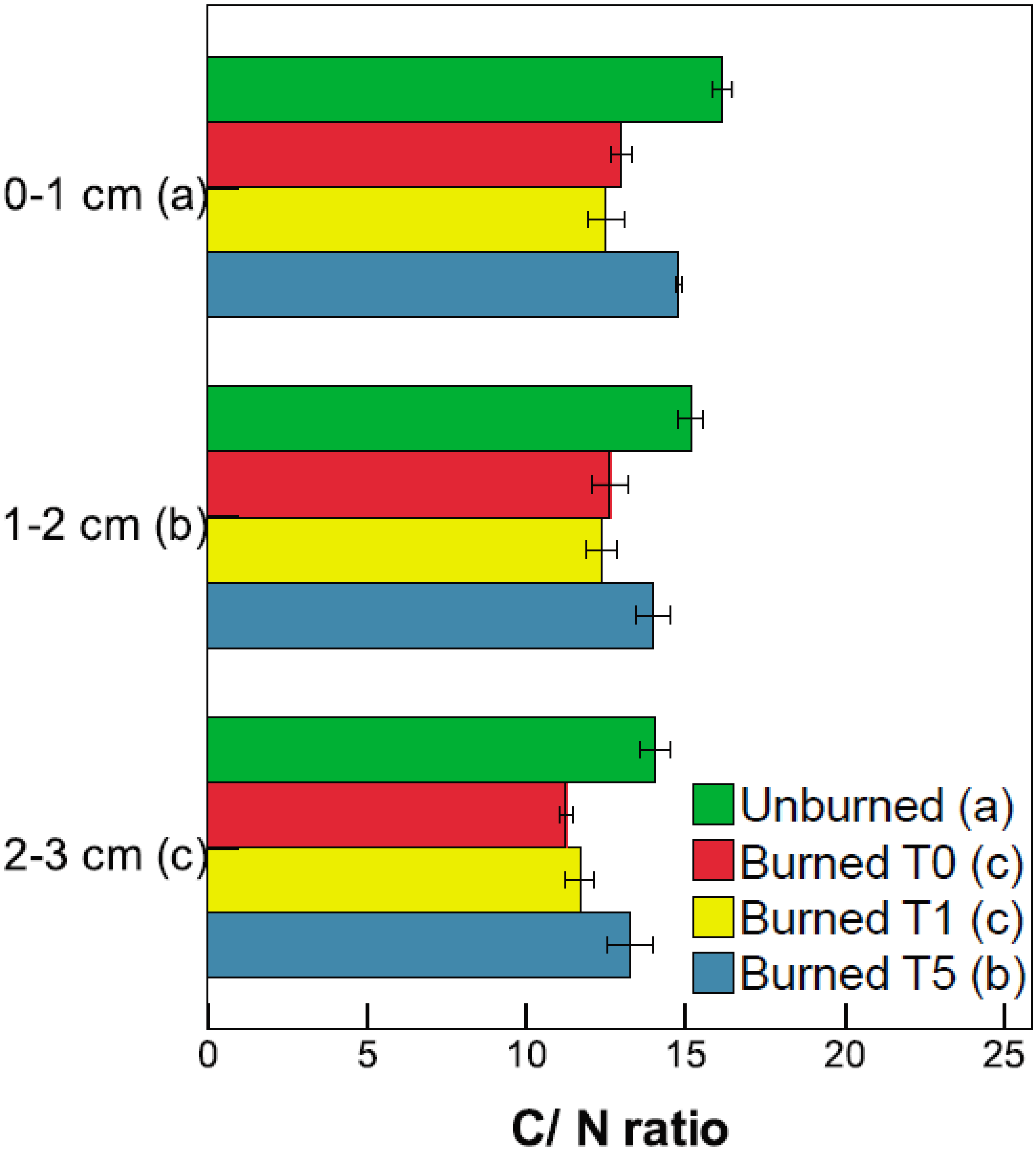
Fig. 1. Views of the study area: (A) experimental plot encroached by *Echinospartum horridum* before the prescribed burning in 2015, (B) the same plot immediately after burning in 2015 (T0) and (C) one year later (T1) and (D) an experimental plot five years after the prescribed burning in 2011 (T5)


0-1 cm (a)
1-2 cm (b)
2-3 cm (c)
Unburned (a)
Burned T0 (b)
Burned T1 (b)
Burned T5 (b)
0
50
100
150
200
250
300
350
400
Total SOC (g/ kg)

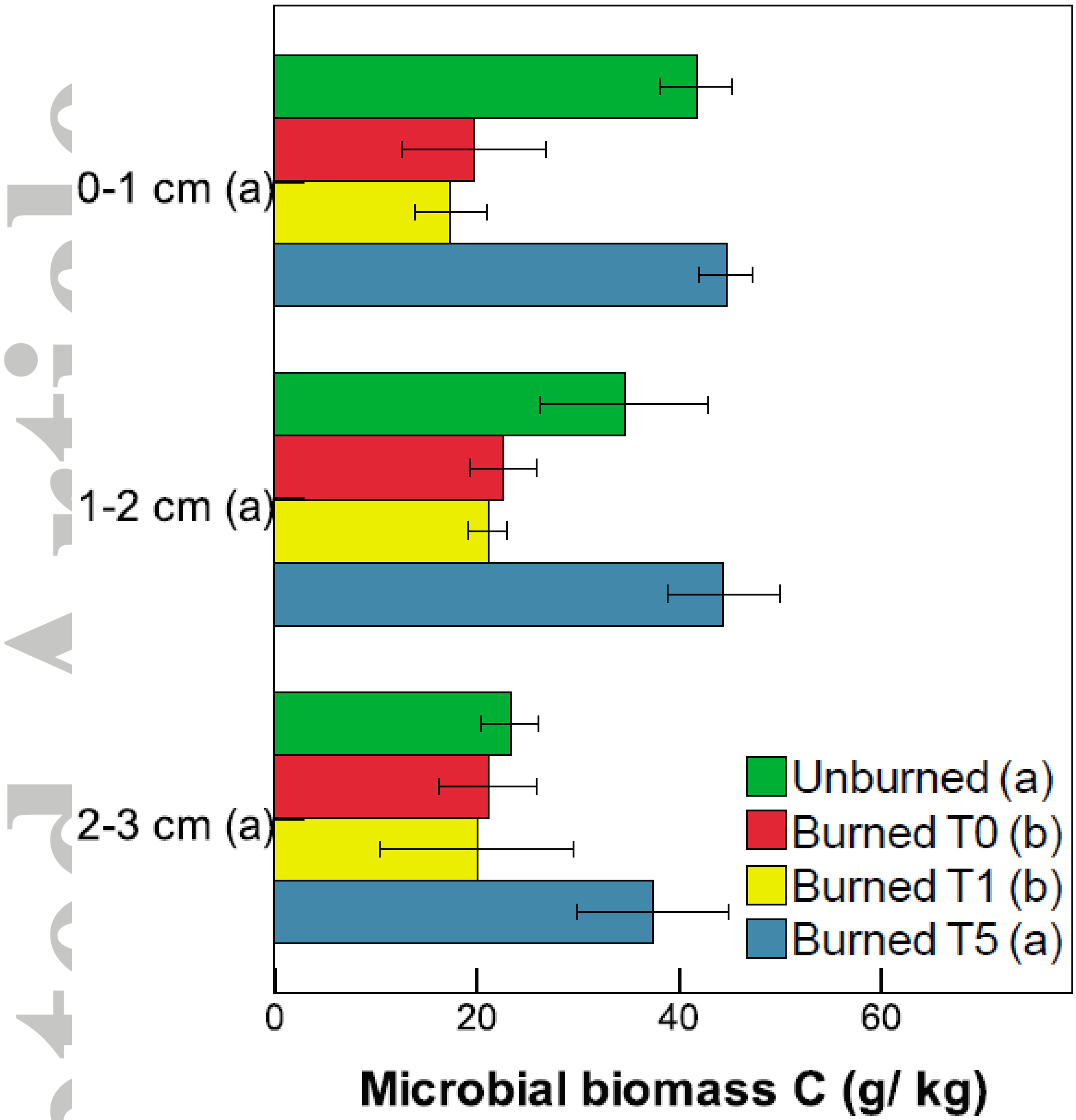
a


0-1 cm (a)
1-2 cm (b)
2-3 cm (c)
Unburned (a)
Burned T0 (b)
Burned T1 (bc)
Burned T5 (c)
5
10
15
20
25
Total N (g/ kg)

b

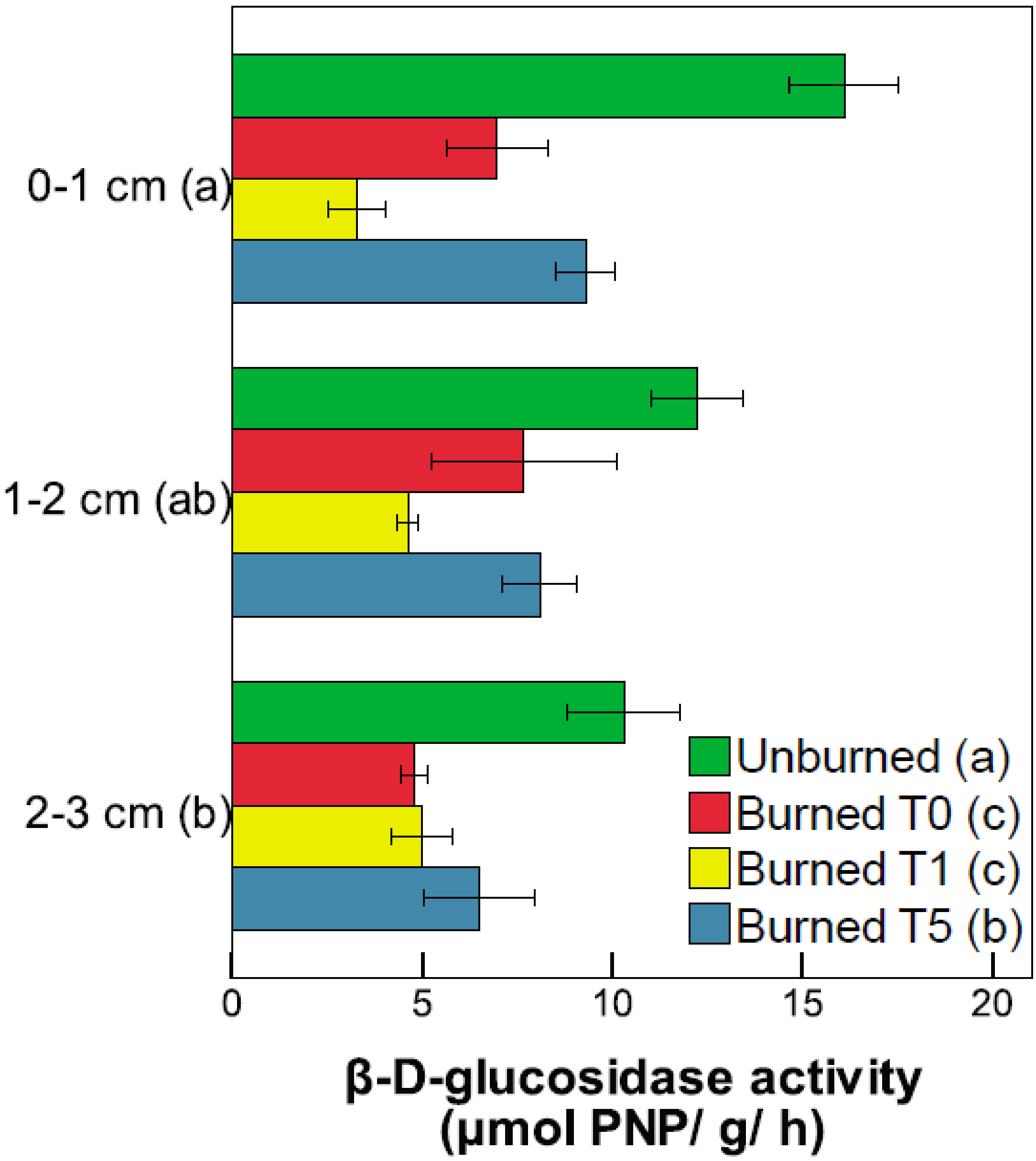

0-1 cm (a)
1-2 cm (b)
2-3 cm (c)
Unburned (a)
Burned T0 (c)
Burned T1 (c)
Burned T5 (b)
0
5
10
15
20
25
C/ N ratio

c

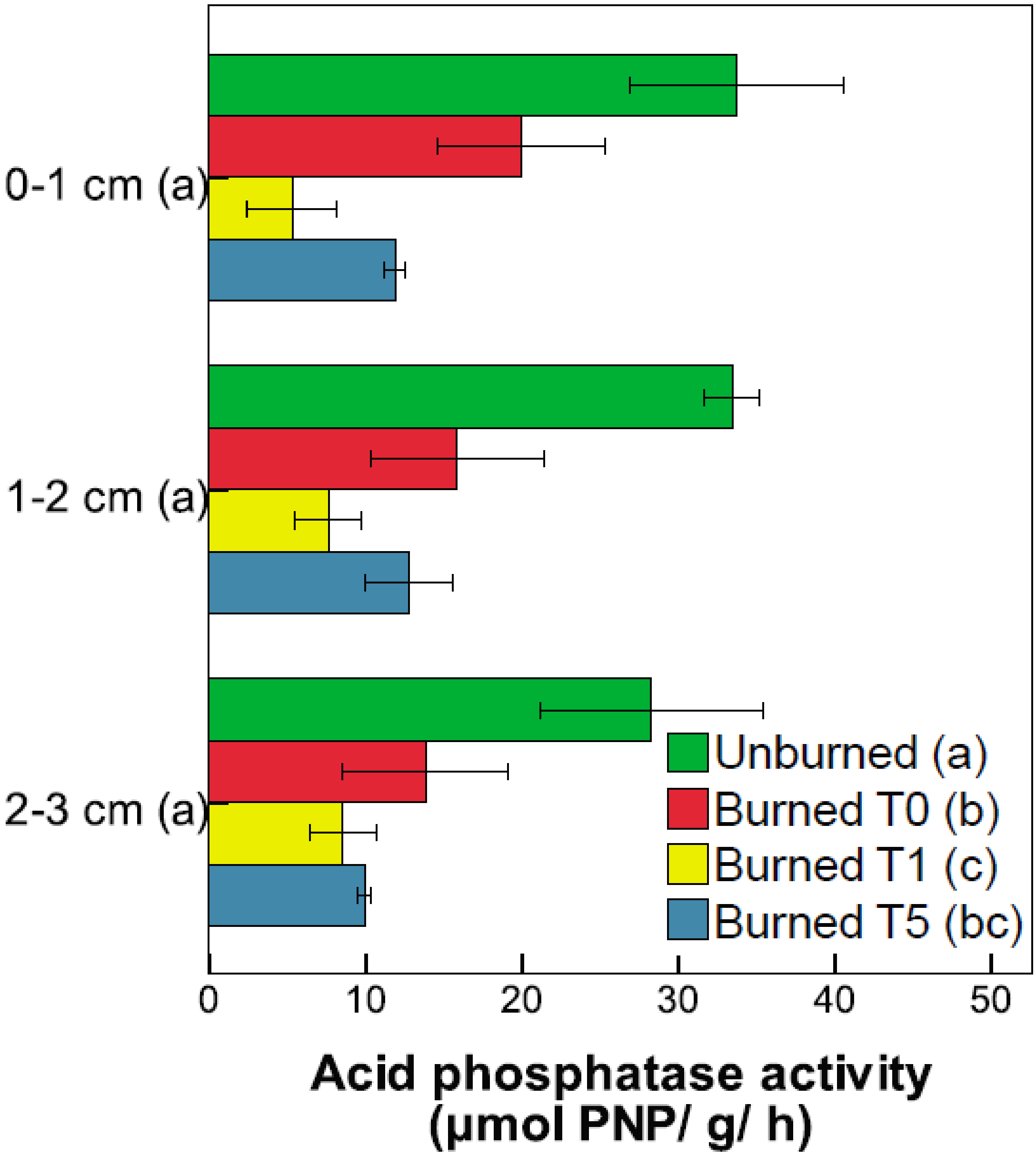


d

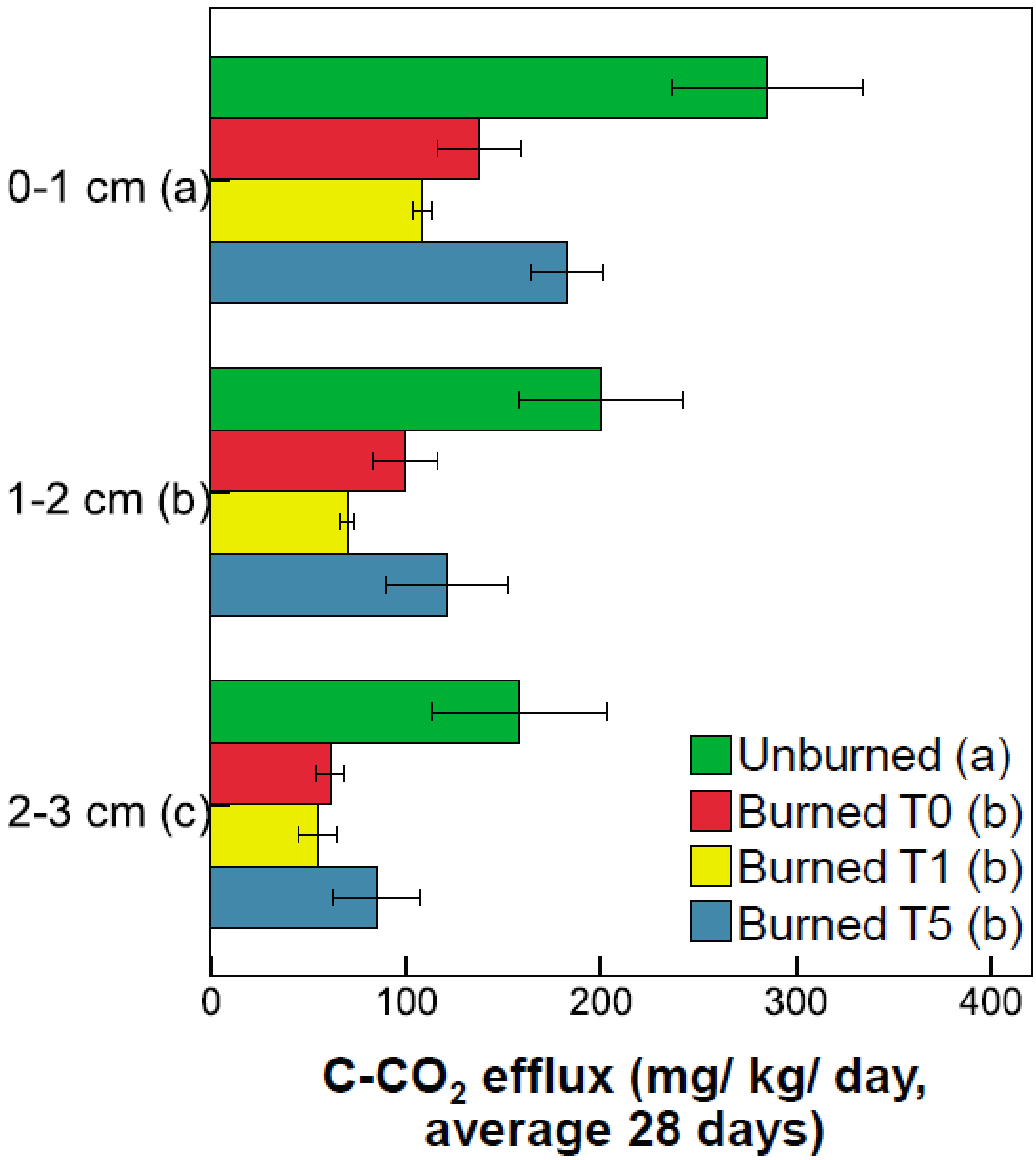
Fig. 2. Temporal evolution of soil organic matter-related properties in the first three centimetres of the soil (mean ± SEM, N=3). (A) SOC, (B) N, (C) C/ N ratio, (D) microbial biomass C. Significant differences (*P* <0.05) between the depths and sampling times are represented by different letters in the axes and legend labels. T0, T1 and T5 are 0, one and five years after burning, respectively.


0-1 cm (a)
1-2 cm (ab)
2-3 cm (b)
Unburned (a)
Burned T0 (c)
Burned T1 (c)
Burned T5 (b)
0
5
10
15
20
β-D-glucosidase activity
(μmol PNP/ g/ h)

a


0-1 cm (a)
1-2 cm (a)
2-3 cm (a)
Unburned (a)
Burned T0 (b)
Burned T1 (c)
Burned T5 (bc)
0
10
20
30
40
50
Acid phosphatase activity
(µmol PNP/ g/ h)

b



c

Fig. 3. Temporal evolution of soil biological properties in the first three centimetres of the soil (mean ± SEM, N=3). (A) β-D-glucosidase activity, (B) acid phosphatase activity, (C) C-CO_2 efflux. Significant differences ($P < 0.05$) between the depths and sampling times are represented by different letters in the y-axis and legend labels. T0, T1 and T5 are 0, one and five years after burning, respectively.

Table 1. Ground cover (%) at the experimental areas burned one (T1) and five (T5) years before. Standard deviation in parentheses; N= 6 (T1), N= 5 (T5)

	T1	T5
Bare soil	42 (4)	5 (7)
Rock and stones	11 (7)	21 (11)
Burned plant residues	24 (8)	18 (6)
Herbaceous plants	14 (9)	43 (6)
Organic debris	9 (3)	13 (6)

Table 2. Repeated measures ANOVA of the investigated soil properties in relation to the time elapsed since the burn, the soil depth and the interaction Time x Depth

		Within subjects			Between subjects		
		Depth	Depth x Time	Error (Depth)	Intercept	Time	Error
df		2	6	16	1	3	8
Total SOC	MS	0.62	0.00	0.01	956.06	0.83	0.17
	F	44.85	0.19		5533.73	4.78	
	P	< **0.001**	0.976		**< 0.001**	**0.034**	
Total N	MS	0.34	0.00	0.01	236.77	0.57	0.11
	F	41.60	0.37		2188.12	5.31	
	P	< **0.001**	0.885		**< 0.001**	**0.026**	
C/ N ratio	MS	7.41	0.28	0.20	6466.57	18.23	1.49
	F	36.46	1.39		4346.01	12.25	
	P	< **0.001**	0.276		**< 0.001**	**0.002**	
Microbial biomass	MS	1.06	0.57	0.73	987.18	9.26	1.45
	F	1.45	0.78		680.79	6.39	
	P	0.264	0.599		**< 0.001**	**0.016**	
β-D-glucosidase	MS	0.14	0.16	0.04	135.62	1.98	0.22
	F	3.98	4.39		615.99	8.98	
	P	**0.040**	**0.008**		**< 0.001**	**0.006**	
Acid phosphatase	MS	0.23	0.37	0.44	539.40	14.69	1.34
	F	0.51	0.83		401.46	10.93	
	P	0.609	0.561		**< 0.001**	**0.003**	
C-CO_2 efflux	MS	1.67	0.01	0.02	800.96	1.64	0.27
	F	70.37	0.43		2972.57	6.10	
	P	< **0.001**	0.849		**< 0.001**	**0.018**	

MS: mean square; df: degrees of freedom

Table 3. Resistance, recovery and resilience after the prescribed burning. T1 and T5 are one and five years after burning, respectively.

	Resistance (%)	Recovery T1 (%)	Recovery T5 (%)	Resilience (%)
Total soil organic C (g kg^{-1})				
0-1 cm	-38.5	-41.8	-52.8	-14.3
1-2 cm	-39.8	-44.0	-51.1	-11.4
2-3 cm	-42.2	-42.4	-46.9	-4.7
average	*-40.2*	*-42.7*	*-50.3*	*-10.1*
Total soil N (g kg^{-1})				
0-1 cm	-23.7	-25.2	-48.3	-24.7
1-2 cm	-28.1	-31.1	-46.9	-18.8
2-3 cm	-27.3	-30.6	-44.1	-16.9
average	*-26.4*	*-29.0*	*-46.4*	*-20.1*
Microbial biomass C (g kg^{-1})				
0-1 cm	-52.7	-58.3	7.1	59.8
1-2 cm	-34.7	-39.1	28.4	63.1
2-3 cm	-9.6	-14.2	60.5	70.0
average	*-32.3*	*-37.2*	*32.0*	*64.3*
β-D-glucosidase (µmol PNF g^{-1} h^{-1})				
0-1 cm	-56.7	-79.8	-46.9	9.8
1-2 cm	-37.4	-62.3	-41.9	-4.4
2-3 cm	-53.6	-51.5	-50.0	3.7
average	*-49.2*	*-64.5*	*-46.3*	*3.03*
Acid phosphatase (µmol PNF g^{-1} h^{-1})				
0-1 cm	-40.9	-84.2	-64.8	-23.9
1-2 cm	-52.5	-77.2	-61.8	-9.3
2-3 cm	-51.2	-69.7	-64.9	-13.7
average	*-48.2*	*-77.0*	*-63.8*	*-15.6*
C-CO_2 efflux (mg kg^{-1} día^{-1}, 28 days)				
0-1 cm	-51.7	-62.0	-37.1	14.5
1-2 cm	-50.4	-65.2	-51.9	-1.5
2-3 cm	-61.5	-65.5	-59.7	1.8
average	*-54.5*	*-64.2*	*-49.6*	*4.93*