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Fire effects on biochemical properties of a semiarid pine forest topsoil at cm-scale

Janielle S. Pereira [a], David Badía [b,*], Clara Martí [b], Juan Luis Mora [c], Vanessa P. Donzeli [a]

[a] Federal University of Lavras, Department of Engineering, Lavras 37200–900, Minas Gerais, Brazil
[b] GEOFOREST – Instituto de Investigación en Ciencias Ambientales (IUCA), Escuela Politécnica Superior de Huesca, Universidad de Zaragoza, Ctra. Cuarte s/n, 22071 Huesca, Spain
[c] GEOFOREST – Instituto de Investigación en Ciencias Ambientales (IUCA), Facultad de Veterinaria, Universidad de Zaragoza, C/Miguel Servet 177, 50013 Zaragoza, Spain

A R T I C L E I N F O

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A B S T R A C T

Forest fires can greatly affect soil properties and processes. In the study of the fire effects on soil, the soil thickness affected by heat depends on the characteristics of the fire and soil itself, but also on the attribute to be measured. The objective of this work is to know to what thickness (up to 1, 2 or 3 cm) various sensitive soil properties are immediately affected by a controlled burning. To achieve this aim, unaltered fresh topsoil (mollic horizon) of a fire-prone Aleppo pine forest in the semiarid Ebro Valley (NE-Spain) were sampled and, without destroying their original structure, burned from the surface in an outdoor combustion tunnel in triplicate. Biological properties are measured, including basal and normalized soil respiration (bSR and nSR), β-D-glucosidase (GLUase) and phosphomonoestarase (PHOase) activities, and related parameters, such as total organic matter (TOM), oxidizable organic C (OxC), nonhydrolyzable carbon (NHC), P-Olsen, pH, soil moisture and soil water repellency (WR). In the unburned soil, most of these properties showed a decreasing gradient with depth which is modified after burning, in some cases inverted (as enzymatic activities and *WR*), in others intensified (P-Olsen) and in most, truncated, with a maximum value in the second cm. The depth of the soil in which changes were recorded varied according to the attibute considered; thus, burning significantly decreased only up to the first cm: bSR (73 %) and TOM (81 %), up to 2 cm: PHOase (89 %), OxC (17 %) and *WR* (96 %) and up to 3 cm depth GLUase (58 %), NHC (24 %) and moisture (73 %). However, P-Olsen and pH both increased after burning up to 1 and 3 cm soil depths, with increases of up to 240 % and 11 %, respectively. In conclusion, fire effects on soil are depth dependent, and this dependency is not uniform across soil properties.

1. Introduction

Forest fires, one of the main disturbances in terrestrial ecosystems (Ford et al., 2021; McGranahan and Wonkka, 2020), have increased lately in number and severity due to climate warming (IPCC, 2022) as well as changes in land use in last decades (Shakesby, 2011; Caon et al., 2014). Severe fires, in addition to the evident loss of forest biomass, lead to drastic consequences for forest environments by modifying their surface microclimate, nutrient cycling, water dynamics and especially soil properties (Barreiro and Díaz-Raviña, 2021). Changes in the soil reaction, the organic matter amount and quality, the nutrient availability, etc. have been reported after wildfires and prescribed fires (Certini et al., 2021; Bento-Gonçalves et al., 2012), the magnitude of which depends on the temperature reached and its residence time (Lombao et al., 2020). However, even focusing on the immediate effects of burning, a wide range of results have been reported (Mataix-Solera et al., 2009; Muñoz-Rojas and Bárcenas-Moreno, 2019). Such variability has been attributed to several factors operating simultaneously: soil moisture, timing of postfire soil sampling, thickness of the sampled soil, type of ecosystem (Lombao et al., 2015; Rodríguez et al., 2018; Lucas-Borja et al., 2019; Huerta et al., 2020), fire severity related to the spatial variability of the fuel plant biomass (Keeley et al., 2012) and the forest soil composition (Alcañiz et al., 2018; Holden et al., 2016; Pivello et al., 2021). This multifactorial variability is common when comparing recently burned areas with adjacent unburned ones, assuming that they were similar before the wildfire (Badía et al., 2014a; Panico et al., 2020;

* Correspondence to: Escuela Politécnica Superior/Technological College, Universidad de Zaragoza, Ctra. de Cuarte s/n, 22071 Huesca, Spain.
E-mail address: badia@unizar.es (D. Badía).

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Table 1
Maximum temperatures (°C) and persistence of temperatures (minutes) recorded in the soil monoliths (mean ± sd of three replicates) during the burning experiment.

Ah-horizon					
Variables		Surface	1 cm	2 cm	3 cm
Maximum temperature (°C)		937 ± 143 °C	293 ± 40	156 ± 39	78 ± 1
Duration (min)	< 100 °C	2.3 ± 1.9	9.7 ± 0.6	12.5 ± 3.5	35 ± 0
	100–200 °C	2.3 ± 1.2	12.7 ± 8.0	16.5 ± 4.9	–
	200–300 °C	6.3 ± 3.8	12.0 ± 5.7	3.0 ± 5.2	–
	300–400 °C	4.3 ± 4.0	1.0 ± 1.7	1.0 ± 1.7	–
	> 400 °C	19.7 ± 3.2	–	–	–

Rodríguez et al., 2018; Sáenz de Miera et al., 2020). To isolate field variables and to discern what effects generate temperature gradients on soil properties, other experimental approaches are used, such as laboratory ovens (Badía and Martí, 2003a; b; Giovannini, 2012; Lombao et al., 2021; Martínez et al., 2021; Pereira et al., 2019a) and controlled surface burns of undisturbed soil blocks (Aznar et al., 2016; Badía-Villas et al., 2014b; Lucas-Borja et al., 2019).

Usually, the immediate wildfire effects on soil properties are limited to the upper centimeters of the soil because of the soil's high thermal inertia (Sacchi et al., 2015; Simon et al., 2016). Thus, in a severe Aleppo pine forest wildfire, no heating effects on the qualitative or quantitative composition of soil organic matter were observed below 2 cm depth (Badía et al., 2014a). In a controlled burn of undisturbed and dry soil blocks, changes in physical and chemical properties were noted in the first centimeter and rarely in the second one, but when that soil was moist, hardly any changes were found, not even in the first cm (Badía et al., 2017). However, biological properties, the most sensitive (Santín and Doerr, 2016) decreased even below 2 cm soil depth in areas of prescribed bush burns carried out under moist conditions (Armas-Herrera et al., 2016; Girona-García et al., 2018). Several meta-analyses (Holden and Treseder, 2013; Pressler et al., 2019), compiled from a large amount of literature, concluded that much of the microbial activity variation after fires is far from being explained.

For all these reasons, the aims of the research were (i) to evaluate the immediate effects of a controlled burn of a fresh topsoil, from a fire-prone Aleppo pine forest, on various microbial and soil enzyme activities and related properties, and (ii) to determine to what depth, on a centimeter scale, each of these properties are affected by heating. The findings of the research can provide innovative information on the differential sensitivity of these soil properties to heating; moreover, this study on biochemical properties complement our previous studies focused on standard physical and chemical properties (Aznar et al., 2016; Badía et al., 2017; Badía-Villas et al., 2014b).

2. Materials and methods

2.1. Experimental design and sampling

The experimental topsoil was sampled in the Montes de Zuera, located within the semiarid Central Ebro Valley (NE-Spain), under a fire-prone Aleppo pine forest (*Pinus halepensis*), at 630 m above sea level (Badía et al., 2013). The topsoil (a mollic horizon from a Rendzic Phaeozem) has a clay-loamy texture, very strong granular structure, high soil aggregate stability and porosity, high organic matter content, high calcium carbonate content, and a basic soil reaction (Badía et al., 2013). Six unaltered topsoil blocks (25 × 25 cm square × 15 cm depth) were carefully removed from the field with a flat shovel and transported to the laboratory in closed containers as described by Badía-Villas et al. (2014b). They were sampled in a homogeneous site (less than 1 m^2) under an old pine forest; the samples intended for burning and control were spatially interspersed. At the time of sampling, the soil blocks collected were moist (412 ± 104 g kg^{-1} in the upper 3 cm). Half of the blocks were burned in an external combustion tunnel, and the other half were preserved (unburned control samples). K-type thermocouples were arranged at four levels: at the surface, at 1 cm, at 2 cm and at 3 cm depth of the horizon Ah, where the temperature was recorded every minute (with a Picotech TC-08 recorder). The burning was caried out with a blowtorch (10 cm in diameter) placed 40 centimeters over each unaltered soil block. The flame was applied until it reached 250 °C at a depth of 1 cm, since it was intended to simulate a severe fire (Hungerford, 1996), which took place at approximately 15–17 min; at that time, the torch was turned off, although the duration of the temperature measurements was extended to 35 min. The maximum temperatures (°C) and the persistence of temperatures (minutes) are reported (Table 1).

2.2. Soil analysis

All soil blocks (three unburned and other three burned) were layered at 0–1, 1–2 and 2–3 cm soil depths, sieved on a 2 mm mesh and packed in plastic bags under refrigeration (4 °C) until all analyses were performed. For the different analytical determinations, the methods described in Page et al. (1982) were followed. Potential microbial activity was measured by capturing CO_2 emitted from the soil in NaOH traps on selected days during an incubation period of 62 days at ½ field capacity and 25 ºC. From this assay, we calculated the basal soil respiration (bSR) or C-CO_2 efflux over this period as well as the normalized soil respiration (nSR) as SR per oxidizable C unit and time, i.e., the rate of organic carbon mineralization (Anderson and Domsch, 1978). Two soil enzyme activities were measured: β-D-glucosidase enzyme activity (GLUase), following Eivazi and Tabatabai (1988), and acid phosphomonoesterase activity (PHOase), following the Tabatabai and Bremner method, modified by Saá et al. (1993).

Given that indirect effects (derived from changes in soil reaction, carbon and phosphorus availability, etc.) may be added to the direct effects of heat, other soil properties have been measured. Thus, the soil reaction was measured in a 1:2.5 soil-to-water ratio (pH 1:2.5); available phosphorus (P-Olsen) was extracted with a 0.5 M sodium bicarbonate and determined colorimetrically by molybdenum-blue method (Page et al., 1982); the total organic matter (TOM) was determined by weight loss on ignition; oxidizable soil organic C (OxC) was determined by wet oxidation with $Cr_2O_7K_2$ 0,4 N; and nonhydrolysable organic carbon (NHC) was determined following 6 M HCl hydrolysis during 18 h at 105 ºC; after washing the excess of Cl^- in the sample, it was dried at 60ºC to determine the oxidizable soil organic carbon (Rovira and Vallejo, 2007). Additionally, the soil water repellency (WR) was determined by the water drop penetration time test (Doerr, 1998). The soil moisture content was determined by gravimetry, drying the samples at 105 °C until they reached a stable weight, then used to calculate all the results on a dry soil basis. As all the blocks were collected at the same point, under the same soil formation factors, differences in the measured properties can be confidently attributed to fire severity.

2.3. Statistical analysis

Kolmogorov-Smirnov test was used to check the normality and homogeneity of variances prior to statistical analysis. The results were transformed as appropriate when the assumption of normality was not fulfilled and then submitted to the analysis of variance (ANOVA). Two-way ANOVA was performed to evaluate the effect of burning and depth, with the means compared by LSD test. When the interaction between factors was significant, treatment (burned versus unburned) and soil depth (0–1, 1–2 and 2–3 cm) were compared separately (Annex 1. Supplementary material) by the Fisher test. Maps of Pearson correlation between the soil properties were performed also using the statistical

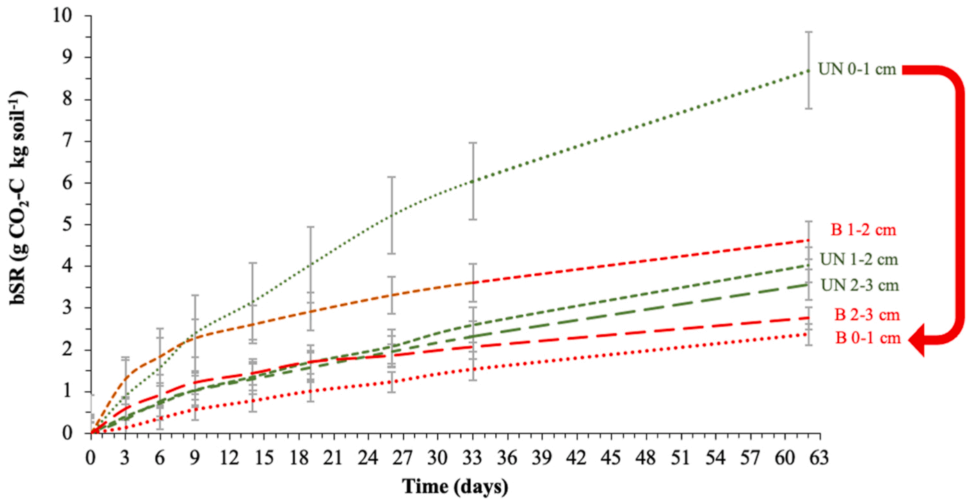

Fig. 1. Basal soil respiration (bSR) in the unburned (UN) and burned (B) samples (g CO_2 -C per kg of soil, accumulated throughout a 62-day incubation trial) by soil depth (0–1, 1–2 and 2–3 cm); the error bars indicate standard deviation of the means (n = 3). The red arrow highlights the drastic reduction in the soil respiration of the uppermost cm of soil after burning.

software XLSTAT (Addinsoft, 2022). The resistance of each soil property to fire (in %) is evaluated with the following unitless formula (Armas-Herrera et al., 2018):

Resistance= −100 [(UN-B)/UN]

Where UN is the value of the variable in the unburned control soil and B is that of the burned soil.

In addition, to obtain a synthetic view of the main gradients of variation in soil properties related to heating and soil depth, a principal component analysis (PCA) was conducted after centering and standardizing the input data by variables, which used temperature and depth as passive (supplementary) variables by means of IBM SPSS Statistics v. 22 (IBM Corporation, Armonk NY, USA).

3. Results and discussion

3.1. Biological activity: bSR and nSR

The cumulative CO_2-C emitted during the incubation assay (basal soil respiration, bSR) followed a logarithmic model (the R values are >0.90 for all log curves) with two phases that represent the consumption of more or less labile fractions of C (Fig. 1); it should be noted that the first phase, in which the most labile C would be consumed, is shorter in burned soils than in unburned soils. Notably, the most biologically active layer (0–1 cm) before burning became the least active after burning (Fig. 1).

The burning of the soil generated a significant reduction in the bSR of the 0–1 cm layer; this parameter was reduced to almost a quarter of its original value, from 8.69 g CO_2 -C kg^{-1} soil in unburned soils to 2.37 g CO_2 -C kg^{-1} soil in burned soils for the 62-day incubation trial (Table 2). The negative influence of wildfires on bSR, proportional to burn severity, has been shown by Holden et al. (2016) in boreal forests. Other works found reductions of this property in the shallowest centimeters of the soil; so, Girona-García et al. (2018), in a prescribed burning of shrublands in Buisán (Central Pyrenees, NE-Spain), found an immediate reduction in bSR up to 2 cm, which was maintained at 6 and 12 months after burning. Armas-Herrera et al. (2016) found that the microbial activity (measured as bSR and GLUase activity), as well as total organic C, of an Eutric Cambisol decreased significantly up to 3 cm measured immediately after a prescribed burning of shrublands in the Tella location, Central Pyrenees. On the other hand, Lombao et al. (2015) found a decrease in bSR, especially at 0–2.5 cm and, to a lesser degree, at 2.5–5 cm in acid soils of two types of recently burned Atlantic forests (*Eucalyptus* and *Quercus*) in the Fragas do Eume Natural Park (NW-Spain) but they were not significant.

This observed reduction in bSR in the topsoil must be sought first in the partial elimination of microorganisms by heat (Bárcenas-Moreno and Bååth, 2009; Santín and Doerr, 2016) and second to the reduction, by combustion, of the C content, an energy source for heterotrophic microbial activity (Holden et al., 2016; Martín-Lorenzo et al., 2021). The temperatures considered critical for soil biota vary according to the authors from 70 °C (Mataix-Solera et al., 2009) to 120 °C (Barreiro and Díaz-Raviña, 2021), with variations in sensitivity between different groups of microorganisms (Lucas-Borja et al., 2019; Sáenz de Miera et al., 2020; Qin and Liu, 2021).

We found bSR to be positively and very significantly correlated with OxC ($r = 0.72$, $p < 0.01$) and with TOM ($r = 0.87$, $p < 0.001$). Given these positive correlations, the nSR was calculated to determine the microbial activity per unit of soil carbon (g CO_2-C emitted per OxC kg^{-1} and day^{-1}). In this regard, soil burning only reduced the nSR at the fist cm by a quarter, whereas the bSR was reduced by three quarters of their initial values (Fig. 2). The nSR decrease, non-significant, as opposed to the bSR, may indicate that quantitative carbon loss by heating dominates over qualitative changes (Aznar et al., 2016; Badía-Villas et al., 2014b; González-Pérez et al., 2004). Thus, Panico et al. (2020), studying the effects of a severe surface wildfire on soil properties in a Leptic Vitric Andosol with different vegetation types in Vesuvius National Park (S-Italy), found a significant decrease in bSR but an increase in the nSR (as well as the C/N ratio), which they attributed to changes in the quality of the organic matter. By heating a calcareous soil in an oven, we had already found differences between both parameters; thus, while bSR (as well as the microbial biomass and the population of bacteria and fungi) progressively decreased with the increase of the applied heat (25, 150, 250 and 500 °C), the nSR only increased significantly at the highest temperature (Badía and Martí, 2003b).

3.2. Enzyme activity: GLUase and PHOase activities

A significant reduction in the GLUase activity was found at all studied thicknesses: practically total (99 %) at 0–1 cm, 78 % at 1–2 cm and 58 % at 2–3 cm (Fig. 3). Similarly, PHOase activity suffered a decrease after burning, significant at 0–1 cm (99 %) and 1–2 cm (89 %) but not at 2–3 cm, although there also decreased up to 40 % (Table 2). Therefore, enzymatic activities are biological properties especially sensitive to burning that show a strong dependence on soil depth (Fig. 3).

The strong reduction of GLUase may be a sign of a potential

Table 2
The effect of fire on biochemical properties (x ± sd) and other related soil properties at various depths. P-values compare the effect of the treatment (Unburned versus Burned) for each soil depth (cm); different lowercase letters indicate significant differences between soil depth (0–1, 1–2 and 2–3 cm) within each treatment, by F-test at 5% probability ($p < 0.05$).

	Unburned	Burned		
Soil depth (cm)	**Soil properties**			Resistance (%)
	bSR (g CO_2-C kg^{-1} soil accumulated 62 d)		*p*	
0–1	8.69 ± 2.10 a	2.37 ± 1.19 b	*0.0105*	-72.7
1–2	4.04 ± 0.42 b	4.62 ± 0.84 a	*0.3415*	+ 14,3
2–3	3.42 ± 0.50 b	2.76 ± 0.36 b	*0.1349*	-19.3
	nSR (g CO_2-C kg^{-1} OxC d^{-1})		*p*	
0–1	0.99 ± 0.34 a	0.71 ± 0.21 a	*0.2714*	-28.3
1–2	0.70 ± 0.17 a	0.87 ± 0.14 a	*0.2943*	+ 24.3
2–3	0.66 ± 0.10 a	0.65 ± 0.05 a	*0.3150*	-1.5
	GLUase (μmol g^{-1} h^{-1})		*p*	
0–1	5.86 ± 1.07 a	0.06 ± 0.05 b	*0.0007*	-99.0
1–2	2.61 ± 0.51 b	0.57 ± 0.51 b	*0.0060*	-78.2
2–3	2.40 ± 0.31 b	1.02 ± 0.39 a	*0.0128*	-57.5
	PHOase (μmol g^{-1} h^{-1})		*p*	
0–1	15.93 ± 6.24 a	0.23 ± 0.16 b	*0.0121*	-98.6
1–2	5.82 ± 0.16 b	0.64 ± 0.79 b	*0.0040*	-89.0
2–3	4.15 ± 1.65 b	2.49 ± 2.27 a	*0.3647*	-40.0
	TOM (g kg^{-1} soil)		*p*	
0–1	314.83 ± 36.88 a	59.63 ± 8.11 b	*0.0030*	-81.1
1–2	184.90 ± 42.44 b	124.43 ± 15.98 a	*0.0820*	-32.7
2–3	150.63 ± 43.54 b	107.73 ± 7.46 a	*0.1679*	-28.5
	OxC (g kg^{-1} soil)		*p*	
0–1	140.67 ± 31.01 a	53.73 ± 6.13 b	*0.0089*	-61.8
1–2	92.50 ± 8.49 b	76.30 ± 5.20 a	*0.0479*	-20.5
2–3	86.53 ± 12.17 b	68.80 ± 4.64 a	*0.0778*	-17.5
	NHC (g kg^{-1} soil)		*p*	
0–1	188.12 ± 17.03 a	49.11 ± 5.64 b	*0.0002*	-73.9
1–2	84.79 ± 4.84 b	56.57 ± 7.35 a	*0.0051*	-33.3
2–3	77.76 ± 9.37 b	59.16 ± 4.25 a	*0.0351*	-23.9
	pH (1:2.5)		*p*	
0–1	7.64 ± 0.32 a	8.51 ± 0.13 a	*0.0123*	+ 11.4
1–2	7.88 ± 0.03 a	8.15 ± 0.05 b	*0.0009*	+ 3.4
2–3	7.90 ± 0.01 a	8.12 ± 0.06 b	*0.0022*	+ 2.8
	P-Olsen (mg kg^{-1})		*p*	
0–1	40.03 ± 30.89 a	136.3 ± 31.88 a	*0.0198*	+ 240.5
1–2	49.95 ± 12.74 a	67.84 ± 10.02 b	*0.1285*	+ 35.8
2–3	37.33 ± 14.78 a	40.92 ± 8.44 b	*0.7284*	+ 9.6
	WR (seconds)		*p*	
0–1	276.3 ± 72.6 a	2.33 ± 2.31 b	*0.0028*	-99.2
1–2	204.0 ± 100.6 ab	8.33 ± 5.50 b	*0.0282*	-95.9
2–3	70.7 ± 39.3 b	99.67 ± 78.70 a	*0.5985*	-40.9
	Moisture (g kg^{-1} soil)		*p*	
0–1	430.8 ± 150.9 a	77.5 ± 54.1 a	*0.0188*	-82.0
1–2	406.7 ± 121.1 a	140.0 ± 92.6 a	*0.0388*	-65.6
2–3	398.3 ± 69.5 a	108.3 ± 84.0 a	*0.0100*	-72.8

Resistance= −100 [(UN-B)/UN]

slowdown of the hydrolysis of oligosaccharides and the degradation of cellulose as a consequence of fire (Huerta et al., 2020; Barreiro and Díaz-Raviña, 2021). In fact, highly significant ($p < 0.001$) positive correlations were found between GLUase and organic matter fractions (TOM, OxC and NHC) as well as bSR. Similar relationships have been found with the PHOase activity, responsible for the hydrolysis of organic P, which highlights its dependence on organic matter availability and soil microorganism activity (Fetzer et al., 2021; Sugier et al., 2013). In any case, the depth-dependent effects of fire on GLUase and PHOase are not the same for both enzyme activities (Fig. 3), as evidenced by other recent scientific articles. Thus, Huerta et al. (2020), exploring the resistance of soil properties (0–3 cm) to wildfire severity shortly after a wildfire in three fire-prone ecosystems (shrubland, heathland, and oak forest), found that GLUase and PHOase activities decreased with burn severity, with greater sensitivity of the latter. Similarly, Lombao et al. (2021) found that the reduction of these enzyme activities was proportional to the heat applied to the soil; they attributed the reduction in PHOase activity, to thermal enzyme denaturalization and/or the elevated available soil P (increase that we have also observed in this work) that inhibits the microbial production of the enzyme. A reduction in GLUase after a wildfire was found in soils of two forest types (*Quercus* and *Eucalyptus* stands) affected by a wildfire in NW Spain (Lombao et al., 2015) for a 0–2.5 cm soil depth, but no changes were found in PHOase activity; furthermore, they found no differences at depths of 2.5–5 cm for any enzyme activity.

In the Sierra de Los Donceles (SE-Spain), Lucas-Borja et al. (2022) reported a significant decrease in GLUase and PHOase (as well as dehydrogenase, urease and protease) when comparing a fire-affected Aleppo pine forest with a nearby unburned forest; since they take samples from a large thickness of soil (0–10 cm) and 8 years after burning, this reduction could be due to both direct (thermal) and indirect effects, if not derived from the usual seasonal variations in biological properties (Gutknecht et al., 2010). García-Carmona et al. (2021), in similar burned forests, also detect a drastic reduction of multiple biochemical parameters in the 0–5 cm of surface soil (enzyme activities, basal soil respiration, nutrients and organic matter to which are significantly correlated) by indirect effects: the erosion of the topsoil after salvage logging. Sáenz de Miera et al. (2020) found that high severity wildfires reduced about 60% the diversity of soil bacterial communities sampling 0–10 cm topsoil in Sierra de la Cabrera (NW-Spain). In

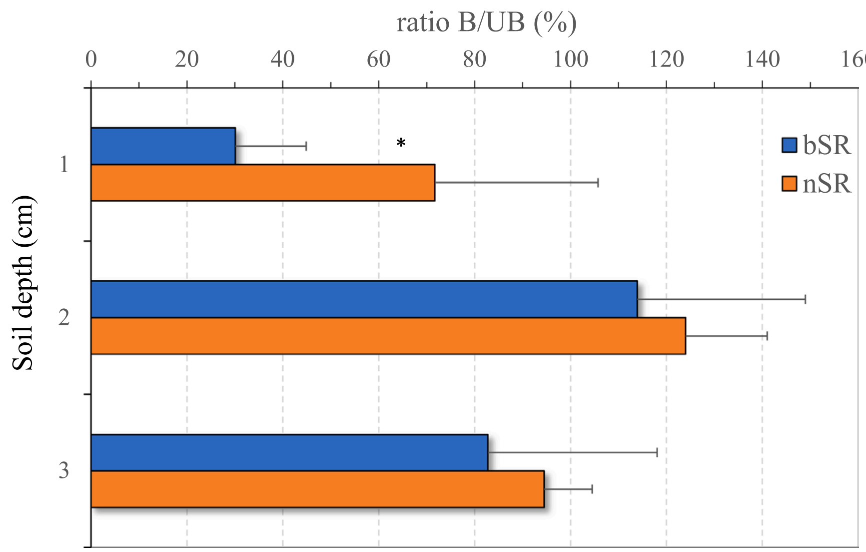

Fig. 2. Effect of fire on microbial activity measured as basal (bSR) and normalized soil respiration (nSR) at different soil depths, expressed as the burned/unburned values ratio (%); values lower than 100 % denote the negative effect and values higher than 100% denote a positive effect of heating treatment. The asterisk indicates significant differences at $P < 0.05$.

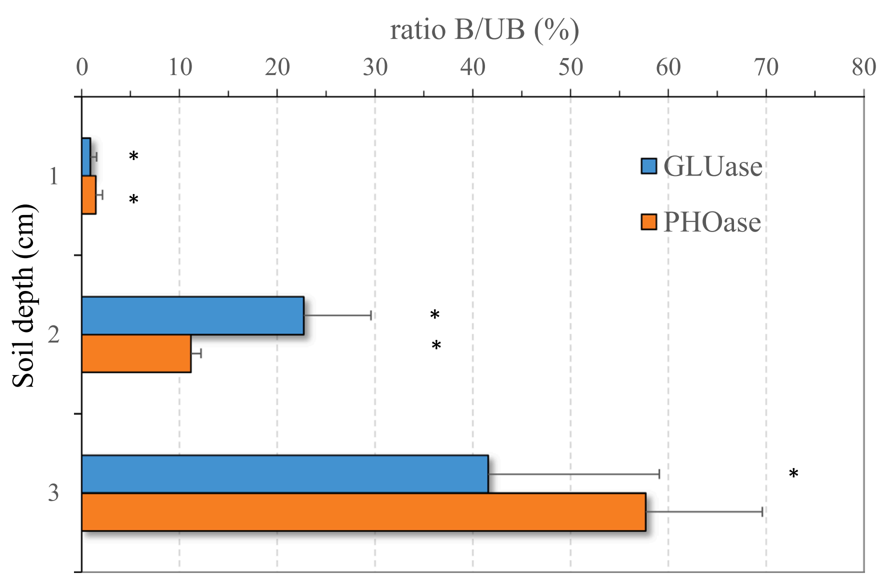

Fig. 3. Effect of fire on enzyme activities (GLUase and PHOase) at different soil depths, expressed as the burned/unburned values ratio (%); values lower than 100% denote the negative effect of heating treatment. The asterisk indicates significant differences at $P < 0.05$.

contrast, burning annual grasses at Santa Cruz mountains in California reduced enzyme activities (including GLUase and PHOase) of a Luvisol (sampling also 10 cm topsoil) by only 10–20%, which is attributed to the low severity of the wildfire (Gutknecht et al., 2010). Neither Lucas-Borja et al. (2019) found significant effects on the biological and chemical properties of a calcareous soil (0–5 cm) under an Aleppo pine forest, subjected to controlled burning in the laboratory; these authors considered that sampling the top 5 cm of soil could have diluted or even canceled out such an impact. However, they found a substantial change in the phylogenetic composition of the soil bacterial communities, which could demonstrate their differential sensitivity to fire (Lucas-Borja et al., 2019). Muñoz-Rojas and Bárcenas-Moreno (2019) also reported a decrease in the enzyme activities and the basal soil respiration (0–5 cm) after different *Pinus halepensis* wildfires in Valencia (E-Spain); these biological properties took 20–25 months to recover to pre-burn levels mediated by the restoration of soil organic C.

3.3. Organic C fractions: TOM, OxC and NHC

All organic fractions have been reduced by burning but each at a different depth: the TOM has been reduced up to the 1st cm (81 %); the OxC both in the 1st (62 %) and in the 2nd cm (18 %); and the NHC for the first (74 %), the second (33 %) and the third cm (24 %). Because of heat, the original gradient with a maximum content on the surface disappeared after burning for each and every one of the three fractions (Table 2). In the present work, we measured a thermal gradient from 900 °C at the soil surface to 78 °C at a depth of 3 cm (Table 1), values that would explain the decrease in the aforementioned organic fractions. At the same time, temperatures in the range of 200–300 °C were maintained for several minutes at the first cm (12`) and even at the second cm (3′). A partial decrease in OxC by heating in the range of 200–300 °C or even the practical total elimination at 500 °C has been previously found (Badía and Martí, 2003b; Sacchi et al., 2015; Terefe et al., 2008). Similar results were observed by Martín-Lorenzo et al.

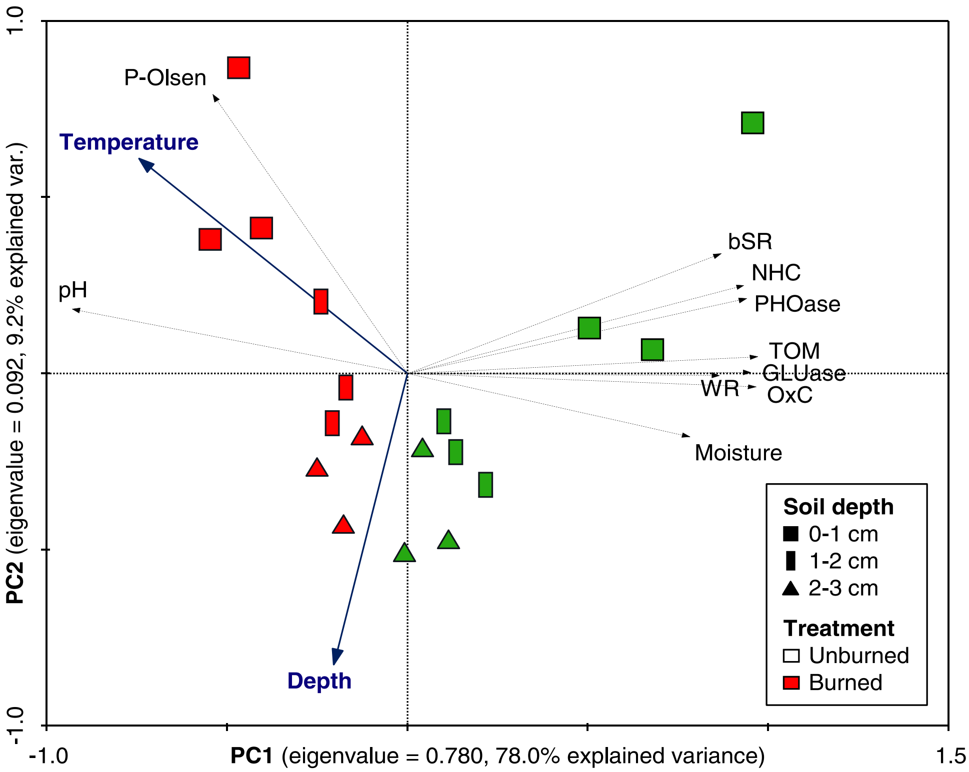

Fig. 4. PCA with soil properties at three soil depths for unburned and burned samples. Temperature and depth are represented as supplementary, "passive" variables, which are not used in the construction of the axes but incorporated post hoc on the basis of their correlation with these axes. bSR = basal soil respiration; TOM = total organic matter; OxC = oxidizable C; NHC = non-HCl-hydrolysable C; GLUase = β-D-glucosidase activity, PHOase = phosphomonoesterase activity; WR = water repellency.

(2021), who detected a loss of 1/3 of the total organic C and ½ of the OxC in the 0–5 cm layer after a cork oak forest wildfire. In other highly destructive Aleppo pine forest wildfire, in midsummer, direct effects on OxC were limited above 2 cm depth (Badía et al., 2014a). Additionally, after burning an ochric horizon (from a pine forest), a significant loss of ¾ of the pre-fire TOC of the litter or O horizon and ½ of the TOC of the upper centimeter of the Ah horizon was found (Aznar et al., 2016). Similarly, the burning of a mollic horizon resulted in the loss of 2/3 of the pre-fire TOC of the litter and 1/3 of the TOC of the upper Ah-cm (Badía et al., 2014a). In addition to the soil organic matter amount, the aforementioned works show that fire also altered its chemical structure, reducing the quantity of typical vegetation markers (terpenes, resinic acids) and lignin markers (methoxyphenols). Long-chain molecular fragmentation by thermal effects is similar to effects caused by biological degradation over long time scales (humus maturity). In any case, all these works show how the high thermal inertia limits the effects of heat to a few superficial centimeters.

On the other hand, the reduction that we have observed in the NHC, up to the 3rd cm, reveals that can serve as proxy of the fire effects on soil; moreover, its strong decrease proves that the resistance of this fraction to laboratory hydrolysis is not parallel to the resistance to heat. As indicated by Greenfield et al. (2013), the NHC fraction includes labile, biodegradable organic compounds, such as carbohydrates, and not only stable organic C. In this same sense, Poirier et al. (2005) observed that NHC from a temperate, loamy, forest soil was subjected to severe biodegradation when changing to agricultural use.

3.4. Other properties

The soil moisture, that reached its maximum value 430 g kg^{-1} in the first centimeter of unburned soils, becomes minimal after burning (Table 2). At all three depths, there was a significant reduction in moisture, with a drastic desiccation of the 0–1 cm layer and a decreased to 1/3 and 1/2 of the initial moisture in the 1–2 and 2–3 cm layers, respectively. These reductions were consistent with the temperatures recorded, which exceeded 100 °C in the 2–3 cm depth range. Although soil moisture can reduce temperature spikes of fire in the upper centimeters of the soil, which can preserve some physical and chemical soil properties (Badía et al., 2017), damage to biota could be greater with humid heat than with dry heat (Wells et al., 1979).

Water repellency practically disappeared in the first two cm of soil as a result of burning (Table 2). Similar block burning experiments have shown that severe burns cause WR to disappear or decrease up to 3 cm on air-dried topsoil but not in wet topsoil (Badía et al., 2017), while moderate burns caused a WR decrease only in the first cm in both mollic (Badía-Villas et al., 2014b) and ochric horizons (Aznar et al., 2016). The WR was found to be very significantly ($p < 0.01$) and positively correlated with soil moisture ($r = 0.64$) and total organic matter ($r = 0.84$), variables that, together with the temperature reached, exert a great influence on WR (Benito et al., 2016; Bodí et al., 2013; Keesstra et al., 2017; Mataix-Solera et al., 2013). The temperature reached in a fire, which varies with soil depth, is the most relevant factor for the variation of the WR (Alcañiz et al., 2018 and references cited therein).

The soil reaction was basic in the unburned calcareous topsoil, with a minimum in the first cm (pH 7.6), which increased with depth (up to 7.9). Severe burning significantly increased the pH of the soil (Table 2) and reversed this gradient, so that the maximum is reached at the first cm (pH 8.5) after burning. The ashes produced in severe fires (derived from the burning of plant biomass, litter and soil organic matter) are usually rich in basic cations and highly alkaline (Badía et al., 2014a; Pereira et al., 2019b). Moreover, high temperatures, such as those measured on the surface (a maximum of 937 °C), can produce the thermal decomposition of $CaCO_3$ and $MgCO_3$ to CaO or MgO and, therefore, increase the pH (Terefe et al., 2008; Rodríguez-Navarro et al., 2009; Giovannini, 2012). Qin and Liu (2021) report that changes in the pH after fire drive differences in soil microbiology.

The controlled burning significantly increased the concentrations of P-Olsen for up to 1 cm soil depth, with values of 3.4 times higher than those recorded before burning (Table 2). Similar increases were found in Aleppo pine wildfires attributed to the dissolution of P from ash beds (Badía et al., 2014; Pereira et al., 2019b; Huerta et al., 2020) or even to the mineralization of organic P related to heating (Badía and Martí,

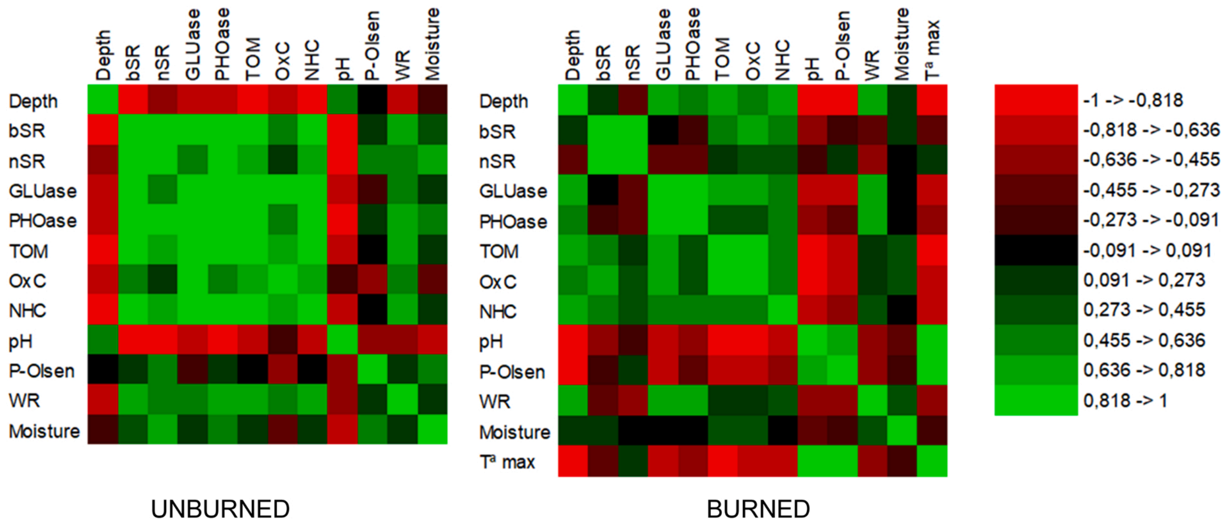

Fig. 5. Heat map of Pearson correlation coefficients (R values) between the soil properties studied for the unburned (left) and burned (right) samples; R values < 0.636 are significant with a P-value< 0.05.

2003a). In a global-scale meta-analysis (174 soil studies under different vegetation and soil types), Butler et al. (2018) found that fire led to a significantly higher concentration of soil mineral P; but, when only coniferous forests are considered, phosphorus was not modified after burning. The P-Olsen content in the topsoil also usually increases after prescribed fires, which are of low severity (Caon et al., 2014; Alcañiz et al., 2018 and references therein). However, Martín-Lorenzo et al. (2021) noted a reduction to a fifth of the original low P-Olsen contents (from 0.5 to 2.6 mg/kg) in the top 5 cm of an Eutric Regosol after a cork oak wildfire passage in the Sierra de Lújar (S-Spain). Pereira et al. (2019b) indicated that P can be volatilized above 700 °C, a temperature that in this study was only reached punctually on the surface (Table 1). P can volatilize above 700 °C (Pereira et al., 2019b) but this temperature in this work was barely reached punctually at the surface (Table 1).

3.5. *Global soil properties relationships*

In the principal component analysis (PCA), the first two dimensions accounted for 87 % of the total variance in the dataset (Fig. 4); component 1 (PC1), with 78 % of variance explained, is related to burning by its visible correlation with the passive variable "temperature". The groups of samples are ordered along PC1 according to the following sequence: unburned 0–1 cm > unburned 1–2 cm > unburned 2–3 cm > burned 1–2 and 2–3 cm > burned 0–1 cm. These groups were clearly separated in the PC space according to the burning effect: the properties located in the first and second quadrants (TOM, OxC, NHC, GLUase, PHOase, bSR, moisture and WR) decreased after burning, but pH and P-Olsen, which increased after burning, were located in the fourth quadrant. Along this sequence and as a result of the fire, the soil organic matter (TOM, OxC and NHC) and the biological properties (GLUase and PHOase activities and the bSR) are reduced, the soil dries out, WR is destroyed, and pH and P increases. PC2, with 9% of variance explained, is related to depth, as seen in the graph in its correlation with the passive variable "depth". Along this second axis, the most non-surface samples soil samples (1–2 and 2–3 cm) of both burned and unburned sites were clustered in the negative direction (Fig. 4). The relationship between the aforementioned properties has been repeatedly found in burned Mediterranean topsoils (Alcañiz et al., 2018; García--Carmona et al., 2021; Mataix-Solera et al., 2009).

Most of the unburned soil properties showed a gradient with depth but burning modified it. This is evidenced by the reversal of the correlations with depth in the heat map, where it is observed how fire reverses the original negative correlations (in red) of unburned samples turning them into positive correlations (in green) in burned ones (Fig. 5).

4. Conclusions

The controlled burning of a calcareous forest topsoil increased the soil pH and the P-Olsen amount and reduced the content of the soil organic matter fractions (TOM, OxC and NHC) as well as the basal soil respiration (bSR) and the activity of soil enzymes responsible for participating in the hydrolysis of the cellulose (GLUase) and the organic phosphate (PHOase). But, in addition, these variations are dependent on: (1) soil depth, given the thermal gradient that fire generates from the surface, and 2) the attribute of the soil considered, according to its sensitivity to heat. Specifically, burning decreased bSR and TOM only up to the 1st cm: PHOase, OxC and WR up to 2 cm: GLUase, NHC and moisture up to 3 cm soil depth. On the other hand, burning increased both P-Olsen, up to the 1st cm, and the pH, up to 3rd cm soil depth. Burning has provoked two opposing responses for these two groups of attributes: (1) the disappearance of the natural gradient that had its maximum values on the soil surface, for those attributes that have decreased, and (2) the exacerbation of this gradient, for those attributes that have increased. These changes, at cm-scale, should be taken into account when monitoring the effects generated by fires on the soil. In conclusion, immediate fire effects on soil are depth dependent, and this dependency is not uniform for all soil properties.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Janielle S. Pereira reports financial support was provided by Conselho Nacional de Desenvolvimento Cientifico e Tecnologico of Brazil.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.pedobi.2022.150860.

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