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The Effects of Fire Intensity on the Biochemical Properties of a Soil Under Scrub in the Pyrenean Subalpine Stage

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Abstract: Fire causes changes in many soil attributes, depending on multiple factors which are difficult to control in the field, such as maximum temperature, heat residence time, charred material incorporation, etc. The objective of this study is to evaluate the effect of a gradient of fire intensities on soils at the cm scale. Undisturbed topsoil monoliths were sampled under scrubs in the subalpine stage in the Southern Pyrenees (NE Spain). They were burned, under controlled conditions in a combustion tunnel, to obtain four charring intensities (CIs), combining two temperatures (50 and 80 °C) and two residence times (12 and 24 min) reached at 1 cm depth from the soil. Unburned soil samples were used as a control. All soils were sampled, cm by cm, up to 3 cm deep. The following soil properties were measured: soil respiration (basal, bSR and normalized, nSR), β-D-glucosidase (GLU), microbial biomass carbon (MBC), glomalin-related soil proteins (GRSPs), soil organic carbon (SOC), labile carbon (DOC), recalcitrant organic carbon (ROC), total nitrogen (TN), soil pH, electrical conductivity (EC) and soil water repellency (SWR). Even at low intensities, GLU, SOC and total GRSP were significantly reduced and, conversely, SWR was enhanced. At the higher CIs, additional soil properties were significantly reduced (MBC and C/N) or increased (DOC, ROC, nSR, easily extractable GRSP). This study demonstrates that there is a differential degree of thermal sensitivity in the measured biochemical soil properties. Furthermore, these properties are more affected at 0–1 cm than at 1–2 and 2–3 cm soil thicknesses.

Keywords: experimental burning; fire intensity; low-severity fires; soil organic matter; soil biological properties; glomalin

Citation: Alfaro-Leranoz, A.; Badía-Villas, D.; Martí-Dalmau, C.; Escuer-Arregui, M.; Quintana-Esteras, S. The Effects of Fire Intensity on the Biochemical Properties of a Soil Under Scrub in the Pyrenean Subalpine Stage. *Fire* **2024**, *7*, 452. https://doi.org/10.3390/fire7120452

Academic Editor: Natasha Ribeiro

Received: 31 October 2024
Revised: 24 November 2024
Accepted: 27 November 2024
Published: 1 December 2024

1. Introduction

Wildfires have caused land degradation across the world, particularly affecting soil properties [1] with a wide range of effects [2]. Although fire has been traditionally used to manage landscapes in forestry and agricultural environments [3], lately, it has been replaced by technical fires performed by professionals. These fires, called prescribed burnings (PBs), are performed under certain edaphic and atmospheric conditions to reduce damage to the soil. However, depending on the temperature and its residence time, some soil properties, particularly the most sensitive ones, like biochemical ones, can be negatively affected by them, especially on the topsoil [2,4,5]. In addition to these direct effects, fire may change the local environment, modifying the soil cover and soil hydrology, and cause indirect effects on those sensitive soil properties [6]. An understanding of the impact of different fire types on soil microbial communities has not yet been mastered, even after the publication of several studies on this topic [6]. It is, therefore, necessary to further study this topic. Also, fires generally do not burn homogeneously and they create complex mosaics of a wide range of burn severities [7]. In general, the variability of fire's effects on soils is high [8]. This heterogeneity in the results can be attributed to several factors that operate simultaneously, like soil atmospheric conditions, post-fire sampling interval, sampled depth, type of ecosystem, spatial variability in fuel and soil type [9,10]. Also, the variability increases when the unburned samples are collected in a different place close to the burned area, assuming that the soils are similar [11].

To avoid dissimilar results in the research of post-fire effects on soils, different approaches have been used to reduce the environmental-related variability and to focus on the thermal effects, such as undisturbed soil monolith burnings. Badía et al. [12] and Pereira et al. [12] studied the effects of undisturbed soil monolith burning under high temperatures (250 °C, at 1 cm) on the physical, chemical and biological properties of mollic horizons within an Aleppo pine forest in the Ebro Basin. They found decreases in all the studied parameters at the O horizon and in the top few cm of the mineral soil. In addition, Badía et al. [13] also studied the effects of high-intensity fire on similar soils, under different moisture conditions, concluding that fire's effects on moist soils are considerably lower compared to dry soils. In gypseous soils in Mediterranean areas, Aznar et al. [14] studied the effects of high-intensity fire (250 °C at 1 cm) via monolith experimental burnings on soil's physical and chemical properties. As in the previously mentioned studies, they found significant decreases in the physical properties and the organic matter content in the O horizon and the upper centimeter of the Ah horizon. Also, in the Mediterranean area, Lucas-Borja et al. [6] performed a controlled burning of calcareous soil monoliths under an Aleppo pine forest, including both living vegetation and dead fuel. Although they did not detect negative changes in any of the chemical properties studied, they found some changes in the composition of the biological community, particularly at the highest intensity.

Furthermore, some scientists select other approaches to reduce factor variability, like field experimental burnings and oven-heating experiments. Hrenović et al. [15] performed an experimental burning in a pasture abandoned for 60 years in the Eastern Mediterranean region. The burning resulted in a moderate-to-high-severity fire and produced decreases in the biological parameters, organic matter and aggregate stability due to the thermal shock, while it produced increases in the water repellency, pH and electrical conductivity after the incorporation of ashes. On the other hand, Badía and Martí [16] oven-heated two different soils (gypseous and calcareous) at temperatures ranging from 25 to 500 ºC and they found that the magnitude of those changed differed in each soil type. Both soils were similarly affected by the different temperatures; in the extreme case of heating to 500 degrees, the properties of both soils, which were very different before burning, degraded in such a way that they converged.

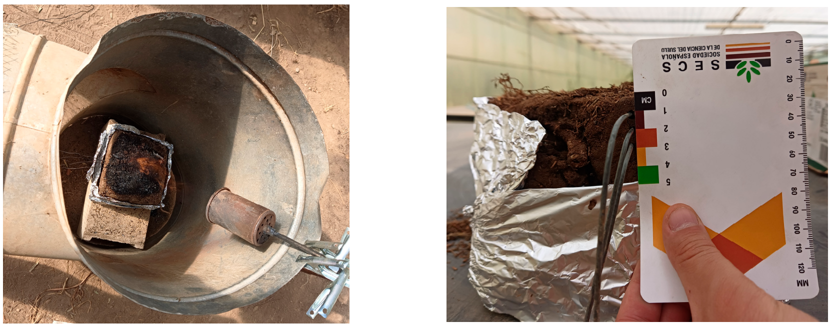
Most of the previously mentioned studies were performed with soils from the Mediterranean region and applying high temperatures and intensities to the soils. Under those conditions (low soil moisture and high burning intensity), the effects on most soil properties were high. The present study aims to fill a knowledge gap, evaluating the thermal-induced effects of different low-to-medium-intensity fires on topsoil properties, simulating those carried out with prescribed bush burning in the subalpine stage in NE Spain. Concretely, the objectives of this work are (a) to evaluate the soil depth that can be affected under different fire intensities; (b) to study the effects of fire intensity on soil organic matter, examining its different fractions; and (c) to analyze the fire intensity effects on soil biological properties. We hypothesize that, under low-to-moderate-intensity burnings applied directly to the mineral soil, only the most sensitive properties, like the biochemical properties, will be affected by the fire, especially close to the soil surface. Additionally, with the removal of plant and litter remains, it will be possible to separate the strictly thermal effects from the effects generated by the incorporation of ashes.

2. Materials and Methods

2.1. Sampling and Experimental Burning

The sampling area is located within the coordinates 42°31′19″ N, 0°6′3″ W, at around 1800 m.a.s.l. in the municipality of Asín de Broto, Central Pyrenees, Spain. Soils in that area, classified as Eutric Endoleptic Cambisols (Loamic, Humic), are slightly acidic, loamy textured and rich in organic matter and biological activity [17]. Fifteen 15 × 15 × 10 cm soil monoliths were sampled from a 120 × 30 cm plot, to avoid effects related to soil spatial variability. Prior to the monolith extraction, both vegetation and litter were cleaned from the soil surface. The monoliths, sampled in early spring, were rapidly transported to the laboratory and were stored at 4 °C.

After the field sampling, the monoliths were burned in controlled conditions, using a blowtorch placed 40 cm from the soil surface (Figure 1a). Three monoliths were selected for each treatment to reflect the heterogeneity of the controlled burns. During each experimental fire, soil temperatures were recorded via Type-K thermocouples placed at the soil surface and at depths of 1, 2 and 3 cm (Figure 1b).



(**a**) (**b**)

Figure 1. (**a**) Experimental burning setup; (**b**) thermocouples' arrangement at the different soil depths: 1, 2 and 3 cm.

Four fire intensities were generated, combining the temperature (T) and the residence time (RT) of that temperature at 1 cm depth. RT was defined as the period (min) during which the target temperature was maintained. To avoid the influence of the ash-induced changes observed in previous studies performed in field conditions [18–21], we removed the aboveground vegetation and the litter and we applied the fire directly to the mineral Ah horizon (Figure 1a). Additionally, three monoliths were kept unburned to have a reference (control) of the soil properties under unburned conditions, leaving a total of five different treatments (Table 1).

Table 1. Temperatures and residence times (RT) used in each treatment within the experiment.

Treatment	Label	T at 1 cm (°C)	RT at 1 cm (min)
Low temperature, short time	LS	50	12
Low temperature, long time	LL	50	24
High temperature, short time	HS	80	12
High temperature, long time	HL	80	24
Unburned	UB	-	-

The thermic characteristics of each type of burn can be seen in Table 2. The charred intensity (CI), which represents the temperature and the duration of the fire [13,22], was used to differentiate the treatments. The temperature threshold for detecting effects on soil biochemical properties [2], the CI for 50 °C, was calculated as the area between the temperature curve and 50 °C (Equation (1)).

$$\mathrm{CI} = \left(\sum_{i=N}^{M} \mathrm{Ti}\right) \times \Delta t \quad (1)$$

where N is the time level at which the temperature first reached 50 °C and M is the time level at which the temperature dropped back to 50 °C.

Table 2. Recorded temperatures, residence times and charred intensity (CI) during the experimental burnings for each treatment (LS, LL, HS, HL) on the soil surface and at different soil depths (cm).

		Residence Time (min)			
Fire Treatment		**Soil Surface**	**1 cm**	**2 cm**	**3 cm**
LS	<50 °C	13.9 ± 4.7	27.3 ± 3.5	40.1 ± 3.3	40.1 ± 3.3
	50–80 °C	5.9 ± 1.1	12.8 ± 0.2	0 ± 0	0 ± 0
	80–100 °C	3.2 ± 1.3	0 ± 0	0 ± 0	0 ± 0
	100–200 °C	6.4 ± 1.7	0 ± 0	0 ± 0	0 ± 0
	200–300 °C	2.8 ± 1.2	0 ± 0	0 ± 0	0 ± 0
	300–400 °C	2 ± 1.1	0 ± 0	0 ± 0	0 ± 0
	400–500 °C	1.5 ± 1	0 ± 0	0 ± 0	0 ± 0
	>500 °C	4.4 ± 2	0 ± 0	0 ± 0	0 ± 0
	Max T (°C)	687.3 ± 101.6	57.7 ± 3.9	37.2 ± 3.9	30.2 ± 7.4
	CI (°C min)	6387.9 ± 1418.6	684.9 ± 26.1	0 ± 0	0 ± 0
LL	<50 °C	4.6 ± 4.7	18.4 ± 1.6	40 ± 2.3	41.9 ± 0.1
	50–80 °C	4.8 ± 3.7	23.5 ± 1.6	2 ± 2.4	0.1 ± 0.1
	80–100 °C	3.5 ± 2.2	0.2 ± 0.3	0 ± 0	0 ± 0
	100–200 °C	14 ± 1.2	0 ± 0	0 ± 0.1	0 ± 0
	200–300 °C	5.9 ± 3.3	0 ± 0	0 ± 0	0 ± 0
	300–400 °C	2.2 ± 1.2	0 ± 0	0 ± 0	0 ± 0
	400–500 °C	1.8 ± 0.6	0 ± 0	0 ± 0	0 ± 0
	>500 °C	5.3 ± 3.8	0 ± 0	0 ± 0	0 ± 0
	Max T (°C)	755.8 ± 68.2	68.5 ± 23.5	81.3 ± 60.8	54.6 ± 28.3
	CI (°C min)	9296.1 ± 2251.8	1363.1 ± 264.0	106.6 ± 118.0	5.2 ± 9.0
HS	<50 °C	6.2 ± 6.1	11.3 ± 6.5	25.2 ± 13.1	33.3 ± 8.9
	50–80 °C	4.9 ± 6.2	18 ± 3.6	16.8 ± 13.1	8.7 ± 8.9
	80–100 °C	1.1 ± 1.5	12.7 ± 4.9	0 ± 0	0 ± 0
	100–200 °C	3.7 ± 1.7	0 ± 0	0 ± 0	0 ± 0
	200–300 °C	3.4 ± 1.2	0 ± 0	0 ± 0	0 ± 0
	300–400 °C	2.9 ± 0.6	0 ± 0	0 ± 0	0 ± 0
	400–500 °C	3.7 ± 1.5	0 ± 0	0 ± 0	0 ± 0
	>500 °C	16.1 ± 7.5	0 ± 0	0 ± 0	0 ± 0
	Max T (°C)	898.2 ± 100.7	85.7 ± 0.6	63.8 ± 11.6	55.7 ± 7.6
	CI (°C min)	17,780.4 ± 6346.1	3624.1 ± 1274.5	2283.8 ± 2000.0	1612.5 ± 1397.5
HL	<50 °C	2.4 ± 2.2	13.7 ± 1.3	21.6 ± 9.3	30.7 ± 8.6
	50–80 °C	0.2 ± 0.3	19.1 ± 6.8	14.9 ± 1.3	11.3 ± 8.6
	80–100 °C	0.1 ± 0.1	9.2 ± 5.4	5.6 ± 7.9	0 ± 0
	100–200 °C	4.3 ± 0.8	0 ± 0	0 ± 0	0 ± 0
	200–300 °C	4.1 ± 2.4	0 ± 0	0 ± 0	0 ± 0
	300–400 °C	2.9 ± 0.7	0 ± 0	0 ± 0	0 ± 0
	400–500 °C	3.8 ± 3.7	0 ± 0	0 ± 0	0 ± 0
	>500 °C	24.3 ± 2.1	0 ± 0	0 ± 0	0 ± 0
	Max T (°C)	897.0 ± 68.5	85.5 ± 1.3	73.3 ± 17.1	56.1 ± 5.9
	CI (°C min)	26,056.6 ± 864.3	4615.8 ± 80.5	3474.2 ± 1049.9	2002.5 ± 457.8

LS: low temperature, short time; LL low temperature, long time; HS: high temperature, short time; HL: high temperature, long time. Values are expressed as mean ± standard deviation.

After fulfilling the temperature and the RT criteria, the monoliths were left to cool down at ambient temperature (25 °C) and then stored at 4 °C for subsequent soil analysis

2.2. Sample Preparation and Laboratory Analysis

Soil samples were taken from each monolith at three intervals from the surface to 3 cm depth, centimeter by centimeter (0–1, 1–2 and 2–3 cm), resulting in a total of 45 samples

(5 treatments × 3 replicates × 3 depths). Samples were kept at 4 °C for biological analysis of soils. Sub-samples were air dried to constant weight and sieved through a 2 mm mesh for chemical and soil water repellency analysis.

Soil moisture content (SMC) was measured gravimetrically, by oven drying the samples at 105 °C until constant weight. The SMC was expressed on a dry soil base (water–soil). The soil reaction (pH) and electric conductivity (EC) were measured in 1:2.5 and 1:5 (*w*/*v*) soil–water suspensions, respectively.

Soil organic carbon (SOC), expressed as g of SOC per kg of dry soil, was measured through chromic oxidation [23]. Recalcitrant organic carbon (ROC), expressed as a fraction of SOC (g of ROC per 100 g of SOC), was obtained from the recalcitrant organic matter (ROM) through the acid hydrolysis (HCl 6N) procedure [24]. Then, ROC was calculated from the ROM using a coefficient of 0.5 [25]. The total nitrogen (TN) content was measured using a CHN Leco 628 elemental analyzer and used to express the C/N ratio (g of SOC per g of TN). The microbial biomass carbon (MBC) was estimated using the fumigation–extraction method [26], using an extraction factor of 0.38 [27], and was expressed in g of MBC per kg of dry soil. The labile or dissolved organic carbon (DOC), expressed as a fraction of SOC (g of DOC per 100 g of SOC), was obtained from the K_2SO_4 soluble organic carbon prior to fumigation. The glomalin-related soil proteins (GRSPs) content was obtained using the [28] and [29] methods. The total GRSPs (T-GRSPs) content was extracted through 6 cycles and expressed as g of T-GRSP per kg of dry soil. Additionally, the easily extractable fraction (EE-GRSP), expressed as a fraction of the T-GRSP, was obtained.

The enzymatic β-D-glucosidase activity (GLU) was determined by the method described in [30] and expressed as μmol of liberated ρ-nitrophenol (PNP) per g of dry soil per hour. The soil microbial activity was measured as the basal soil respiration (bSR) in an incubation assay (28 days) of soil samples under optimal temperature (25 °C) and moisture (75% water-holding capacity) conditions. The released CO_2 was captured by soda traps [31], measured at intervals of 7 days during the incubation period and expressed as mg of C-CO_2 per kg of soil per day. Additionally, the bSR was normalized by the SOC (nSR or coefficient of mineralization) and expressed as mg of C-CO_2 per g of SOC per day.

The soil water repellency (SWR) was estimated by the water drop penetration time test, consisting of applying droplets of distilled water onto the soil's surface and measuring the time until it had completed infiltration [32]. The analysis was conducted in non-sieved and air-dried soil samples. The results were categorized using the classes proposed in [33].

2.3. Statistical Analysis

The values reported in the text are expressed as the mean ± standard deviation unless otherwise noted. To identify the differences in the studied soil properties related to the fire intensity treatments (UB, LS, LL, HS and HL) as well as soil depth, a two-factorial ANOVA was run to assess significant variance in soil response and the interactions among the two factors (treatment × depth). A pairwise comparison using Tukey's HSD test ($p < 0.05$) was also used to evaluate the statistical significance of the differences in the response variables. To satisfy the assumptions of the statistical tests (homogeneity of variance and normal distribution), the data were subjected to normality and homoscedasticity tests and were transformed whenever necessary, using the boxcox function ("MASS" library). In addition, an ANOVA–simultaneous component analysis (ASCA), using the "limpca" package [34], was also conducted to obtain a synthesized view of the distribution of the samples depending on the different fire and soil factors and to quantify the contribution of each measured variable to the total variation. All statistical analyses were performed using the Rstudio 4.3.0 open-source software.

3. Results and Discussion

3.1. Fire Intensity Effects on the Soil Properties

The experimental burning of soil monoliths at four different charred intensities produced a gradient of effects, particularly in the most heat-sensitive properties. Moreover, the negative effects of fire were particularly concentrated close to the soil surface (0–1 cm) for some of the affected properties. These facts highlight that the fire severity ranged from low to moderate, proportionally to the intensity.

All burn types caused a significant decrease in soil organic carbon (SOC) at the shallowest depth (0–1 cm), except for the least intense one (LS), where the decrease was non-significant (Figure 2a). This behavior could be attributed to the heat sensitivity of SOC, which starts to be consumed at around 300 °C, as reported by Santín and Doerr [2], which was by far the highest temperature reached on the soil surface of any of the soil monoliths (Table 2). Previous controlled burning experiments showed significant decreases in SOC under high fire intensities [9,12,14], matching the findings of the present study. However, unlike in the present work, Pereira et al. [9], simulating more extreme fire intensities, observed this reduction up to a depth of 3 cm. The absence of effects below 1 cm in the present study highlights the high soil thermal inertia, as the latent heat of vaporization keeps soil temperatures close to 100 °C until all of the water has completely evaporated, as previously demonstrated by Badía et al. [13], who pointed out the effect of soil moisture conditions on the effects of burning on topsoil. On the contrary, in studies where vegetation was present during low-intensity burnings, no immediate changes or even increases in SOC have been reported, due to the incorporation of partially charred organic matter from the litter and the plant covers [8,35].

The labile or dissolved carbon fraction (DOC) suffered a significant increase in HL and HS, compared to UB, for all the soil depths studied (Figure 2b). High temperatures cause SOM to break down and release simpler organic compounds. Soluble sugars and other low-molecular-weight organic compounds are among the primary products of this decomposition process, contributing to the pool of DOC. As organic matter is partially decomposed, it becomes more soluble, which leads to a rise in the concentration of DOC, including soluble sugars and other labile carbon forms [36,37]. In PBs performed in similar environments, no significant immediate decreasing tendencies were reported due to the combustion of the soil organic layer [17,38,39]. However, in an experiment that tested the effects of different burn severities in a forest in Northeastern Russia, Ludwig et al. [40] found that the post-fire concentrations of DOC increased with burn severity, as in the present study. Additionally, Dou et al. [41] found post-fire DOC increases after a PB of a pine forest in China, but those changes were not immediately significant in the topsoil layer and peaked almost a year post-fire. Both hypothesized that the fire might have caused a solubilization of SOM due to the incomplete combustion of SOM. The recalcitrant organic carbon fraction (ROC) showed the same trend, but it was only significant for HS (Figure 2c). The incomplete combustion of SOM might also be responsible for the formation of recalcitrant pyrogenic organic matter [42]. According to Salgado et al. [43], who studied the impact of fire on SOC fractions, fire can increase SOM recalcitrance. Ludwig et al. [40] did not directly measure the ROM in their controlled experiment, but they found that there was a high concentration of highly aromatic and hydrophobic carbon after the fire, which they related to an increase in ROC.

The chemical oxidation of soil organic matter induced by burning significatively altered carbon (C) and nitrogen (N) transformation processes, changing the C/N ratio by the most intense burn (HL) across all of the soil depths studied (Figure 2d).

Generally, due to its low volatilization temperature, most topsoil N content is lost to the atmosphere, even in low-to-moderate-intensity fires [44]. However, this loss is often compensated for by the release of available N forms during the combustion of SOM [35]. Moreover, in prescribed burnings and wildfires, post-fire increases in N content have been reported due to N-rich ash being incorporated into the soil. Girona-García et al. [27] explained that, when ash is not incorporated into the soil, no significant increases in N stocks can be detected after prescribed burnings. Knicker et al. [36] explains that, as in the present study, soils subjected to moderate heating do not usually experience major C/N differences compared to the unburned soils because the formation of significant amounts of recalcitrant N forms requires high temperatures. On the other hand, under more intense fires, burning increases the N content of SOM, which is mainly immobilized into recalcitrant forms [36,37]. This was the case for Armas-Herrera et al. [45], who found an immediate post-fire decrease in the C/N ratio after a high intensity prescribed burning in the subalpine stage in the Central Pyrenees. These changes in the C/N ratio are consistent with the previously discussed results for DOC and ROC.

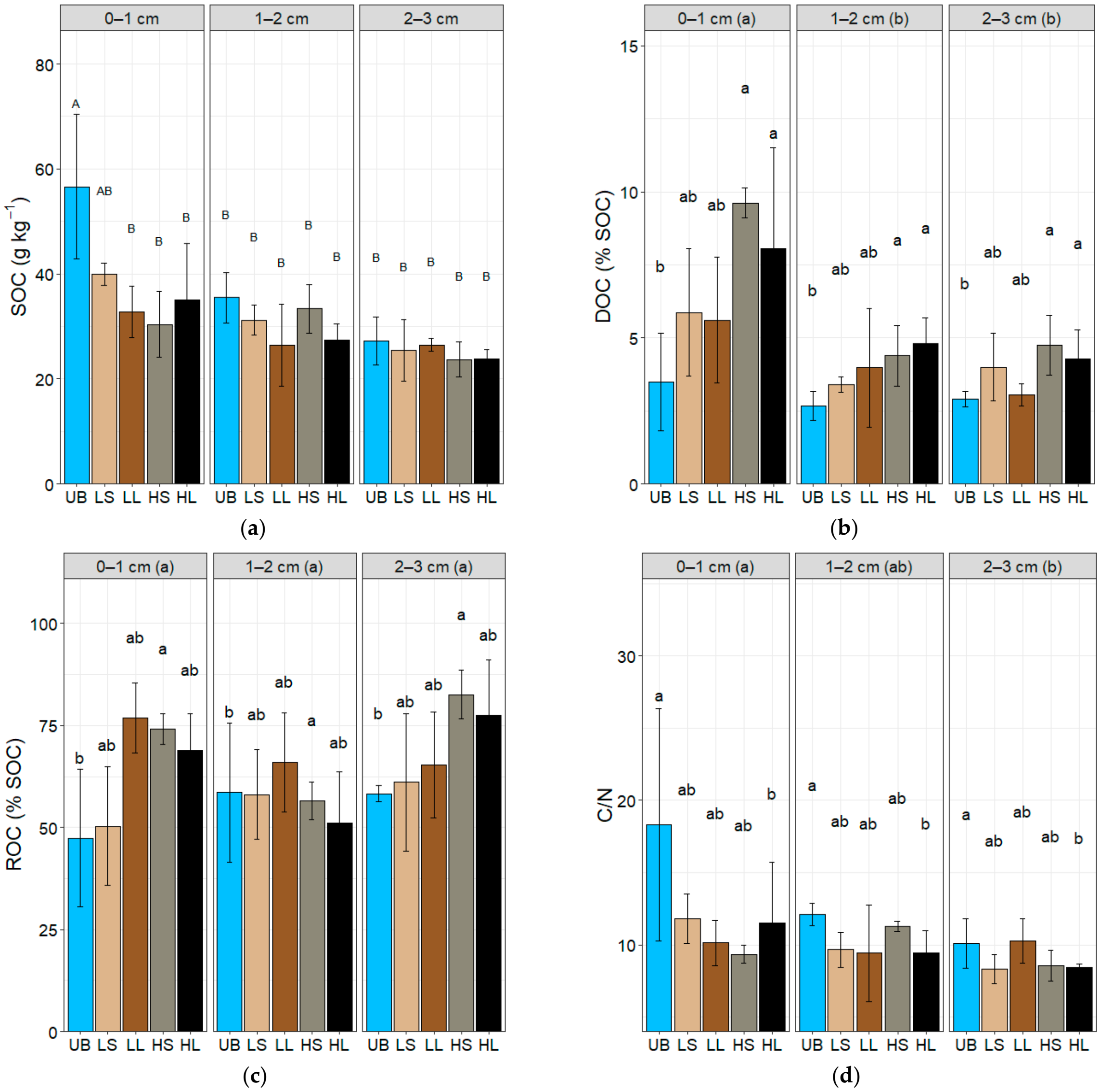


Figure 2. Fire's effects on (**a**) soil organic matter: soil organic carbon, (**b**) labile carbon, (**c**) recalcitrant organic carbon and (**d**) C/N ratio. Lowercase letters on top of the bars indicate significant differences between treatments and those between brackets between depths ($p < 0.05$). Uppercase letters on top of the bars indicate significant differences within all samples when the interaction between treatment and depth was significant. In each bar, the mean ($n = 3$) and the standard deviation are represented.

The effects on the measured biological parameters were diverse. The fire only significantly affected the microbial biomass carbon (MBC) in the most intense burn type (HL), but the effects on this parameter were quite diverse and the variability among replicates was high (Figure 3a). As Santín and Doerr [2] pointed out, fire usually affects the MBC, especially at temperatures above 80 °C. Previous work studying PBs' effects on this parameter in the Central Pyrenees showed that, depending on the temperatures reached in the soil, the effects were diverse. When the temperatures reached were high, the PB produced a partial sterilization of the soil immediately post-fire [38,39]. On the other hand, at lower temperatures, other works reported no changes in this parameter [17,27].

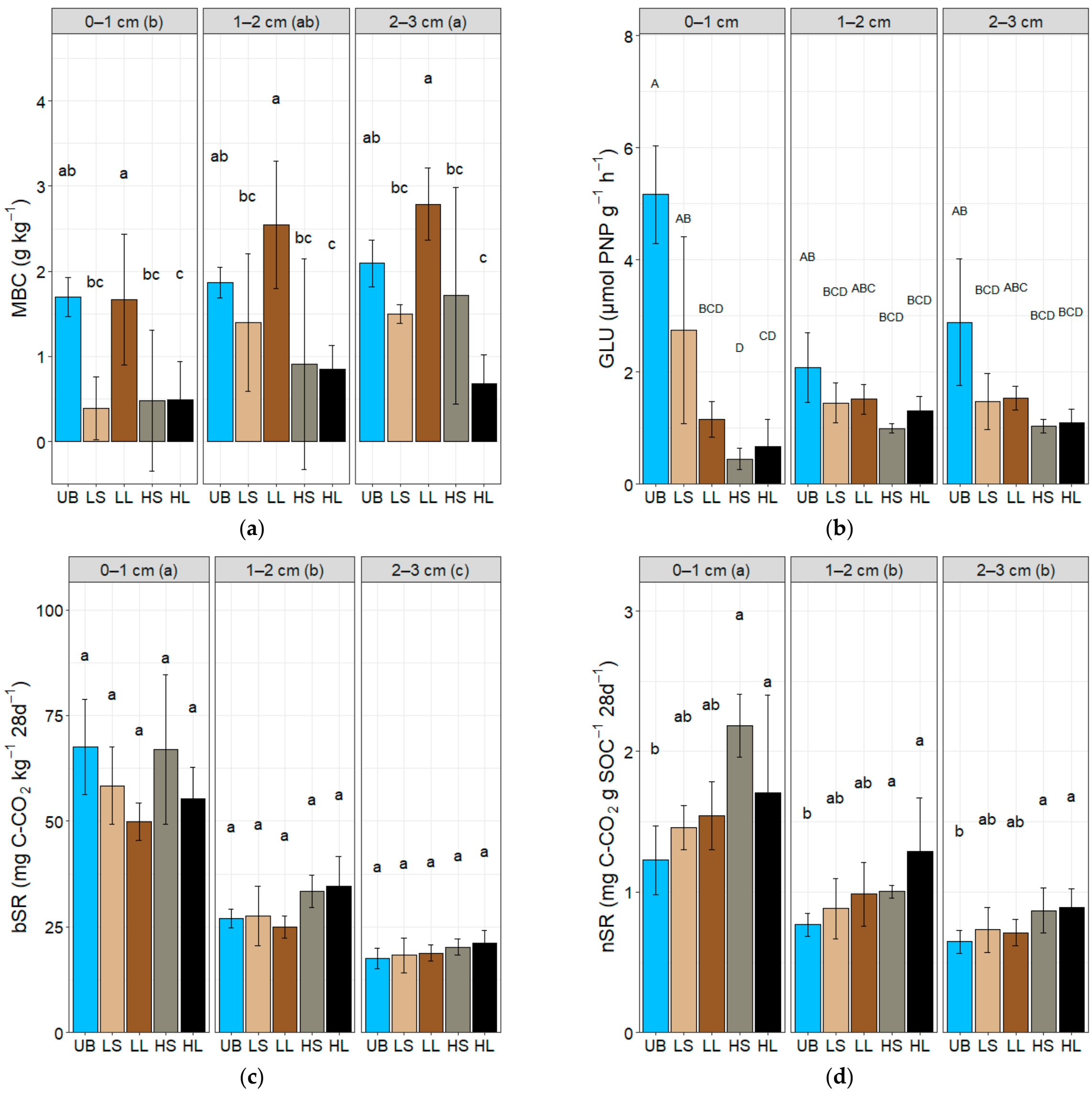


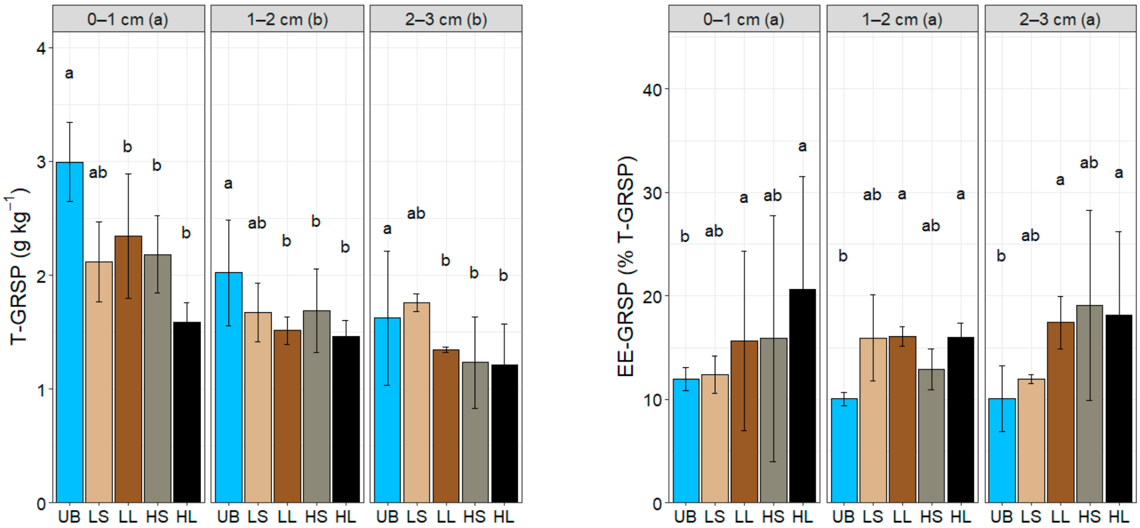
Figure 3. Fire's effects on soil biological properties: (**a**) microbial biomass carbon (MBC), (**b**) β-D-glucosidase activity (GLU), (**c**) basal soil respiration (bSR) and (**d**) normalized soil respiration (nSR). Lowercase letters on top of the bars indicate significant differences between treatments and those between brackets between depths ($p < 0.05$). Uppercase letters on top of the bars indicate significant differences within all samples when the interaction between treatment and depth was significant. In each bar, the mean ($n = 3$) and the standard deviation are represented.

On the contrary, the β-D-glucosidase activity (GLU) was significantly affected by all types of burns at the 0 to 1 cm soil depth (Figure 3b). This hydrolytic extracellular enzyme is involved in the C cycle, particularly in cellulose degradation, and it is particularly sensitive to thermal shock [46]. The effects on this parameter were especially severe in the most intense burn types (HS, HL). This effect has been found in previous studies performed in shrub PBs in the Central Pyrenees, even under very low fire intensities [17,39], as well as under higher ones [27,38], and it has generally been attributed to thermal extracellular enzyme denaturation [36]. A laboratory experiment testing different fire severities also found reductions in GLU activity even at the lowest severities, but they blamed the fire-induced changes in substrate availability [40]. Additionally, in another laboratory-based burning under controlled conditions, Pereira et al. [9] concluded that enzymatic activities are particularly sensitive to burning and show a strong dependance on soil depth. As in the present study, they found a strong decrease in GLU at the 0–1 cm depth, and even at the 1–2 cm depth, but the temperatures reached in their study were much higher than those in the present work.

The soil microbial activity, measured as the basal soil respiration (bSR), was not affected by the fire (Figure 3c), denoting that the microbial community was barely affected. However, the respiration normalized to the SOC content (nSR) was significantly higher after the most intense burns (HL and HS) than in the UB (Figure 3d). This is a symptom of stress in the microbial community [4], which has maintained a stable activity stable despite the decrease in its size (Figure 3a) and resources (Figure 2a). Even though the DOC was higher in those burns (Figure 2c) and it is known to stimulate the microbial activity [17,47], in previous studies based on experimental burns, the response of soil microbial activity was dependent on the fire intensities that were applied to the soil. Unlike in our study, in Pereira et al.'s [9] work, the soil was heated up to 250 °C at 1 cm and the bSR was severely reduced at the 0–1 cm depth due to the direct thermal impact on microorganisms and the indirect effects of the SOC reduction. In the same study, the nSR was not significantly reduced, indicating that quantitative C losses dominated over the qualitative ones. Other similar works reached the same conclusion [12,14]. In our study, at lower intensities and opposed to the previously mentioned findings, we can hypothesize that the qualitative changes dominated over the quantitative ones, as happened in Fernández et al.'s study [48], who quantified changes in organic matter chemical composition in soil samples heated in a laboratory at 150, 220, 350 and 490 °C. In the case of PBs in the Central Pyrenees, the post-fire response of the soil microbial activity also varies depending on the reached fire intensities. When the measured temperatures and residence times were high, a significant reduction in bSR was found [27,38] but, when the recorded intensity was lower, no major immediate changes were found for this parameter [17,27].

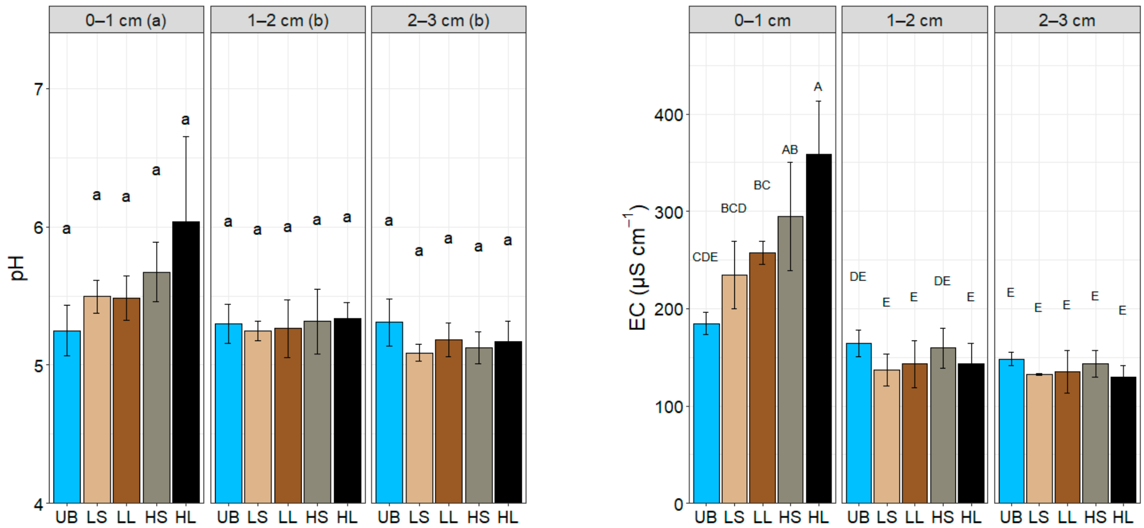
Glomalin-related soil proteins (T-GRSPs) are an indicator of the fire's perturbation of arbuscular mycorrhizae fungi [49], which are responsible for the production of those proteins [49]. T-GRSPs were significantly decreased by the fire in all the burn types, except in the least intense ones (LS) within all the depths studied (Figure 4a). This confirms that fire's effects on GRSPs are dependent on both the temperature reached in the soil and its residence time [50]. On the other hand, an increase in the labile GRSP fraction (EE-GRSP) was observed for all the depths studied, which was significant for LL and HL (Figure 4b), following the same trend as the DOC (Figure 2c) and showing a relationship between SOC quality and GRSP, which was also reported by Lozano et al. [51] and Rillig et al. [52].

None of the different fire intensities provoked any significant changes in soil pH (Figure 5a). Normally, changes in pH are related to the presence of ash rich in basifying cations [53]. However, in this experimental burning, the vegetation and litter were removed from the mineral soil surface prior to burning. Previous studies have also reported the absence of significant changes in pH after prescribed burnings. According to Alfaro-Leranoz et al. [17] and Fontúrbel et al. [21], when the ash does not become incorporated into the mineral soil, no significant changes in soil pH are produced. However, a significant increase in soil electric conductivity (EC) from 0 to 1 cm could be observed between the most intense burns (HS, HL) and the control (UB), and between HL and LS (Figure 5b). The increase in EC could have been caused by the partial combustion of SOM at the shallowest soil depth, as well as the combustion fine roots, that liberates soluble nutrients into the soil [54]. Badía et al. [13], did not find significant changes in pH after high intensity burning applied directly to the mineral Ah horizon; unlike in the present study, their soils were highly calcareous, with an important buffer capacity. However, like in the present work, an increase in EC was found at the shallowest depth and higher post-fire concentrations of soluble ions were found [13]. The same behavior of pH and EC was reported by Hrenović et al. [15] and they related it to both the soluble ions release and the formation of oxides during the combustion of SOM.



(**a**) (**b**)

Figure 4. Fire's effects on glomalin-related soil proteins fractions (GRSP): (**a**) total fraction (T) and (**b**) relative labile fraction (EE). Lowercase letters on top of the bars indicate significant differences between treatments and those between brackets between depths ($p < 0.05$). In each bar, the mean ($n = 3$) and the standard deviation are represented.



(**a**) (**b**)

Figure 5. Fire's effects on (**a**) soil pH and (**b**) soil electric conductivity (EC). Lowercase letters on top of the bars indicate significant differences between treatments and those between brackets between depths ($p < 0.05$). Uppercase letters on top of the bars indicate significant differences within all samples when the interaction between treatment and depth was significant. In each bar, the mean ($n = 3$) and the standard deviation are represented.

Related to biochemical properties is soil water repellency (SWR), which is greatly influenced by the quantity and quality of soil organic matter, which in turn is derived from the type and biomass of plants and litter [55,56]. In this study, SWR was higher on the soil surface and showed a negative relationship with depth, except for during the most intense fire (HL), which showed lower water repellency at the surface than at the 1–3 cm depth (Figure 6). The intermediate intensities (LL and HS) showed the same SWR at the 0–1 and 1–2 cm depths. Moreover, a positive relationship with fire intensity was found for all the burn types except for the HL burn, which showed the lowest SWR at the 0–1 cm depth. The highest SWR values (0–3 cm) were found in HS, coinciding with the highest ROC values and confirming the hypothesis formulated in [40]. Natural SWR is normal in many soils, as reported previously in several publications [13,57,58]. After low-to-medium-intensity fires, SWR can increase due to the production of hydrophobic compounds during the SOM combustion [36,58]. This was the case for a low intensity fire performed in Central Portugal, which induced a post-fire increase in SWR [59]. When the fire intensity is higher, the SWR tends to disappear due to the destruction of SOM and, therefore, the hydrophobic organic substances, as reported in several studies. Specifically, Aznar et al. [14], Badía-Villas et al. [12] and Pereira et al. [12] reported the destruction of SWR at high fire intensities. Even in a low severity PB in Central Pyrenees, in field conditions, Girona-García et al. [60] reported a decrease in SWR in an originally water-repellent soil, from 0 to 2 cm; according to them, the SOC content was directly related to SWR and its reduction was caused by the loss of SOC [60].

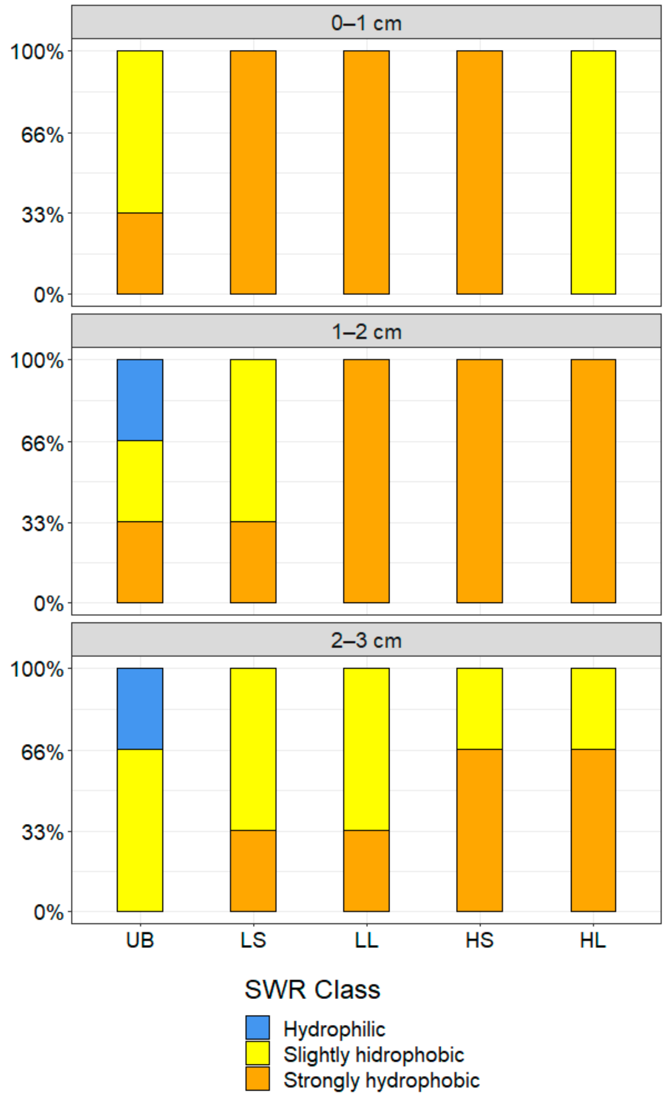


Figure 6. Occurrence (%) of soil water repellency (SWR) according to the Water Drop Penetration Time (WDPT) test for the unburned (UB) and burned (LS, LL, HS, HL) samples, within the different soil depths (0–1, 1–2 and 2–3 cm). SWR classes defined by [33].

3.2. Interrelation Between Parameters: ANOVA Simultaneous Component Analysis (ASCA)

The score and loading plots in Figure 7 show the results from the multivariate analysis. ASCA showed significant differences in both treatments ($p < 0.01$) and depths ($p < 0.01$), and in their interaction ($p = 0.01$). Most of the variance was explained by the depth

(32.6%), while the treatment and the interaction between factors explained the 22.6% and 15.1%, respectively.

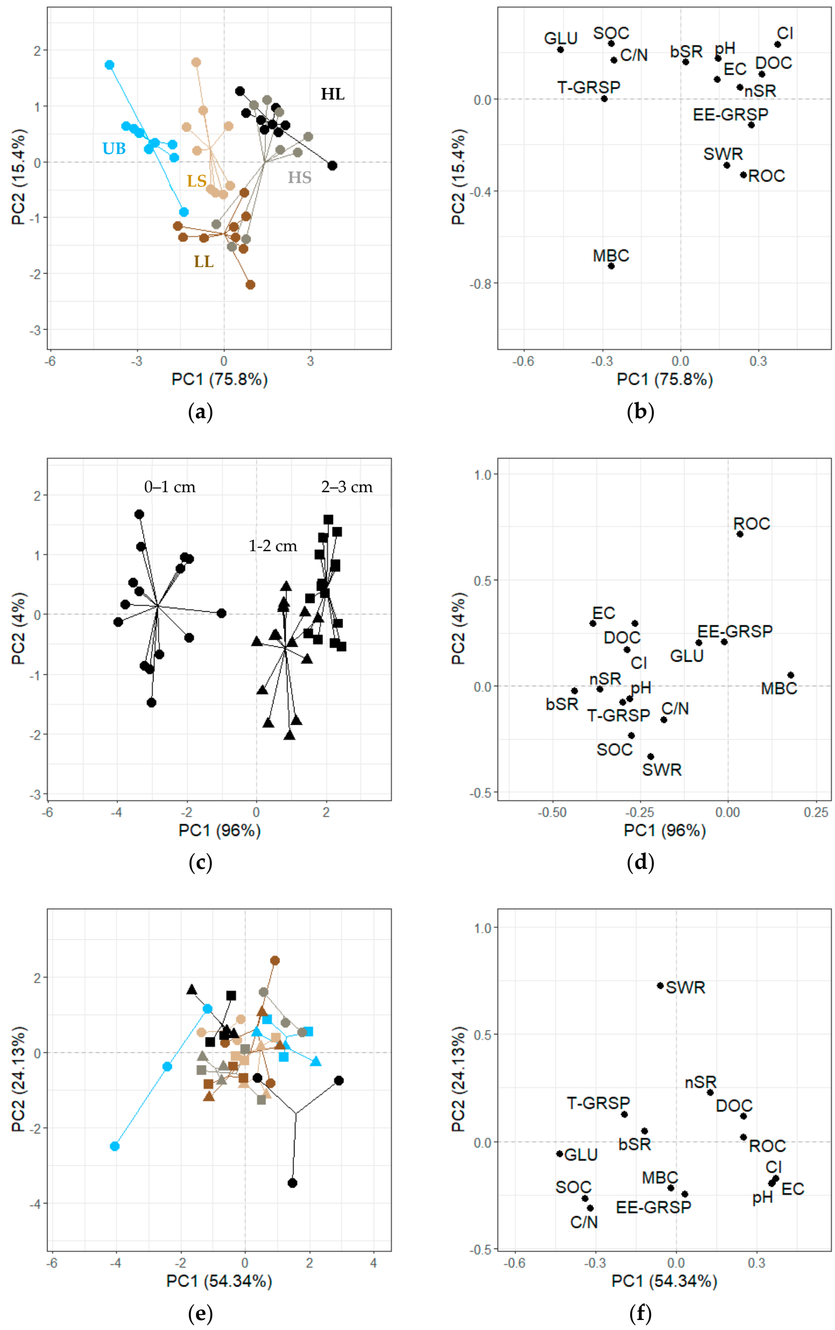


Figure 7. Score and loading plots of the ANOVA simultaneous component analysis (ASCA). (**a**) Scores and (**b**) loadings for treatment: charred intensity (CI); (**c**) scores and (**d**) loadings for soil depth: circles (0–1 cm), triangles (1–2 cm) and squares (2–3 cm); and (**e**) scores and (**f**) loadings for the interaction between treatment and depth. Blue dots from score plots refer to unburned (UB), light brown to low temperature and short time (LS), dark brown to low temperature and long time (LL), gray to high temperature and short time (HS) and black to high temperature and long time (HL). Abbreviations from loading plots refer to soil organic carbon (SOC), labile or dissolved organic carbon (DOC), recalcitrant organic carbon (ROC), total glomalin-related soil proteins (T-GRSPs), labile GRSP (EE-GRSPs), microbial biomass carbon (MBC), β-D-glucosidase activity (GLU), basal soil respiration (bSR), normalized soil respiration (nSR), electrical conductivity (EC) and soil water repellency (SWR).

3.2.1. Thermal Shock Effects

For the treatment, the first two principal components accounted for 91.2% of the variance (Figure 7a,b). Principal Component 1 (PC1) explained 75.8% of the variance and was related to the temperature reached. Principal Component 2 (PC2) explained the remaining 15.4% of the variance.

PC1 distributed the samples from the UB ones, with negative scores, to the HS and HL, with the highest positive scores, leaving LS and LL in between, with scores close to 0 (Figure 7a). All samples, except the LL ones, had positive PC2 loadings, which seems to suggest that the effect of the residence time was only relevant in the low temperature burns, making no difference when the temperature was high. The variables in Figure 7b are distributed depending on their response to the thermal shock, with the ones that suffered significant decreases with the highest negative PC1 loadings (GLU, T-GRSP, SOC, C/N) and more related to the UB samples. On the contrary, the variables that experienced post-fire increases (CI, DOC, EE-GRSP, ROC, nSR, SWR, EC, pH) have the highest PC1 loadings and are more related to the HS and HL samples. MBC has both high negative PC1 and PC2 loadings and it is slightly related to the LL samples. It is also a parameter that met its highest values with this burn type, combining a low temperature (50 °C) and a long time (24 min), which suggests that a low temperature combined with a long residence time might trigger microbial growth.

Some of the hypotheses from the discussion are supported by the loadings of PC1 shown in Figure 7b. For example, DOC and ROC, which are parameters that were related to the destruction of SOM, are located close to the CI. Also, the SWR is located close to ROC, showing a strong relationship between them. In addition, nSR, which was hypothesized to be more related to SOM quality that quantity, is close to both DOC and ROC, and considerably far from SOC. Moreover, the C/N ratio has opposite loadings to ROC, which could indicate a relationship between the N content and the ROC.

3.2.2. Soil Depth Affected by Fire

The first two PCs accounted for 100% of the variability, with PC1 being the one that explains most of it (Figure 7c,d). The 0–1 cm soil samples had the highest negative scores and were very differentiated from the rest, while the 1–2 and 2–3 cm ones had lower positive scores and were close to each other (Figure 7c). The variables are distributed from left to right, depending on their values (Figure 7d). Almost all of them that have the highest values at the shallowest depth have the highest negative loadings and are related to the 0–1 cm samples, while the ones that do not show differences between depths (EE-GRSP, ROM) have neutral loadings. Only the MBC, which has its lowest values at 0–1 cm, is more related with the 1–3 cm depths and has the highest positive loading.

3.2.3. Interaction Treatment: Depth

In the case of the interaction between treatment and depth, the first two PCs explained 78.4% of the variability (Figure 7e,f). PC1 accounted for 54.3% and the PC2 explained the remaining 24.1%. In this case, the UB 0–1 cm samples, with the highest negative scores of PC1, and the HL 0–1 cm samples, with the highest positive scores of PC1, could be clearly differentiated from the rest (Figure 7e). Also, the unburned (UB) samples show a gradient according to the depth (left to right), differentiating the 0–1 cm samples from the 1–2 and 2–3 cm ones. With the lowest fire intensity (LS), this difference is less noticeable, and for the rest of the burn types, the samples are distributed oppositely. This loss of the original soil properties gradient with depth due to fire has been previously demonstrated [12].

This distribution suggests that the PC1 shorts the samples by the combined effect of depth and fire severity. In other words, the higher the scores are for the 0–1 samples compared to the 1–3 cm ones, the higher the severity was. This highlights the correctness of the experimental design as the four types of burns created a gradient of fire severity on the soil properties studied.

In Figure 7f, the variables that experienced increases due to the fire effect from 0 to 1 cm have the highest positive PC1 loadings and can be related to the HL 0–1 cm samples. On the other hand, the variables that were negatively affected by the fire from 0 to 1 cm have the highest negative PC1 loadings and can be related to the UB 0–1 cm samples.

4. Conclusions

Laboratory-controlled burnings of soil monoliths, producing four different charring intensities, caused a gradient of effects on most of the soil properties analyzed, and allowed us to differentiate properties that are more sensitive and more resistant to heat. In particular, GLU, SOC and total GRSPs were significantly reduced even at the lowest charring intensity. These properties can, therefore, be used as indicators of changes in soil health due to disturbances such as wildfires or prescribed burning. Most of the heat-driven changes under the least intense treatment were only detectable in the shallowest soil cm (0–1 cm). At higher CIs (HL and HS), additional soil properties were significantly modified, decreasing (MBC, C/N) or increasing (DOC, ROC, nSR, easily extractable: total GRSP) even at greater soil depths. It is noteworthy that, in addition to the loss of soil organic matter quantity, there was a change in its quality with increases in the labile and recalcitrant fractions, reducing the intermediate one, especially during the most intense burns. These changes can affect other soil properties in the short and long term, altering the soil carbon cycle. This fact highlights the importance of choosing the appropriate environmental conditions for carrying out prescribed burns, to avoid reaching high temperatures or long residence times and to minimize alterations in the ecosystem balance. To do so, it is very important to carry burns out without the flame remaining for too long in the same spot and with the soil moist to minimize heat transmission. The lack of the application of fuel management practices could lead to the occurrence of big forest fires in drier and uncontrolled conditions, reaching higher temperatures for longer periods and exerting considerable effects on the soil ecosystem.

Author Contributions: Conceptualization, A.A.-L., M.E.-A. and D.B.-V.; methodology, A.A.-L., M.E.-A. and C.M.-D.; software, A.A.-L.; validation, A.A.-L., C.M.-D. and D.B.-V.; formal analysis, A.A.-L.; investigation, S.Q.-E., A.A.-L. and C.M.-D.; resources, D.B.-V., S.Q.-E. and A.A.-L.; data curation, A.A.-L., C.M.-D. and D.B.-V.; writing—original draft preparation, A.A.-L. and D.B.-V.; writing—review and editing, D.B.-V., C.M.-D. and A.A.-L.; visualization, D.B.-V.; supervision, D.B.-V.; project administration, D.B.-V.; funding acquisition, A.A.-L., M.E.-A. and D.B.-V. All authors have read and agreed to the published version of the manuscript.

Funding: A. Alfaro-Leranoz was funded by a DGA predoctoral research grant (BOA20200713012), financed by the Government of Aragón. This research received a "Félix de Azara" Research Award 2022, from the Diputación Provincial of Huesca.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflict of interest.

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