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A Burning Intensity Gradient Modifies Sensitive Soil Properties Depending on Sampled Soil Depth and the Time Since Fire

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‖ In memoriam.

Abstract

The effects of wildfires and prescribed burnings on soil are highly variable. In order to evaluate the effects of different burning intensities on soil properties, a surface-controlled burn of undisturbed soil monoliths was carried out by combining temperatures (50 and 80 °C) and residence times (12 and 24 min). The effects of this burning gradient are evaluated at two soil depths (0–1 and 1–3 cm), with time (just after burning or immediate effects, T0, and five months later, T5), as well as the influence of ash (presence or absence). The results indicate that most soil properties were affected by the burning gradient applied only in the most superficial cm (0–1 cm), with few effects at greater depths. The most intense burn had the strongest immediate impact, reducing soil organic carbon, recalcitrant organic carbon, and microbial biomass carbon, as well as increasing the labile organic carbon and the microbial activity. On the other hand, this burning caused a strong decrease in soil water repellency at a 0–1 cm depth and increased it at 1–3 cm. In contrast, medium-intensity burning caused the opposite effect, increasing water repellency at the soil surface and reducing it at 1–3 cm. As a result of the mineralization of organic matter, the EC and pH increased significantly in all burning combinations and both soil depths studied. After five months (T5), several of these parameters tended to approach the values of unburned soil.

Keywords: laboratory burning; fire intensity; soil organic carbon; microbial activity; microbial biomass; soil water repellency; shallow near-surface samples; 0 and 5 months after burning

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Academic Editor: Grant Williamson

Received: 31 July 2025

Revised: 18 August 2025

Accepted: 25 August 2025

Published: 3 September 2025

Citation: Escuer-Arregui, M.; Alfaro-Leranoz, A.; Badía-Villas, D.; Conte-Domínguez, A.P.; Martí-Dalmau, C.; Ortiz-Perpiñá, O. A Burning Intensity Gradient Modifies Sensitive Soil Properties Depending on Sampled Soil Depth and the Time Since Fire. *Fire* **2025**, *8*, 351. https://doi.org/10.3390/fire8090351

1. Introduction

Grazing has had a primary role in traditional landscape management in the Central Pyrenees (NE Spain) for centuries [1]; however, since the mid-20th century, the grazing activity has been decreasing due to several socioeconomic factors, like rural exodus [2–4] and the intensification of agricultural systems [5–7]. As a consequence, several Pyrenean grasslands have been encroached upon by shrub communities [8,9], especially by *Echinospartum horridum* (Vahls) Rothm (Fabaceae) [10,11], a thorny scrub that forms nearly monotypic communities [12] with high flammability [13] and high colonization rates [14]. Landscape changes due to vegetation succession occur relatively quickly [15], with the rate dependent on environmental conditions and human management [16].

For these reasons, prescribed burnings and mechanical cuttings have been performed since the 1990s in order to restore pasture lands [17]. Therefore, prescribed burns could be an effective method to control the grassland encroachment by some shrubs, if combined with grazing [10,18]. In fact, this practice was quite common in the region among shepherds in the past, who used to burn shrubs regularly in order to keep their pasture lands in optimum conditions [19]. However, the occasional burning of *Echinospartum horridum*, a pyrophyte species with a great post-fire germination capacity, is sometimes not entirely effective by itself [20]. For this reason, repeated prescribed burns of the same type of scrub are occasionally being carried out, which increases the chances of negatively affecting the soil. Moreover, its pillow-like shape and the large amount of dry biomass close to the ground is capable of generating high temperatures on the soil surface when it burns [13,21–24].

One of the ecosystem's most-sensitive components impacted by fires is soil [25], and numerous studies have reported diverse and heterogeneous, fire-induced changes to its properties, depending on the variables considered [26–29]. Although the effects of prescribed burns are often superficial, due to soil's thermal inertia and moisture content [30], their impact can vary depending on the maximum temperatures and the burn duration [31]. Some of those heat-sensitive soil properties, like soil organic matter (SOM), soil water repellency (SWR), and soil microbiology, are closely related [27,32,33]. Similar to what happens during wildfires, prescribed burnings can affect the quality and quantity of the SOM [34,35] Even if degradation only affects the first few centimeters of soil [36], it is within this layer that the majority of organic matter resides and, depending on the fire's severity, the SOM can disappear almost entirely or even increase due to charcoal input [37]. Since SOM is essential for soil fertility, structure, and biological activity, its reduction or modification can affect survival and recolonization of soil organisms [26] and increase susceptibility to erosion due to the loss of aggregate stability [32]. Also, understanding the effects of burning in mountain soils is crucial, given these soils store large amounts of organic carbon [38].

Furthermore, SWR can be eliminated by fire if the soil is naturally water-repellent, as has been reported by other studies performed in the Central Pyrenees [31], or it can be induced by the volatilization of certain (normally lipidic) organic compounds that migrate to deeper soil layers and become condensed there [39,40]. Some studies performed in the same area have observed the immediate elimination and posterior reappearance of SWR after one year, even at higher values than before burning [31]. In addition, the formation of those hydrophobic layers can reduce the soil infiltration capacity, facilitating runoff and soil erosion [41], or increment the soil aggregate stability [32].

The microorganisms residing in soil drive 80–90% of all soil processes, maintaining soil fertility and quality [42], and fire's effects on them can endanger the soil ecosystem's recovery [43,44]. They respond immediately to fire, suffering damage even from low temperatures and short-duration burns [28], which eliminate a significant portion of the microbial biomass in the uppermost centimeters of soil [45]. In deeper layers, no changes or even increases in microbial activity are often observed due to the rise in soluble carbon and nutrients from combusted SOM, included microorganisms, from the upper layers [46–48].

Since the results obtained to date with prescribed burns in the field, carried out under very similar conditions, have been heterogeneous when the analysis is performed at the centimeter level of the soil, we have considered conducting laboratory burns (as in previous studies [21–24,49]). Our hypothesis is that the effects of prescribed burns on the topsoil properties are the result of the combination of maximum temperature and heat residence time reached at a given depth or soil thickness. If we combine both variables, we will be able to discern which plays a more determining role in altering sensitive soil properties. In addition, ashes and the time elapsed since burning are included as variables to be analyzed.

2. Materials and Methods

2.1. Sampling Area

Soil monoliths were collected (in October 2020) under a dense cover of mature thorn scrubs (*Echinospartum horridum*). The sampled area is located in Puerto Sobás, within the municipality of Yebra de Basa (NE-Spain) (Appendix A, Figure A1) whose characteristics are summarized in Table 1. Soils, developed over Eocene-age conglomeratic parent material, are characterized by a loam to sandy loam textures, elevated levels of soil organic matter, high aggregate stability, high cation exchange capacity and pH values approaching neutrality. According to the World Reference Base for Soil Resources (WRB) [50], soils are classified as *Eutric Endoleptic Cambisols* (Loamic, Humic).

Table 1. General characteristics of the study site. Source: [21].

Coordinates	42° 30′ 55.0″ N 0° 15′ 47.9″ W
Altitude (masl)	1480
Mean Annual Temperature (°C)	8.3
Mean Annual Precipitation (mm)	1015
Aspect	South
Mean slope (%)	5
Echinospartum horridum cover (%)	75
Aerial biomass (kg/m^2)	9.24
Litter: Oi + Oe (kg/m^2)	1.62

Twenty-five intact soil monoliths (15 × 15 cm in surface area and 10 cm in depth) were collected, corresponding to a total area of 0.56 m^2. All monoliths were obtained continuously from the same place (Appendix A, Figure A2), to avoid soil spatial variability, and they were extracted using a flat spade. To preserve their structural integrity and prevent disaggregation, each monolith was immediately wrapped in a layer of heavy-duty aluminum foil and transported rapidly to the laboratory to be burned.

2.2. Experimental Design of Burnings

To obtain a range of heat intensities, different burning temperatures and durations were applied to the surface of the undisturbed soil monoliths, where the thorn scrub biomass observed under field conditions was deposited. This configuration produced five series (Figure 1) of soil monoliths. The five series were named, according to the combination of temperature and burning duration, as follows:

- HL set, high temperature and long duration: high-intensity burning.
- HS set, high temperature and short duration: moderate intensity by temperature.
- LL set, low temperature and long duration: moderate intensity by duration.
- LS set, low temperature and short duration: low-intensity burning.
- UB set, unburned or control soil, representative of pre-burn conditions.

In addition to the variable burning intensity, our experimental design included two other variables: the time elapsed (T) and the presence (+) or absence (−) of ashes and charred biomass on the burned soil during that time. In this way, five of the twenty-five soil monoliths, one per burn type, were selected for the T0 sampling, to assess the immediate effects of burning. The remaining twenty were stored and sampled five months later, to assess the short-term (5 months) effects of burning. Half of them were stored intact (T5+), with partially charred biomass and ash on the surface (to approximate field conditions), two per burn type. The other half had the ashes removed before storage (T5−), also two per burn type.

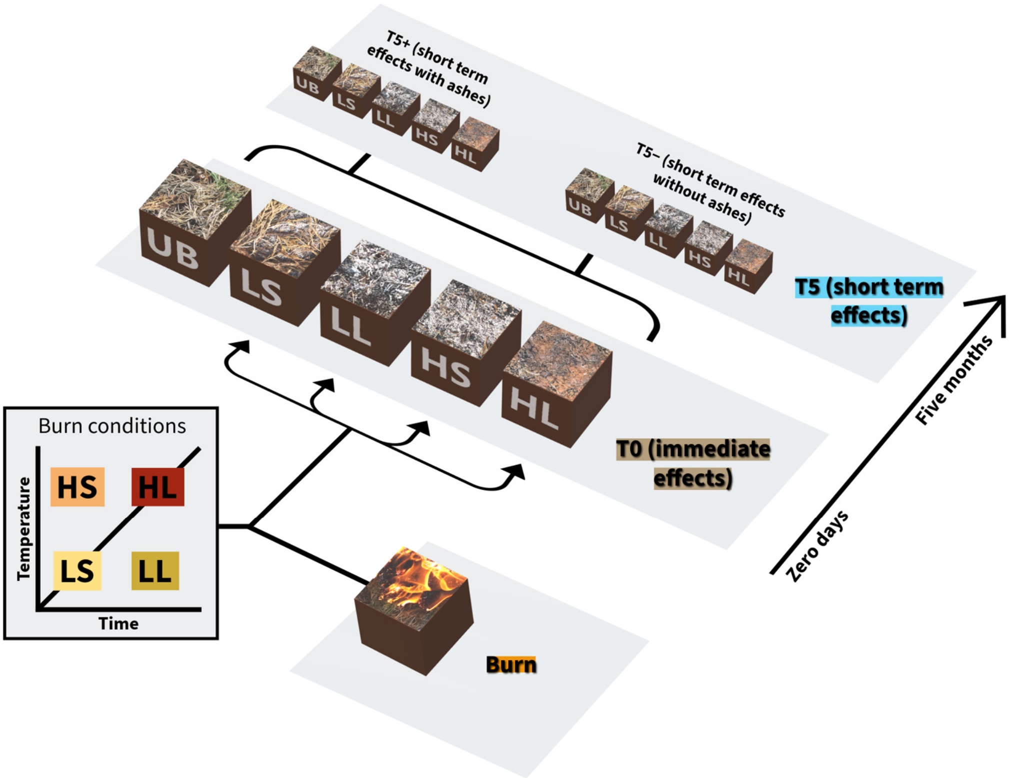


Figure 1. Design of the experimental soil burning. Abbreviations: UB (unburned soil), LS (low temperature and short duration), LL (low temperature and long duration), HS (high temperature and short duration) and HL (high temperature and long duration).

To achieve the different-intensity gradients, a low (50 °C) and high (80 °C) temperature to be reached at 1 cm of soil depth was chosen (Table 2). The low temperature was set at 50 °C, since this is the temperature at which changes in soil biological attributes begin. The high temperature was set at 80 °C, since at this level, most of the fine roots have perished, significantly affecting the bacterial and fungal biomass [1]. The residence times are defined as the time during which the temperature criteria were maintained. Empirical and modeling studies show that soil heating at ~1 cm depth often unfolds over minutes to tens of minutes, with substantial variation driven by fuel load, slope/wind (head vs. backing fire), and smoldering of duff layers. Heat and moisture transport models [51] likewise reproduce these minute- to multi-minute profiles. Consequently, we set 12 min to represent rapid-spread conditions, with brief but intense heating pulses, typical of head fires; and 24 min (2×) to emulate slower-spread/backing or smoldering-influenced scenarios, where elevated temperatures persist longer. These criteria are based on previous prescribed burning studies performed in the same area [21,23,24,26,28].

Table 2. Temperature criteria and their residence times at 1 cm depth for the experimental burnings.

Treatment	Label	Temperature Criteria at 1 cm (°C)	Residence Time of the Temperature Criteria at 1 cm (min)
High temperature, long time	HL	80	24
High temperature, short time	HS	80	12
Low temperature, long time	LL	50	24
Low temperature, short time	LS	50	12
Unburned	UB	-	-

2.3. Experimental Burning Execution

Before conducting the burns, the monoliths were randomly grouped into sets of five, which were subjected to a burn treatment of varying intensity, including a control. Subsequently, the biomass of *Echinospartum horridum* to be applied on the surface of each monolith was calculated to replicate field conditions. For this, the collected shrub biomass (of *Echinospartum horridum*) was divided into three fractions based on the diameter of its stems and branches: fine (ø < 0.5 cm), medium (0.5 < ø < 1.0 cm), and coarse (ø > 1.0 cm). The weight of each fraction and the total biomass were recorded to determine the percentage that each fraction represented of the total. To calculate the total shrub biomass corresponding to each monolith, the surface area of each monolith was measured. Then, the biomass equivalent to the sampled field area was calculated with the aerial biomass provided by [21]. Finally, the total biomass assigned to each soil monolith was distributed homogeneously over its surface, maintaining the proportion of the three diameter-based fractions.

Thereupon, the monoliths were burned by placing them in a combustion chamber with the corresponding shrub biomass on their surface, using an industrial torch to regulate the burn intensity according to the specified temperature and duration criteria. To prevent localized overheating, the torch was systematically moved across the monolith surface, ensuring a uniform and consistent burn. More details of this type of burning have been previously described [49,52,53].

To control the temperature during the burning process, the temperature was recorded using K-type thermocouples and a TC-08 data logger from Pico Technology (Pico Technology, James House, Colmworth Business Park, St. Neots, Cambridgeshire, UK) at one-second intervals on the soil surface and at depths of 1 cm, 2 cm and 3 cm. To check whether the different types of burning had effectively created a gradient of intensities, the following indicators were obtained based on the temperature records for each burn (Table 3): the maximum temperature reached during each monolith's burn (T max), the residence time above 50 °C (Time 50), 100 °C (Time 100), 300 °C (Time 300), and 500 °C (Time 500), as well as the charred index (CI), calculated as the integral of the temperature curve over time, only when the temperature exceeded 50 °C (Equation (1)).

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

In this equation, 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.

After the burn, the T0 sets were taken directly to the laboratory for immediate analysis. The T5 monoliths were kept in a greenhouse for five months with daily irrigation, at temperatures ranging from 9 to 15 °C and a relative humidity of approximately 75%, until the time of analysis. In the T5− monoliths, ash and charred plant material was carefully removed from the soil surface using tweezers and a brush prior to the storage period.

Table 3. Recorded temperatures, residence times and charred intensity (CI) during the experimental burnings for each treatment (LS, LL, HS, HL) and soil depth (0–1 and 1–3 cm). For each parameter the mean (n = 5) and the standard deviation are shown.

Soil Depth	Label	T Max (°C)	Time (min) >50 °C	>100 °C	>300 °C	>500 °C	CI (°C/min)
0–1 cm	HL	431 ± 100	57.0 ± 18.1	33.5 ± 9.2	19.8 ± 12.8	2.3 ± 4.8	13,478 ± 5974
	HS	503 ± 51	39.8 ± 18.9	27.9 ± 13.7	20.6 ± 10.9	0.8 ± 1.7	11,456 ± 5893
	LL	254 ± 168	24.7 ± 8.8	11.8 ± 13.7	4.9 ± 10.2	3.4 ± 7.3	4103 ± 3234
	LS	305 ± 131	22.1 ± 4.7	12.4 ± 7.9	3.4 ± 5.5	0 ± 0	3598 ± 2392
	UB	16 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1–3 cm	HL	76 ± 7	57.5 ± 21.3	0 ± 0	0 ± 0	0 ± 0	3999 ± 1537
	HS	62 ± 14	26.9 ± 24.8	0 ± 0	0 ± 0	0 ± 0	2262 ± 1257
	LL	47 ± 6	0.01 ± 0.01	0 ± 0	0 ± 0	0 ± 0	1041 ± 218
	LS	43 ± 6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	391 ± 120
	UB	15 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

2.4. Methods of Analysis of Soil Properties

To prepare the samples, ashes and charred plant residues were removed from the surface of the monoliths. Sampling was conducted at depths of 0–1 cm and 1–3 cm, based on previous studies indicating that soil properties show no significant changes at greater depths following prescribed burns in similar locations [21–24,31,54]. A portion of the fresh sample was set aside for biological property analysis within a maximum of 24 h, while the remaining sample was air-dried until constant weight and then sieved to 2 mm.

From the T0 monoliths (5 monoliths), a total of 20 samples were obtained by splitting each monolith in half and collecting samples from two depths (0–1 and 1–3 cm). For the T5 monoliths (20 monoliths), a total of 40 samples were obtained by collecting samples at the same two depths (0–1 and 1–3 cm) from each monolith.

Soil organic carbon (SOC) content was determined using the wet oxidation method with chromic acid [55]. Dissolved or labile organic carbon (DOC) was extracted with K_2SO_4 and measured using the wet oxidation method with chromic acid [56]. Recalcitrant organic carbon (ROC) was extracted using the method for estimating non-hydrolyzable organic carbon [57,58] and was also measured by the wet oxidation method with chromic acid. To determine microbial biomass carbon (MBC), the fumigation–extraction method with chloroform established by [56] was employed, considering a calibration factor (Kc) of 0.38 [21]. Soil respiration was measured by incubating the samples under optimal conditions of temperature (25 °C) and moisture (50% field capacity). The emitted CO_2 was captured using NaOH traps [59] and measured every 7 days during 28 days. Soil respiration was expressed in terms of basal soil respiration (bSR). The persistence of soil water repellency (SWR) was determined in the sieved samples at 2 mm using the water drop penetration time test (WDPT) defined by [60]. SWR was categorized into the 5 classes established by [61]. The pH and electrical conductivity (EC) were measured in 1:2.5 w/v and 1:5 w/v soil aqueous suspensions, respectively, using the method proposed by [62]. Soil moisture content (SMC) was determined gravimetrically after air-drying the samples at 105 °C to a constant weight.

2.5. Statistical Analysis

Statistical analyses were performed using RStudio (4.4.3 version) open-source software [63]. A one-way analysis of variance (ANOVA) was conducted for the burning types (5 levels: LS, LL, HS, HL, and UB), separating the data by depth and sampling time. 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 Box–Cox function from the "MASS" library. The pairwise comparison using Fisher's HSD test ($p < 0.05$) was also applied to evaluate the statistical significance of the differences in the response variables. Additionally, an ANOVA-simultaneous component analysis (ASCA), using the "limpca" package [64], was conducted to explore patterns of distribution of the samples among the different fire and soil factors, and to quantify the contribution of each measured variable to the total variation. Furthermore, a Pearson correlation was performed to identify relationships among the studied soil properties.

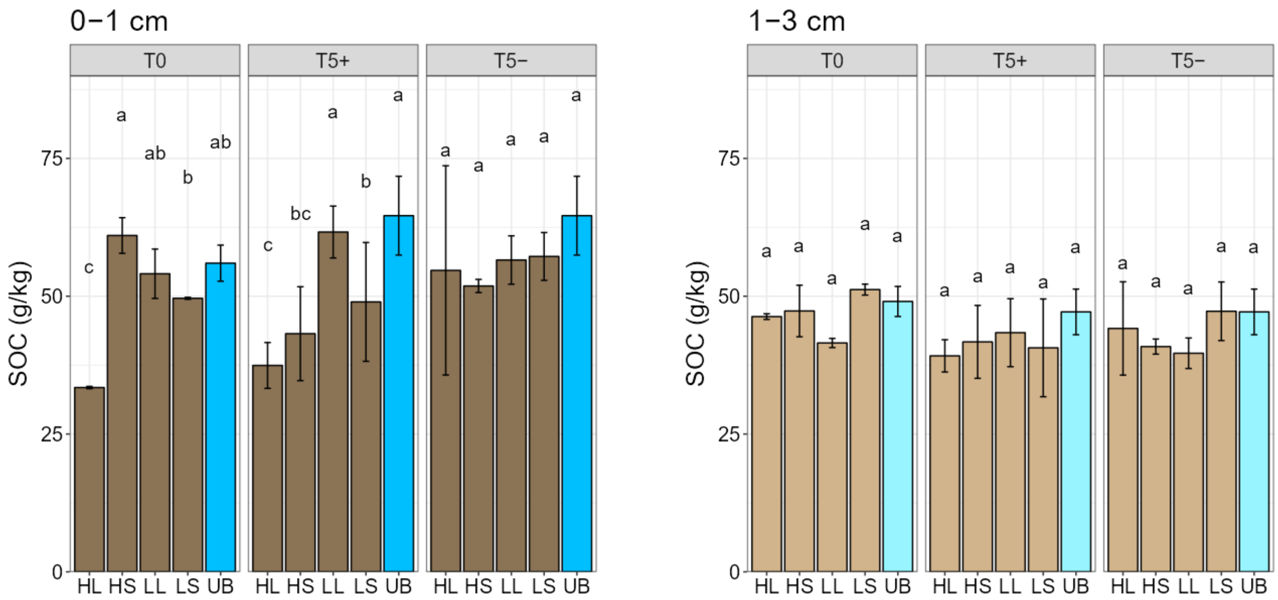
3. Results

3.1. Fire-Intensity Effects on Quantity and Quality of Soil Organic Carbon Fractions

The burning only caused a significant loss of SOC ($p = 0.01$) in the most intense burn (HL) in T0 (Figure 2a), where the content was reduced from 56.00 ± 3.28 g/kg in UB to 33.43 ± 0.21 g/kg. In contrast, the SOC content at a depth of 1–3 cm (Figure 2b) was not affected by any type of burning.

In the case of T5+, a lower content of SOC was observed in 0–1 cm for all burns except for LL, with HL showing the lowest values (37.44 ± 4.16 g/kg), followed by HS (43.20 ± 8.52 g/kg). At a depth of 1–3 cm, no differences were observed compared to UB. This was also the case for T5− at both sampled soil depths.

The burning led to a general increase in DOC as a direct effect (Figure 2c), with significant differences observed only in the HL and HS burns, which increased from 3.0 ± 0.23 g/kg (UB) to 7.68 ± 0.36 g/kg and 7.36 ± 1.62 g/kg, respectively. This increase was also seen at a soil depth of 1–3 cm (Figure 2d), where there was a significant increase in HL (7.96 ± 0.54 g/kg) and HS (3.60 ± 0.21 g/kg), and a decrease in LL (1.88 ± 0.22 g/kg), from 1.88 ± 0.21 g/kg (UB). For T5+, significant differences were only observed at the surface for HL, where the most significant decrease in labile carbon content occurred (from 2.35 ± 0.87 g/kg to 1.01 ± 0.65 g/kg). At 1–3 cm depth, no significant differences were observed. In T5−, there were no significant differences between the four burn types and UB at any depth.



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

Figure 2. *Cont.*

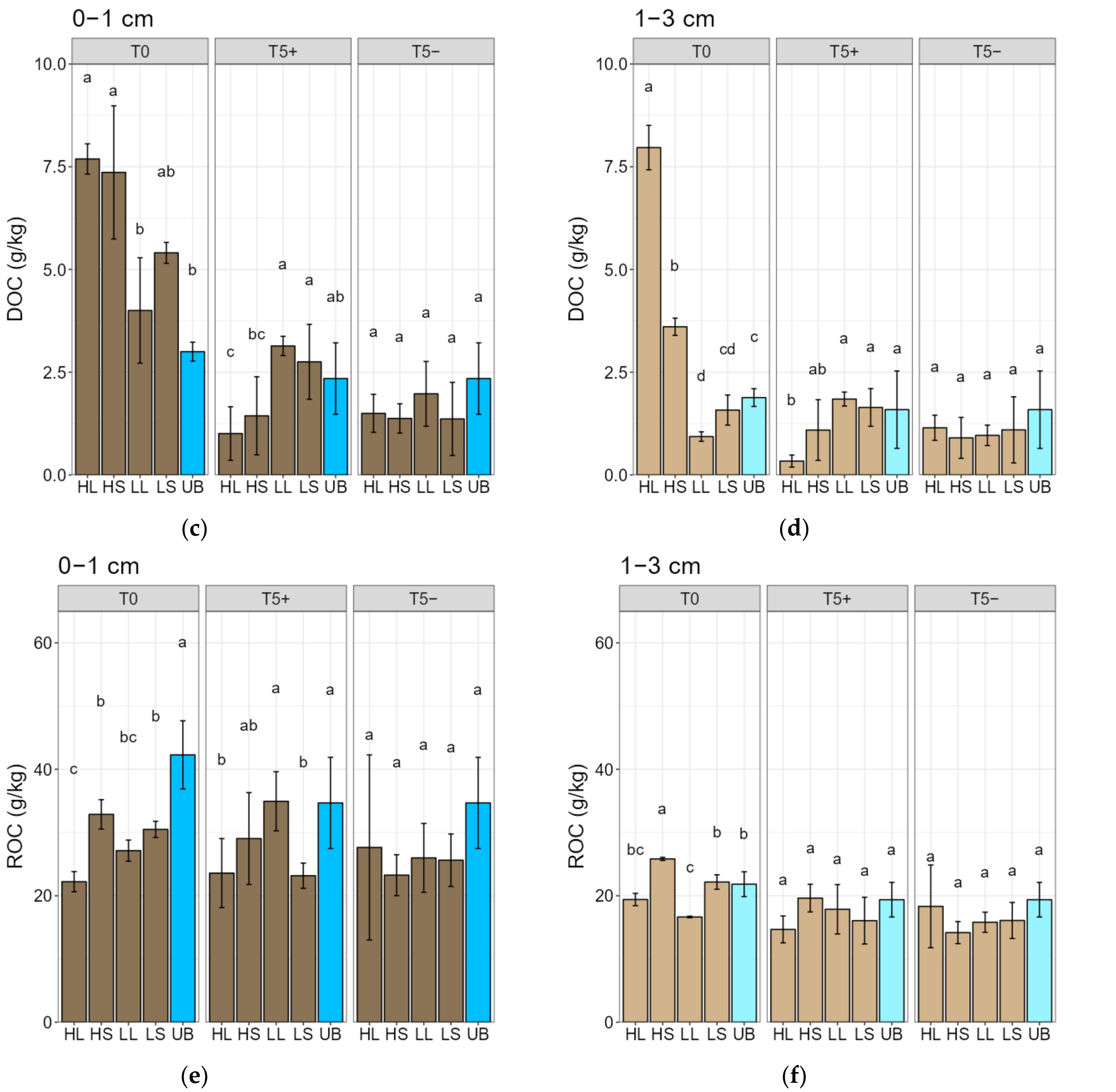


Figure 2. Fire effects on soil organic carbon fractions: soil organic carbon (SOC) at 0–1 cm (**a**), soil organic carbon at 1–3 cm (**b**), labile organic carbon (DOC) at 0–1 cm (**c**), labile organic carbon at 1–3 cm (**d**), recalcitrant organic carbon (ROC) at 0–1 cm (**e**) and recalcitrant organic carbon at 1–3 cm (**f**). In each bar, the mean and the standard deviation are represented. Letters on top of the bars indicate significant differences ($p < 0.05$) among burning treatments for each time period: just after burning (T0), and five months later (with ashes, T5+ or without ashes, T5−).

The direct effect of the fire on the surface resulted in a generalized decrease in ROC (Figure 2e) compared to UB (42.28 ± 5.38 g C/kg). This effect appeared to be more pronounced for the high-intensity burn (22.23 ± 1.60 g C/kg). At 1–3 depth (Figure 2f), there were two notable effects ($p < 0.01$): an increase in HS (25.83 ± 0.24 g C/kg) and a decrease in LL (16.64 ± 0.12 g C/kg). For T5+ and T5−, there were no differences between burned and UB.

3.2. Fire-Intensity Effects on Other Chemical Soil Properties: pH and EC

For T0, UB soil pH at a depth of 0–1 cm (Figure 3a) was 6.04 ± 0.13, and at 1–3 cm, it was 5.94 ± 0.23 (Figure 3b).

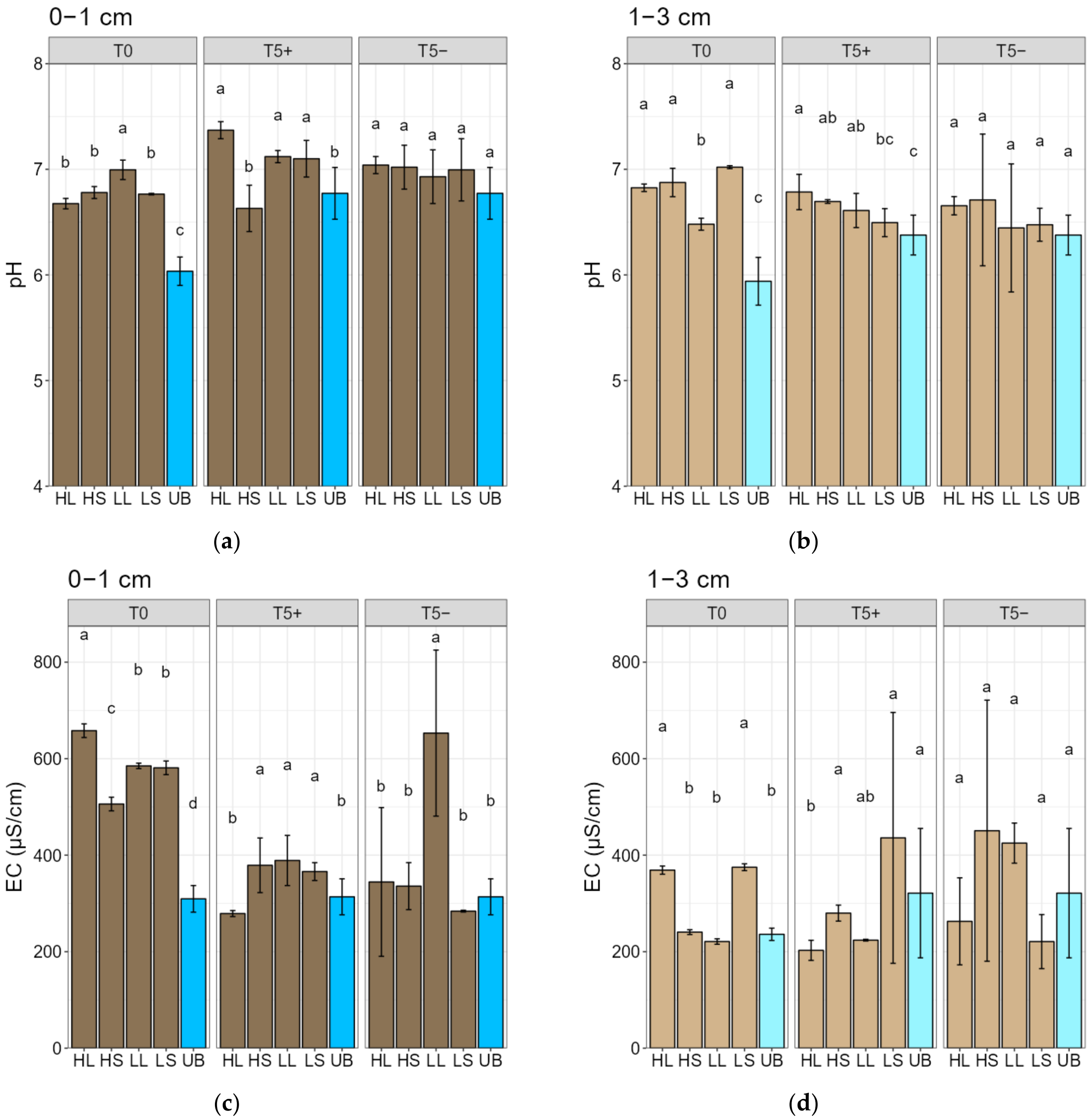


Figure 3. Fire effects on other soil properties: pH at 0–1 cm (**a**), pH at 1–3 cm (**b**), electrical conductivity (EC) at 0–1 cm (**c**) and electrical conductivity at 1–3 cm (**d**). In each bar, the mean and the standard deviation are represented. Letters on top of the bars indicate significant differences ($p < 0.05$) among burning treatments for each time period: just after burning (T0), and five months later (with ashes, T5+ or without ashes, T5−).

After burning, a general increase in pH was observed at both depths. The effect of burning on surface EC (UB = 309.0 ± 27.6 µS/cm) led to a significant increase ($p < 0.01$) in EC (Figure 3c) overall, with the most substantial rise in HL (658 ± 14.1 µS/cm). At a depth of 1–3 cm (Figure 3d), only HL (369 ± 8.5 µS/cm) and LS (375 ± 7.1 µS/cm) showed a significant increase compared to UB (236 ± 12.7 µS/cm). After five months, the pH in T5− showed no significant differences from UB at any depth (6.77 ± 0.3 and 6.38 ± 0.2), and T5+ also showed no differences at 1–3 cm; however, it presented significant differences at the surface in all burn types except HS. For EC in T5+ and T5−, no differences were observed from UB at 0–1 cm (313.7 ± 40.3 µS/cm) or at 1–3 cm (321.3 ± 144.8 µS/cm).

3.3. Fire-Intensity Effects on Soil Microbial Biomass Carbon and Soil Respiration

The burning process caused two direct notable effects in MBC, with significant differences observed compared to UB (7.87 ± 1.18 g/kg) (Figure 4a). First, an increase in MBC was detected in LL, with a value of 12.94 ± 1.91 g/kg. Second, MBC was undetectable in HL and very low in HS (0.11 ± 0.16 g/kg). In the 1–3 cm layer (Figure 4b), MBC was undetectable in HS, and significantly lower than UB (7.48 ± 1.08 g/kg) in HL (2.89 ± 0.45 g/kg) and LS (5.77 ± 0.24 g/kg).

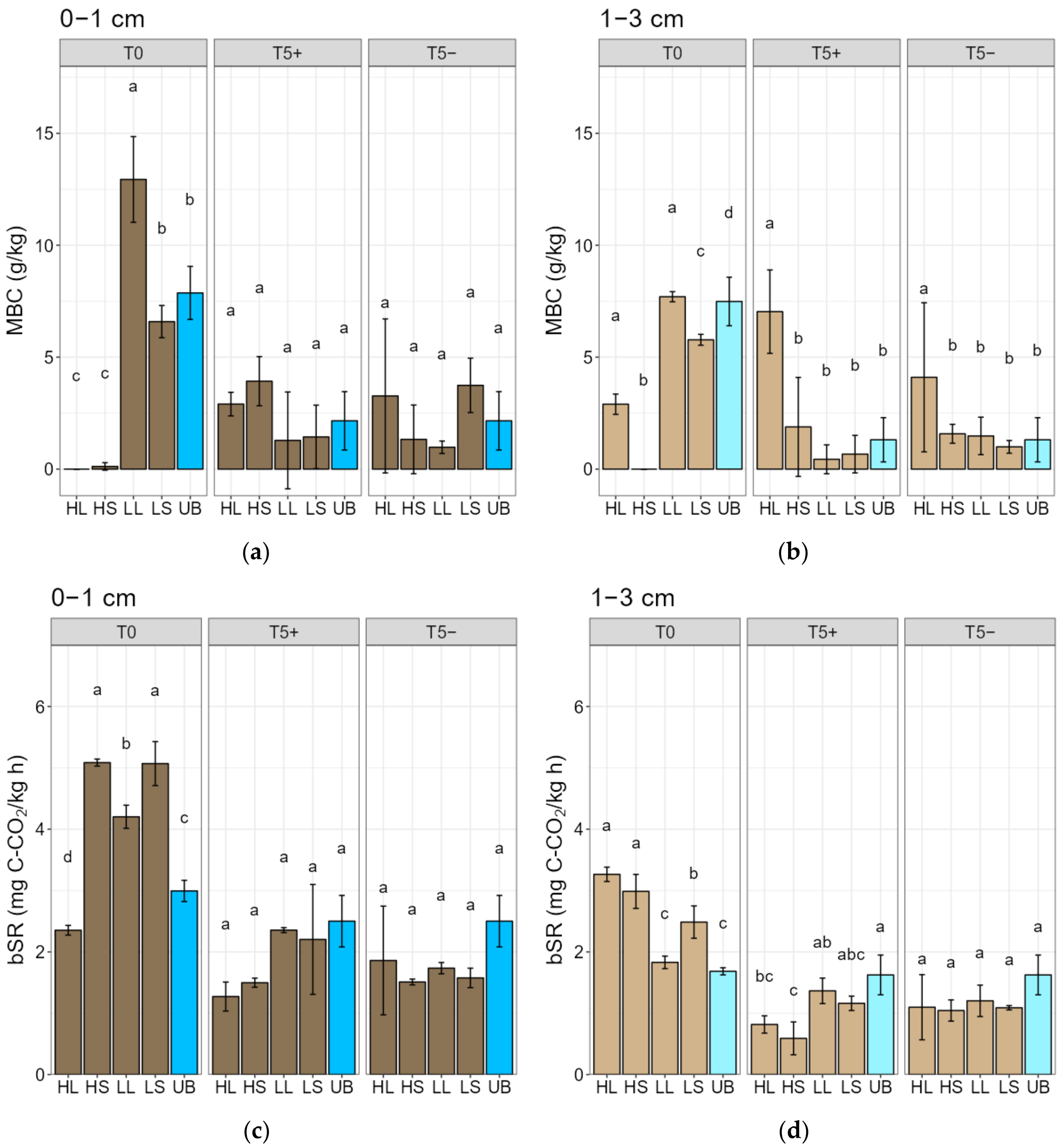


Figure 4. Fire effects on soil biological properties: microbial biomass carbon (MBC) at 0–1 cm (**a**) and at 1–3 cm (**b**), and basal soil respiration (bSR) at 0–1 cm (**c**) and at 1–3 cm (**d**). In each bar, the mean and the standard deviation are represented. Letters on top of the bars indicate significant differences ($p < 0.05$) among burning treatments for each time period: just after burning (T0), and five months later (with ashes, T5+ or without ashes, T5−).

For bSR, there was a significant increase in LS, LL and HS, and a significant decrease in HL, compared to UB (2.99 ± 0.17 mg C/kg h) (Figure 4c). The increase was most pronounced in HS and LS burn types, where values were very similar (5.09 ± 0.06 mg C/kg h and 5.07 ± 0.36 mg C/kg h, respectively). An increase was also noted in the 1–3 cm layer (Figure 4d) for HS (3.26 ± 0.11 mg C/kg h) and LS (2.98 ± 0.27 mg C/kg h), and even in HL (2.48 ± 0.26 mg C/kg h).

After five months, all parameters exhibited a similar behavior and generally showed no significant differences compared to UB. In the case of MBC, UB had a value of 2.16 ± 1.3 g/kg for 0 to 1 cm and 1.30 ± 1.06 g/kg for 1 to 3 cm. Only HL in T5+ and T5− showed differences at the 1–3 cm depth, with values significantly higher than UB ($p < 0.01$), recording 7.03 ± 0.23 g/kg and 4.10 ± 3.33 g/kg, respectively. The bSR showed no significant differences in any burn type compared to UB at the surface (2.58 ± 0.46 mg C/kg h), while at the 1–3 cm depth, HL (1.624 ± 0.35 mg C/kg h) and HS (0.59 ± 0.32 mg C/kg h) exhibited significant differences ($p = 0.02$). In T5−, there were no significant differences among the four burn types and UB at any depth.

3.4. Fire-Intensity Effects on Physical Soil Properties: Water Repellency and Moisture Content

UB samples exhibited 20.5% severe water repellency and 70.5% strong water repellency (Figure 5), while, at greater depths, they predominantly displayed strong water repellency. Following the controlled burns, a complete loss of water repellency was observed at the surface for HL (93.8% hydrophilic), alongside a significant increase in water repellency with depth (93.8% extremely water-repellent). In the case of HS, the opposite effect was observed at the surface, showing an increase in repellency (68.8% severely water-repellent and 22.5% extremely water-repellent). Less severe burns, LL and LS, showed a slight loss of repellency at the surface compared to UB, both 100% strongly water-repellent, whereas from 1 to 3 cm, minimal effects were observed. In T5, the control soil from 0 to 1 cm was predominantly slightly water-repellent (64.1%). At depths of 1 to 3 cm, it was slightly more hydrophilic (42.2%) and slightly water-repellent (46.9%). In T5+, the effects of the burns on HL and HS at both surface and depth were not observed, with the soil being predominantly hydrophilic. In contrast, for LL and LS, SWR appeared to have decreased, being predominantly slightly water-repellent. In T5−, a similar effect was observed as in T5+, with a general reduction in repellency for all burn types; however, for HL from 0 to 1 cm, the hydrophilic class was less represented (37.5%) compared to T5+. Notably, for HS from 1 to 3 cm, the entirety of the samples fell within the hydrophilic class, indicating a complete loss of SWR.

In the case of soil moisture content (SMC), initial values for unburned (UB) samples at both surface and depth (Figure 6a,b) at T0 were 0.54 ± 0.003 g/g and 0.43 ± 0.007 g/g, respectively. At the surface level, all burn treatments showed significant differences from UB ($p < 0.01$), with the highest evaporation observed in HL, followed by LS (0.37 ± 0.007 g/g) and HS (0.38 ± 0.004 g/g), which did not show significant differences among them. The same trend was observed at the 1–3 cm depth, except this time HS (0.44 ± 0.01 g/g) did not differ significantly from UB. After five months, UB values were 0.64 ± 0.47 g/g in the surface and 0.50 ± 0.08 g/g in depth. Only in T5+ at the surface, there were significant differences between the UB and all burn treatments.

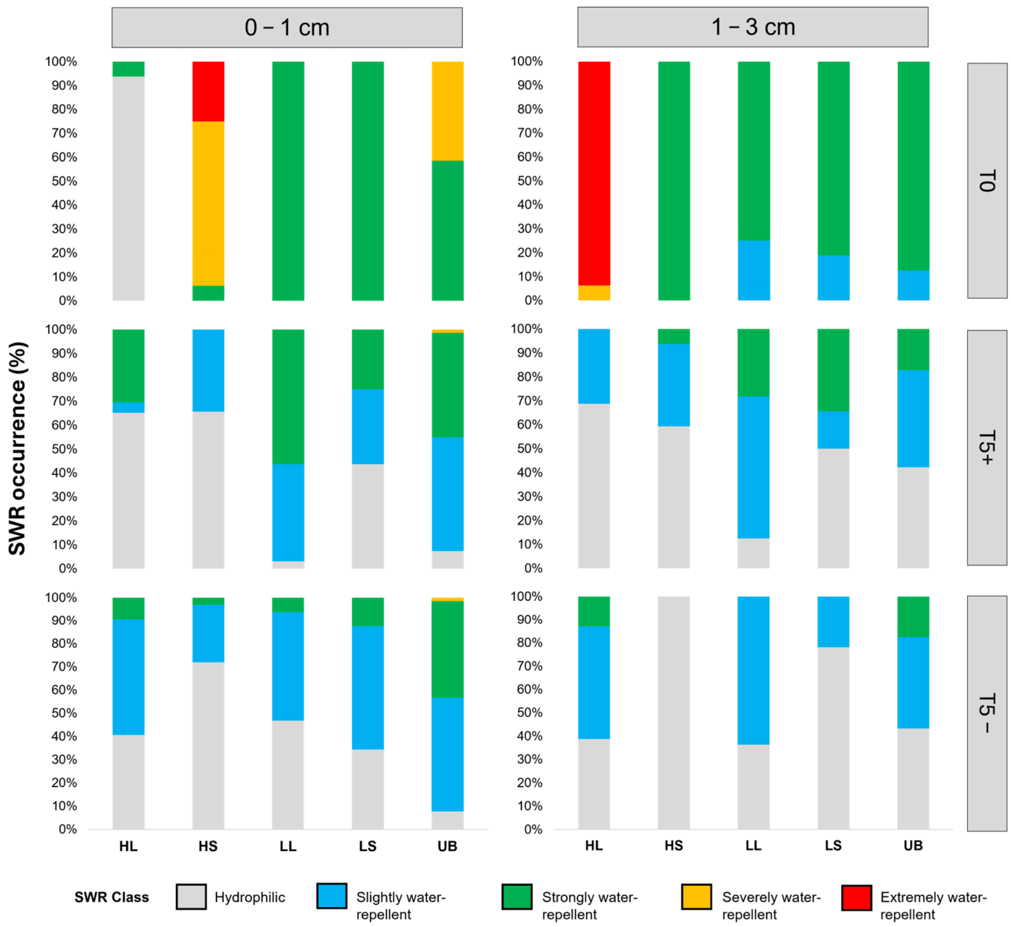


Figure 5. Fire effects on soil water repellency (SWR) at 0–1 cm and 1–3 cm soil depths, according to the classes (occurrence, in %) of the Water Drop Penetration Time test: just after burning (T0), and five months later (with ashes, T5+ or without ashes, T5−).

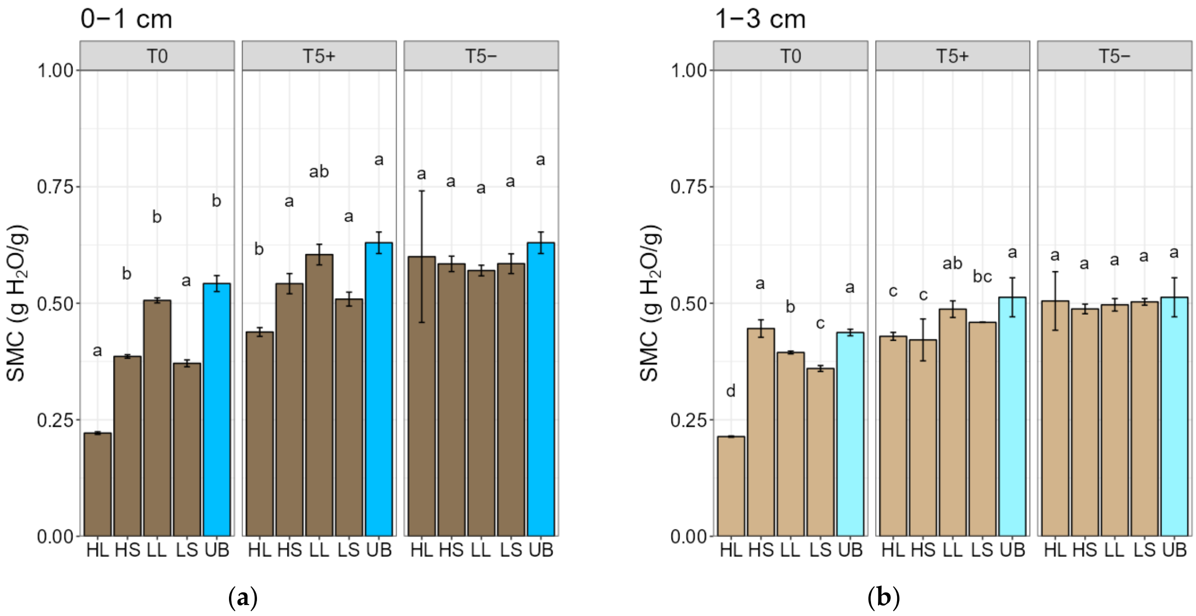


Figure 6. Fire effects on soil moisture content (SMC) at 0–1 cm (**a**) and 1–3 cm (**b**) soil depths. In each bar, the mean and the standard deviation are represented. Letters on top of the bars indicate significant differences ($p < 0.05$) among burning treatments for each time period: just after burning (T0), and five months later (with ashes, T5+ or without ashes, T5−).

3.5. Relationship Between Soil Properties and Burning Parameters: ASCA

In Figure 7, the scores and loadings of the ASCA are shown. The multivariate analysis outlined that there were significant differences between the different burn types ($p < 0.01$) and times ($p < 0.01$).

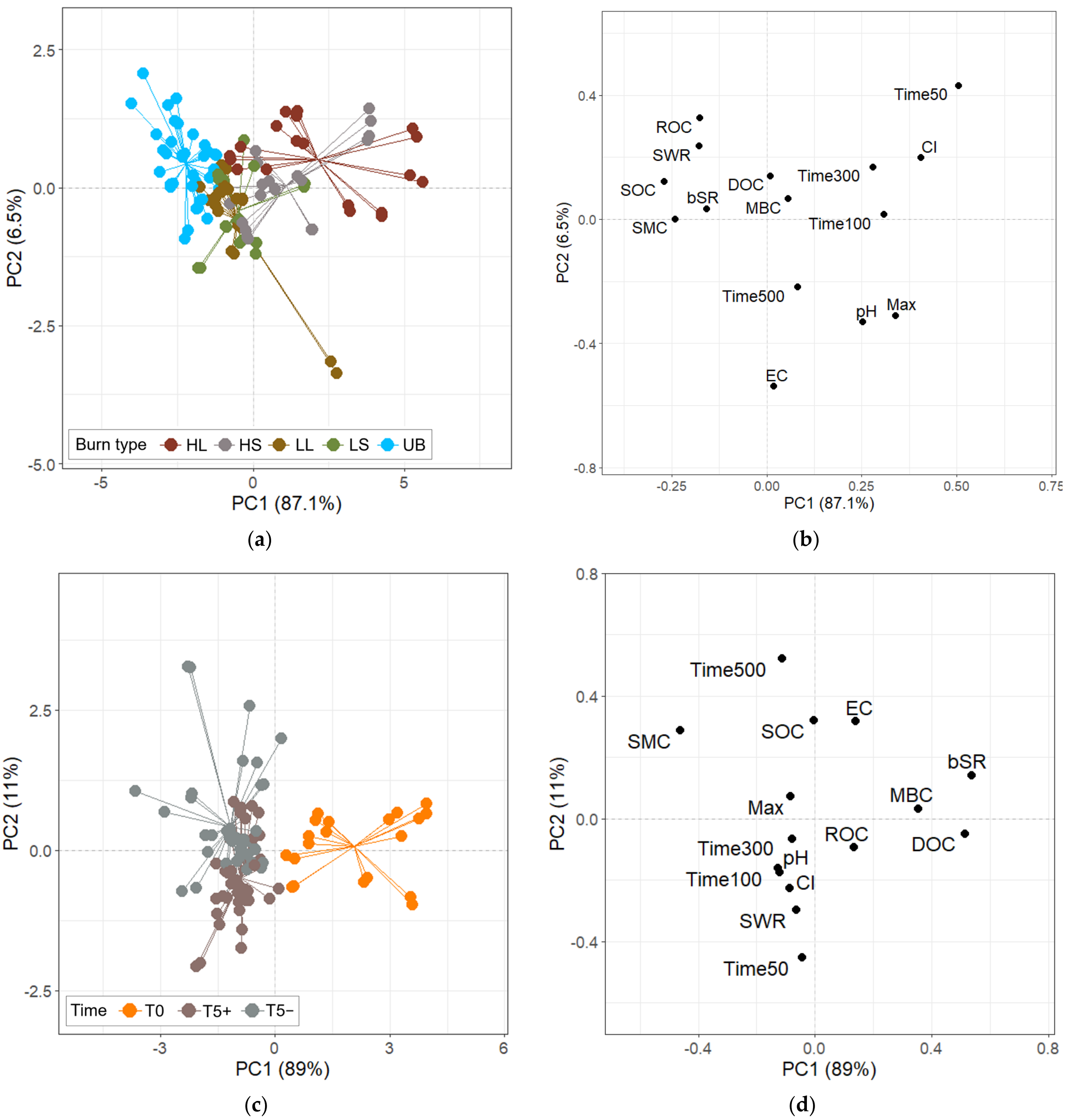


Figure 7. Score and loading plots of the ANOVA simultaneous component analysis (ASCA): scores (**a**) and loadings (**b**) for burning treatments; scores (**c**) and loadings (**d**) for time periods (T0, T5). All abbreviations are provided within the text.

For the burn type, the first two principal components (PCs) accounted for 93.6% of the variance (Figure 7a,b). PC1 explained most of the variance (87.1%) and we hypothesized that it was related to the temperature of the fire intensity applied to the soil in the different burn types. As can be seen in Figure 7a, the samples were distributed along the x axis depending on the fire intensity, following a gradient from the negative scores, corresponding to the UB ones, to the ones with the highest scores, corresponding with the most intense burn (HL). The other burn types were located in between. LS and LL were not differentiated from each other, but they were differentiated from both the control (UB) and the higher-intensity burns (HS and HL). HS and HL were more differentiated than LS and LL, but were quite similar. As can be seen in Figure 7b, the burn characterization variables (CI, Max, Time50, Time100, Time300 and Time500) had the highest PC1 loadings and were related to the HL and HS burn types. The most sensitive variables (TOC, SMC, ROC, bSR, SWR), that were more affected by the burns, were related to the UB samples and had the lowest PC1 loadings. The variables that were positively affected by the low–moderate-intensity burns (DOC, MBC, EC) were related to them and showed close to neutral loadings.

For the time, PC1 explained 89% of the variability and PC2 only explained 11% (Figure 7c,d). In this case, the samples were distributed along the x depending on the time (Figure 7c). However, we related PC1 to this factor. T0 samples were clearly differentiated from T5 samples and showed the highest scores. However, no differences attributed to the persistence of ash were observed and T5+ and T5− samples showed very similar negative score values. In Figure 7d, the SMC had the highest negative PC1 loadings and was related to the T5 samples, when it experienced its highest values. In contrast to the SMC, the variables demonstrated immediate post-fire increases, where microbial activity (bSR), DOC, and MBC showed the highest PC1 loadings and were related to the T0 samples.

4. Discussion

4.1. Fire-Intensity Effects on Quantity and Quality of Soil Organic Carbon

Changes in the amount of soil organic matter (SOM) could have resulted from two different fire effects: the removal of most of the litter layer and organic compounds from the upper centimeters of the mineral soil when high temperatures were reached, resulting in their oxidation and the production of volatiles and gases; and the addition of charred substances from incomplete combustion, generating pyrogenic organic matter (PyOM) [65,66]. These effects normally occur at temperatures above 200 °C, where combustion begins, and the formation of PyOM takes place [28]. Combustion of SOM below 450 °C is incomplete [66], while combustion above this temperature is total [37]. In this study, temperatures exceeding 450 °C were reached in both HL and HS; however, the immediate effect of reduced SOC appears to be observed only in the most intense burn (HL), likely due to its longer duration. This also explains the lack of significant effects of fire on SOC in depth, as these temperatures were not reached in any type of burn at 1–3 cm. In LS and LL, it is likely that an effect on the quality rather than the quantity of soil organic carbon (SOC) occurred, as temperatures reached 200 °C but did not exceed 450 °C.

These findings are consistent with reports by various authors, who observed considerable superficial reductions in this parameter in soils under *Echinospartum horridum* and *Buxus sempervirens* L. (Buxaceae), in areas close to the sampling sites, immediately after burns with parameters corresponding to those of HL and HS in our study [21–23,54].

After five months, it was difficult to determine whether there was any trend in the variation in SOC. This may have been due to the spatial variability of the samples, which makes it difficult to determine whether there was any trend in the variation in SOC. Normally, over the long term, there is an increase above pre-fire levels of SOC [26]. However, Girona-García et al. [22] did not observe any variation after 6 and 12 months, attributing this to a low incorporation of ashes and charred materials. Neither Alfaro-Leranoz et al. [24] nor Girona-García et al. [21] found significant differences between the control and the immediately post-burn or the short- to medium- term post-burn periods. Specifically, the samples used in [21] correspond to the same extraction area as the monoliths used.

On the other hand, we hypothesized that qualitative changes in SOM occurred due to the generation of PyOM. This encompasses all organic compounds formed or transformed by the effects of fire, such as partially charred biomass, charcoal, and soot [66,67]. They represent a continuum of combustion products, characterized by high carbon content, heterogeneous chemical composition, and dominated by aromatic structures, ranging from minimally thermally altered plant residues, which are easily biodegradable, to highly condensed polycyclic compounds like soot [68,69].

Typically, labile organic compounds in soil SOM can be transformed into aromatic or heteroaromatic structures, highly resistant to biological degradation due to carbonization. However, thermal shock can also transform SOM by breaking down complex organic substances, converting them into products with lower molecular weight and biochemically simpler structures, a process generically referred to as pyrolysis [35,70,71]. Pyrolysis could explain the increase in dissolved organic carbon (DOC), although the production of PyOM at low temperatures typically results in more labile carbon fractions than those produced at high temperatures [72]. However, authors such as Choromanska and DeLuca [73] observed an almost twofold increase in the content of soluble sugars as the temperature rose from 160 °C to 380 °C, suggesting that this could be a consequence of the release of carbohydrates from the lysis of microbial and plant tissues, and the distillation of partially decomposed litter. It appears that, for DOC, there was a prevailing effect of high temperatures, as HS and HL showed significantly higher values at the surface. Also, in HL, there was sufficient time to transmit higher temperatures deeper into the soil, resulting in values higher than those at the surface.

The reduction in these labile forms of SOM after five months in both UB and burned samples aligns with the observations made in previous studies, [22,24], whose authors also did not observe immediate effects, although they did report a reduction in the short- to medium- term following the fire. They attributed this behavior to the assimilation or mineralization of those labile forms of SOM by a recolonizing and actively growing microbial biomass following the fire, or to the separate or combined action of leaching, as well as to a reduction in soil organic matter inputs from the organic layers.

In the case of leaching, the vertical transport of pyrogenic organic carbon (PyOC) by water flow may occur, depending on the intrinsic properties of the compound and the soil [72], which was feasible here considering the moisture content of the monoliths after five months. The hypothesis of microbial utilization of labile carbon formed during the burn was supported by a positive correlation (Appendix A, Table A1) between DOC and bSR (r = 0.72), suggesting that this type of carbon was utilized by microorganisms until nearly depleted over the five months following the burn. Alfaro-Leranoz et al. [24] also observed a strong positive correlation among these parameters.

One of the compounds formed during the production of PyOM is black carbon (BC). This term refers to structures of organic carbon generated by the incomplete combustion (carbonization) of SOM and woody residues during a fire, originated at temperatures between 250 and 500 °C [26,74,75]. As the carbonization temperature and fire intensity increase, its aromaticity also increases [69,71], and it generally has a longer residence time in the soil than the SOM from which it derives [68,75].

It is worth highlighting the high content of recalcitrant carbon in UB at T0. Given that Mediterranean ecosystems are prone to wildfires and are commonly managed using traditional fire-based practices, the content of BC in their soils under pyrophytic species is expected to be notable [76]. In soils that have experienced frequent fires, BC can account for 35 to 40% of the total organic carbon. This suggests that the high content of recalcitrant carbon in UB samples at T0 might have been due to the BC formed through the historical fire management in the area [10,77,78].

Our findings at T0 diverged from the typical increases observed in this fraction of SOC following a fire [26]. One explanation for this discrepancy could be the loss of carbon through emissions into the atmosphere, forming the atmospheric fraction of PyOC, which corresponds to the smaller particle size fractions that are more recalcitrant in these products [72]. This volatilization effect would likely be more pronounced in the high-intensity treatment (HL) due to the formation of a hydrophobic layer at depth. Furthermore, the transformation of ROC into labile C may have occurred through the pyrolysis process associated with DOC, considering that no loss of SOC was recorded in LS, LL and HS treatments. Another possible explanation for the loss of recalcitrant carbon in samples subjected to higher-intensity burnings is that it may have undergone aromatization to such an extent that it no longer reacted with chromic acid. Knicker et al. [79] have suggested that methodologies employed to estimate the BC content of SOM through strong acid oxidation are questionable, owing to the resistance of BC to destructive chemical methods, such as potassium dichromate oxidation, due to its refractory nature. Certini et al. [80] observed in a study on the impact of fire in Mediterranean pine forests that the fractions resistant to dichromate oxidation primarily consisted of aromatic carbon, with a lesser contribution from alkyl C and O-alkyl C derived from PyOC. This could also account for the observed reduction in SOC in the HL treatment, as these two properties had a high positive correlation ($r = 0{,}72$). Additionally, Velasco-Molina et al. [81] observed in an Umbric Ferrasol in Brazil that, at depth, a reduction in the recalcitrance of PyOC occurred due to a high substitution of aromatic molecules by carboxyl groups, thus increasing the efficiency of oxidation through dichromate. This suggests that, in this study, the fire may have led to new aromatization into compounds resistant to acid oxidation, and that the observed difference compared to UB was due to the latter containing more oxidizable recalcitrant forms due to the passage of time, thereby allowing for higher levels of recalcitrant carbon to be detected through the method used.

While our data do not permit a definitive conclusion regarding whether the observed phenomenon represents a genuine loss of recalcitrant carbon or merely a reduction in its chemical reactivity to dichromate, there is indirect evidence suggesting carbonization, as indicated by the darker soil color and the presence of charred remnants in the most intense burned monoliths [66].

If the quantification of recalcitrant carbon was accurately conducted, the short-term invariability suggests the following two possibilities: (i) that recalcitrant forms originating from PyOC remained unaltered in the short term, exhibiting resistance to microbial degradation; (ii) if fire, indeed, reduced this parameter or if no incorporation of this type of PyOC occurred through ashes, the soil's carbon sink potential within the top three centimeters could have been diminished. This is significant as many pyrogenic processes increase SOC recalcitrance, thereby extending its residence time (decades to centuries [82]) compared to its precursors [28]. Additionally, it is worth considering that perhaps insufficient time had passed for ashes, potentially containing BC, to integrate into the soil.

4.2. Fire-Intensity Effects on Other Chemical Soil Properties: pH and EC

EC generally increases due to a rise in soluble ions released by SOM combustion, followed by a reduction over time due to precipitation, salt fixation, and leaching. Meanwhile, the increase in pH was attributed to the alkaline nature of the ashes, with a return to pre-fire values depending on fire intensity [83]. Short, medium, and long-term fire effects on pH and EC in soils from the same region show varied patterns [23,24,54]. In this study, the similarity among burn types for these parameters may allow for discrimination between burned and unburned samples but apparently does not enable differentiation among burn intensities. For instance, the increase in EC as a result of fire may have not been directly proportional to its intensity, as observed by Badía et al. [84], who reported an EC increase up to 250 °C followed by a decline at 500 °C in gypseous and calcareous soils from the Ebro Valley. These authors observed a significant correlation of EC with total soluble ions [84].

4.3. Fire-Intensity Effects on Soil Microbial Biomass Carbon and Soil Respiration

The soil properties primarily affected at temperatures below 200 °C are the biological ones, including the death of fine roots, loss of microbial biomass, and reduction in the seed bank [28]. Typically, the zone of greatest biological activity coincides with the depth that experiences the highest temperatures during a fire, which corresponds to the upper 2.5 cm of the soil. The immediate effect of fire on soil microorganisms is the reduction in their biomass [36], which can lead to a sterilization effect [26,85], as observed in HL at 0–1 cm and in HS at 1–3 cm. Complete sterilization of metabolically active organisms in the uppermost soil layers can occur; however, inactive forms may survive due to the formation of spores and adaptation to drought stress [36]. Furthermore, the effects of fire on soil biological properties can also be attributed, not only to the direct impact of fire, but to changes in both SOM quality and microbial communities [21]. Thus, the increase in bSR as an immediate effect of fire may have been produced due to the rapid microbial recolonization stimulated by the incorporation of DOC and the increased nutrient availability [71].

In calcareous soils, Badía et al. [86] also found that intermediate heating (150 °C and 250 °C) stimulated bSR; nevertheless, at the highest experimental temperature (500 °C), both bSR, as well as other biological properties (metabolic quotient or specific soil respiration and colony forming units of both bacteria and fungi), were significantly reduced.

In bSR, a reduction effect was only observed in HL, likely because the sterilizing effect on the soil outweighed the significant release of DOC from pyrolysis. For the remaining burn treatments, an increase in bSR was observed, potentially linked to the increase in DOC content (as previously discussed) and the fact that microbial biomass was only slightly affected. For LL, a significant increase in MBC was observed, while LS showed no changes regarding to UB, which is consistent with findings in low-intensity or short-duration burns [87]. Thus, for MBC, high temperatures appeared to be more influential than residence time, given the lack of differences noted between them, while the effect of time was more pronounced at lower temperatures. In contrast, for bSR, a predominant effect of time was observed, as evidenced by lower values (HL) or smaller increases relative to other burns (LL) in those of longer duration.

The effect on microbial parameters after five months suggested that, once labile carbon began to become scarce at some point during this period, microorganisms may have experienced a significant decline due to their inability to utilize recalcitrant and intermediate carbon. Consequently, there may have been sufficient labile carbon for the remaining microorganisms to proliferate in the more intense burns, potentially reaching biomass levels similar to those in the less intense burns at T0, until the scarcity of labile carbon triggered the same reduction process seen in the less intense burns. This process was observed by Bárcenas-Moreno at al. [88] after a fire in an area of Mediterranean sclerophyllous vegetation. They identified different phases of microbiota post-fire recovery that align with those observed in this study and they hypothesized that recolonization of vegetation could further normalize the data.

Similar effects on the reductions in microbial biomass and microbial respiration after a period of time following fire were also described by Fontúrbel et al. [87] and D'Ascoli et al. [89], attributing this to microbial immobilization and the consumption of easily biodegradable nutrients and materials released after burning, as well as changes in nutrient inputs due to the removal of vegetation cover. De Marco et al. [90], in burns in Mediterranean maquis with similar characteristics to LL and LS, observed a short-term increase in respiration and endogenous mineralization coefficient, attributing it to changes in water content, soluble C, and nutrient availability. They also noted that microbial biomass after fires could be dominated by r-strategists, which have high energy consumption per unit of biomass, and are highly efficient in decomposition, but less efficient in biomass generation. On the other hand, the reduction in MBC values for UB after five months may have been a response to the alteration of the microhabitat created by the plant cover and biomass of shrubs for soil microorganisms [21].

The effects of fire on soil biological properties in similar zones observed in previous studies are varied. Our results align with those reported by Armas-Herrera et al. [54], who observed a reduction in MBC and bSR in the 0–1 cm depth. Conversely, in the same zone, Girona-García et al. [21] noted no direct fire effects on bSR or MBC but observed an increase relative to control soil after five months.

4.4. Fire-Intensity Effects on Physical Soil Properties: Water Repellency and Moisture Content

Fire-induced changes in SWR are caused by various factors, including the coating of mineral particles with hydrophobic microbial products, leachates from organic materials, or irreversible desiccation of SOM. Generally, fire can produce the following two effects: an intensification of existing repellency or its emergence through the partial combustion of SOM, or destruction via oxidation on the surface and/or development in deeper layers through translocation [91–94].

The relationship between temperature and SWR was described by Debano [95] as follows: minor changes occur below 175 °C; between 175 °C and 200 °C, intense repellency is developed; and between 280 °C and 400 °C, repellency is destroyed. In our study, we observed both typical effects, as follows: the formation of a repellent layer in depth and its almost complete elimination at the surface in the case of HL, and an increase at the surface in HS. The HL results align with those reported by DeBano et al. [96], where organic materials were translocated deeper after an experimental burn, and SWR was partially destroyed in the upper layer. The temperatures that cause repellency destruction were reached in all burns, which could explain the surface reduction in HL, LL, and LS but not the intensification in HS. This suggests that repellency destruction depends on the combined effect of high temperatures and long burn times. Krames and DeBano [97] found that repellency could either be intensified or destroyed under extreme temperature conditions during a fire, depending on the duration: 300 °C for 30 min is enough for surface destruction, whereas approximately 200 °C for 10 min may intensify it.

On the other hand, the process explaining the formation of the hydrophobic layer in depth in HL is the vaporization of organic substances at the surface, which diffuse in a gaseous state into deeper layers following temperature gradients until they encounter cooler layers where they condense [27,98]. The formation of this layer is more influenced by the presence of a sharp temperature gradient than by the absolute temperature reached, as the vaporized substances move according to these gradients in a process similar to distillation [98]. Additionally, for the hydrophobic substance to bind and cause extreme repellency, further heating after condensation is necessary [96]. Only the HL burn at depth showed, along with a strong temperature gradient, an extended duration above 50 °C in depth.

Girona-García et al. [31] observed that, in the control samples, water repellency ranged from strongly to extremely repellent, which was reduced to slightly repellent in the 0–1 cm layer following fire. They identified a relationship between the increase in SWR and the loss of SOC, attributing this effect to the destruction of natural hydrophobic SOC. However, in their regression analysis, they noted that SOC levels did not explain SWR variability in samples immediately post-fire or six months later, suggesting that the quality of SOC and fire-induced changes might have also contributed to this variability. Other researchers have similarly indicated that the quality of SOM is more crucial than its quantity for SWR [35,99]. In this context, we found a positive correlation between SWR and DOC (r = 0.52), suggesting that the PyOC contributing to or intensifying repellency may be labile in nature [33].

After five months, it appears that the effects of the controlled burns had either disappeared or significantly diminished. This trend is common when fire causes or increases soil water repellency [100,101]. This attenuation may have happened due to continuous moistening of the soil monoliths in the greenhouse, subsequent leaching, chemical transformation of organic substances that induce repellency, and/or microbial consumption. Notably, soils can lose repellency during extended wet periods as amphiphilic molecules coating solid components reorient themselves [91]. Additionally, the oxidation of hydrophobic PyOM on the surface can facilitate its transfer to the soil solution [81,102], where consistent water flow can remove it via leaching [103]. In this context, a negative correlation was observed between this property and SMC (r = −0.4).

Microbial biomass activity is also capable of mineralizing a fraction of hydrophobic PyOM [101,104]. A practical example of this is the use of microbial community inoculation in water-repellent soils as a remediation method. Specifically, Lowe at al. [105] observed that microbial activity was essential for eliminating repellency in a severely water-repellent soil, and that solely applying physical treatments would have been insufficient for the permanent removal of repellency.

The natural surface moisture values seemed slightly higher than those recorded in December at the same site by Girona-García et al. [21]. The more significant moisture loss observed at depth in HL can be explained by the longer residence time of this burn type. High moisture content in the samples can limit heat transfer to deeper layers, as the energy required for vaporizing water prevents temperature increases until complete evaporation occurs [106]. Badía et al. [107] confirmed that the increase in the thermal capacity of moist soil (at field capacity) plays a more important role than the increase in thermal conductivity in heat transfer in burned soils [106].

Only T5+ at the surface showed differences compared to UB, suggesting that ash presence may have reduced infiltration, possibly due to its high water-storage capacity [66].

5. Conclusions

The burning gradient generated by the combination of temperatures and the duration of the burns has allowed us to observe a range of effects on most soil properties. In general, the results showed that temperature is more influent than the heating duration in the post-fire effects, especially at low temperatures. Most of the fire effects were found at 0 to 1 cm, while at 1 to 3 cm soil depth, hardly any changes were observed. This finding highlights the need for finely stratified sampling in studies of this nature, as broader surface samples can dilute detectable fire effects. The degree to which the thermal gradient affects the soil varies with the property analyzed. Therefore, fire impacted both the quantity and quality of soil organic carbon. Quantitatively, it caused a significant loss of organic carbon at 0–1 cm. Qualitatively, fire generally increased labile carbon levels and reduced recalcitrant carbon in this surface layer. Microbial properties showed a diverse response to the different fire intensities. One of the most notable impacts was the near-total elimination of microbial biomass carbon in high-temperature burns. However, microbial activity was stimulated by low-severity burnings, which was related to the increase in labile carbon, easily available to microorganisms, which increased significantly with this type of burning.

Regarding soil water repellency, it is very notable that before burning, soil under shrub cover exhibited slight natural water repellency, which was removed by low-temperature burns. In contrast, high-temperature burns developed significant water repellency in the surface or the subsurface layers, depending on the burn duration. Moreover, pH and electrical conductivity seemed to remain stable among all burned types.

After five months, burn effects largely disappeared across most parameters in both ash-preserved and ash-free monoliths, likely due to continuous moisture exposure, leaching, and/or microbial consumption. In these conditions, leaving or not leaving ash on the surface was not relevant for most soil properties.

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

Funding: This project was supported by a research grant funded by the Provincial Council of Huesca for the project entitled "Prescribed burning as a tool to prevent large forest fires in the Central Pyrenees (XXV edition of Félix de Azara Research Project Call 2022)". Moreover, Andoni Alfaro-Leranoz was funded with a predoctoral research scholarship (BOA20200713012) granted by the Government of Aragón.

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

Acknowledgments: We are grateful for the support received from the technicians in the Soils Laboratory of the Escuela Politécnica Superior (Technological College) of Huesca, University of Zaragoza (Spain), where this research work was conducted. The experimental design of this project was conceived by Oriol Ortíz-Perpiñá, who passed away on 24 June 2023, to whom we dedicate this work.

Conflicts of 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.

Appendix A

Figure A1. Location of sampling area. (**A**): Spain; (**B**): Central Pyrenees; (**C**): Puerto Sobás.

Table A1. Pearson correlation coefficients, showing the degree of linear association (negative and positive) between soil properties, for all treatments, times and sampled depths. * $p < 0.05$, ** $p < 0.01$.

Variable	SOC	DOC	ROC	MBC	bSR	SWR	pH	EC
DOC	0.16							
ROC	0.72 **	0.30 **						
MBC	0.04	−0.06	0.16					
bSR	0.48 **	0.72 **	0.53 **	0.20 *				
SWR	0.02	0.52 **	−0.02	−0.04	0.30 **			
pH	0.17	0.02	0.18	−0.01	0.13	0.04		
EC	0.16	0.35 **	0.24 **	−0.01	0.30 **	0.06	−0.04	
SMC	0.63 **	−0.36 **	0.48 **	−0.07	−0.02	−0.40 **	0.12	−0.07

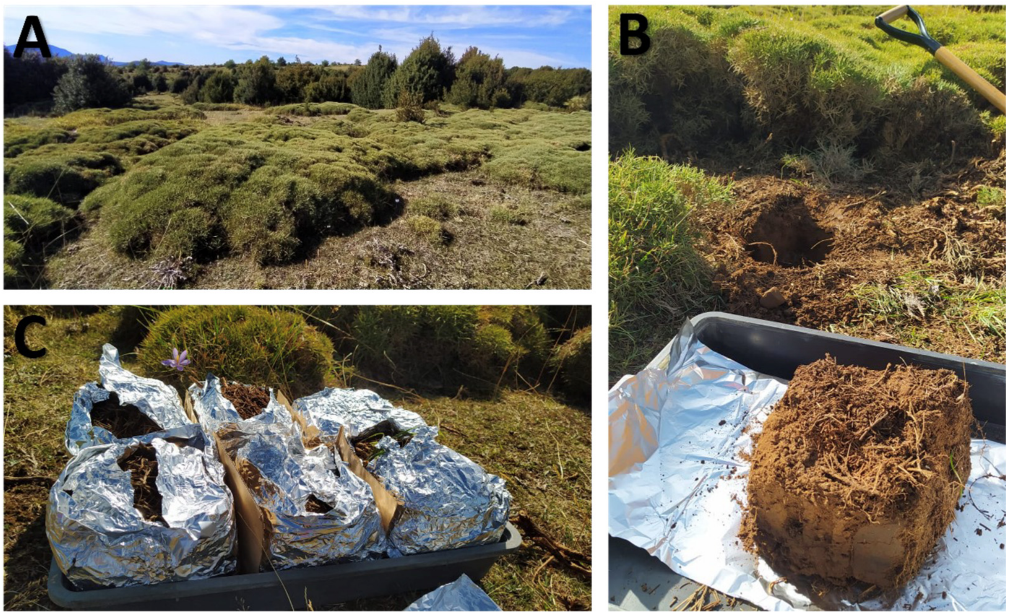


Figure A2. Block sampling procedure. (**A**): Sampling area; (**B**): extracted soil block and extraction area with the surface free of shrubs; (**C**): blocks prepared for transport.

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