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Impact of burn severity on soil properties in a *Pinus pinaster* ecosystem immediately after fire

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Abstract. We analyse the effects of burn severity on individual soil properties and soil quotients in Mediterranean fire-prone pine forests immediately after a wildfire. Burn severity was measured in the field through the substrate stratum of the Composite Burn Index and soil samples were taken 7–9 days after a wildfire occurred in a *Pinus pinaster* Ait. ecosystem. In each soil sample, we analysed physical (size of soil aggregates), chemical (pH, organic C, total N and available P) and biological (microbial biomass C, β-glucosidase, urease and acid phosphatase activities) properties. Size of aggregates decreased in the areas affected by high burn severity. Additionally, moderate and high severities were associated with increases in pH and available P concentration and with decreases in organic C concentration. Microbial biomass C showed similar patterns to organic C along the burn severity gradient. The enzymatic activities of phosphatase and β-glucosidase showed the highest sensitivity to burn severity, as they strongly decreased from the low-severity scenarios. Among the studied soil quotients, the C : N ratio, microbial quotient and β-glucosidase : microbial biomass C quotient decreased with burn severity. This work provides valuable information on the impact of burn severity on the functioning of sandy siliceous soils in fire-prone pine ecosystems.

Additional keywords: biological properties, chemical properties, maritime pine, physical properties, wildfire.

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Introduction

Wildfires are a determining factor in the functioning and structure of fire-prone forests (Keeley *et al.* 2012). In general, these forests are adapted to natural fire regimes, recovering under a wide range of fire frequencies and severities (Noss *et al.* 2006). However, burn severity is increasing in many parts of the world because of changes in land-use and climatic conditions (Lindner *et al.* 2008; Moreira *et al.* 2011; Doblas-Miranda *et al.* 2017). This is the case in the Mediterranean Basin, where there is major concern about the ecological consequences of high-severity fires on soils in fire-prone pine ecosystems (Pausas *et al.* 2008), which are the forests most affected by fire in this region (Moreira *et al.* 2012).

Burn severity is used to describe the loss of or change in biomass caused by fire in the ecosystem (Key and Benson 2006; Keeley 2009; Fernández-García *et al.* 2018*a*). The most common approach for estimating burn severity in forest soils is through visual evidence of the loss of litter and changes in soil upper layers, such as alterations in soil colour and structure, soil char or ash depth (Parsons *et al.* 2010). This evidence is highly valuable for managers because it is related to other physical, chemical and biological changes in soils (Vega *et al.* 2013) and to some extent to ecosystem responses after fire, such as soil erosion (Shakesby 2011; Vieira *et al.* 2015) or vegetation recovery (Fernández-García *et al.* 2018*b*). However, other factors such as vegetation and soil type mediate changes caused by fire in ecosystems as well as potential responses after fire, thus limiting the predictive capacity of burn severity measurements (Keeley 2009). It is therefore necessary to study the relationships between burn severity and changes in soil status and processes in different types of ecosystems and especially in those prone to fire. A better understanding of the impact of burn severity on ecosystems may help to clarify its value as a tool to identify target areas in which to implement emergency stabilisation strategies (Merino *et al.* 2018). Some studies have analysed changes in soils related to burn severity (Jordán *et al.* 2011; Jain *et al.* 2012; Pingree *et al.* 2012; Dzwonko *et al.* 2015; Miesel *et al.* 2015; Moody *et al.* 2016), but few analyse the effects of burn severity on soil properties immediately after a wildfire (Vega *et al.* 2013).

Several physical, chemical and biological soil properties provide relevant information on soil status and functioning in relation to fire (Certini 2005). Among physical properties that have shown to be affected by fire are those related to soil structure (e.g. aggregate size) (Jordán et al. 2011; Mataix-Solera et al. 2011). Changes in soil structure can play an important role in hydrological, biological and gas-exchange processes (Neary et al. 1999; Puglisi et al. 2006). Soil structure also provides information on soil resistance to external factors, and could thus be indicative of soil vulnerability to erosion (Cerdá and Jordan 2010). Additionally, several soil chemical properties such as pH and nutrient-supplying capacity (carbon (C), nitrogen (N) and phosphorus (P)) are usually affected by fire (Certini 2005). These soil properties show a close relationship with ecosystem productivity (Arshad and Martin 2002), as well as plant and microbial diversity (Roem and Berendse 2000). Nevertheless, biological properties tend to be affected by lower-intensity disturbances than chemical parameters (Paz-Ferreiro and Fu 2016; Alcañiz et al. 2018). Fire-induced changes in microbial biomass also involve impacts on nutrient cycling and soil detoxification capacity as microorganisms are both a source and a sink of nutrients, and they participate in the degradation of xenobiotics and in the immobilisation of heavy metals (Gil-Sotres et al. 2005; Lagomarsino et al. 2009). Likewise, the microbial biomass, with plant and animal residues are the main sources of soil enzymes, responsible for the catalysis of soil biochemical reactions (Tabatabai 1994; Li et al. 2009). As proteins, soil enzymes are denatured by fairly low temperatures (60–70°C) (Tabatabai 1994), and can therefore be significantly affected immediately after a fire, depending on burn severity (Vega et al. 2013). Among soil enzymes, those involved in C, N and P cycles are considered especially relevant in ecosystem functioning, because they allow soil biota to obtain the major nutrients from complex organic substrates (Lagomarsino et al. 2009; Li et al. 2009; Adetunji et al. 2017).

According to Bastida et al. (2008) and Lagomarsino et al. (2009), the study of changes in soil quotients (ratios between two different soil properties) can provide additional information on impacts on soil status and functioning. Fire impacts on soil quotients may be more consistent among different soils because they are relativised. One of the most used soil quotients is the C : N ratio (Badía et al. 2014; Schnecker et al. 2015), which controls organic matter mineralisation and the development of microorganisms (Wild 1992). This quotient, which is sensitive to fire (Vega et al. 2013), shows low variability in undisturbed forest soils (Wild 1992), and in therefore likely to be highly generalisable. Another soil quotient commonly analysed after disturbances is the microbial quotient (Q_{mic}), a ratio of microbial biomass C and soil organic C. This quotient may be more informative about changes in organic matter than the single assessment of soil organic C and microbial biomass C separately (Piao et al. 2001). Thus, Q_{mic} provides an idea of substrate availability for microorganisms (Lagomarsino et al. 2009; Paz-Ferreiro and Fu 2016) and organic matter stabilisation (Piao et al. 2001). Additionally, specific activities of soil enzymes (enzyme activity per unit of microbial biomass C) have been proposed as indicators of the physiological capacity of the microbial community (Waldrop et al. 2000; Bastida et al. 2008; Lagomarsino et al. 2009).

The objective of the present study is to analyse the effects of field-estimated burn severity on individual soil properties, as well on as soil quotients indicative of soil status and processes in a *Pinus pinaster* forest immediately after fire. Specifically, we aim to analyse the effects of burn severity on (i) physical (mean weight diameter), chemical (pH, organic C, total N, available P) and biological (microbial biomass C, β-glucosidase, urease and acid phosphatase activities) soil properties, as well as on (ii) soil quotients (C : N, Q_{mic} and the activity of β-glucosidase urease and acid phosphatase per microbial biomass C unit), incorporating the study of the specific activity of soil enzymes into the fire ecology discipline. According to prior studies focused on the effects of fire on soils, we hypothesised that physical (Jordán et al. 2011; Mataix-Solera et al. 2011), chemical (Marcos et al. 2007; Badía et al. 2014) and biological (Neary et al. 2008; Vega et al. 2013) properties and soil quotients (Vega et al. 2013) would be affected at different severities. We expected biological properties to be affected at lower severities than physical and chemical properties (Santín and Doerr 2016). Besides, as soil quotients combine the information of two different soil properties (Bastida et al. 2008; Lagomarsino et al. 2009) we expected them to be more sensitive to burn severity than single soil properties.

Material and Methods

Study site

The study was conducted within a fire scar that burned on 21 July 2015 in Sierra del Teleno (León Province, north-west Iberian Peninsula) (Fig. 1). This wildfire affected a *Pinus pinaster* Ait. (maritime pine) ecosystem, which is the type of forest most affected by fire on the Iberian Peninsula (Área de Defensa contra Incendios Forestales del Ministerio de Agricultura, Alimentación y Medio Ambiente 2012; Instituto da Conservação da Natureza e das Florestas 2015).

The wildfire scar is an area of 16 ha located on a south-east-facing hillside with a slight slope (5%) and an average altitude of 1025 m. The soils are characterised by a dark acid surface horizon rich in organic matter, weakly developed subsurface horizons, free-draining conditions and absence of carbonates. These soils are classified as Haplic Umbrisols according to the World Reference Base for Soil Resources (WRB) system (Jones et al. 2005). The soil textural class in the study area is sandy loam. The parent material is silt, clay, sand, boulders and conglomerates from the Neogene period (GEODE 2018). The climate is Mediterranean, Csb type according to Köppen, and classified by the Spanish meteorological survey as temperate with dry temperate summers (Agencia Estatal de Meteorología and Instituto de Meteorologia de Portugal 2011). Mean annual precipitation is ∼685 mm and mean annual temperature is 10°C (Ninyerola et al. 2005). The understorey of the *P. pinaster* forest is dominated by *Erica australis* L., *Halimium lasianthum* (Lam.) Spach. and *Pterospartum tridentatum* (L.) Willk.

Data collection

Field sampling

Field sampling was carried out on the 7th, 8th and 9th day after the wildfire. In the period between the wildfire and field sampling, there was no precipitation in the study area, and the atmospheric conditions were warm (mean temperature of 20.65°C), dry (mean relative humidity of 49.62%) and not windy (mean wind speed of 11.75 km h^{-1}) (National Oceanic and Atmospheric Administration 2018). Additionally, soils were

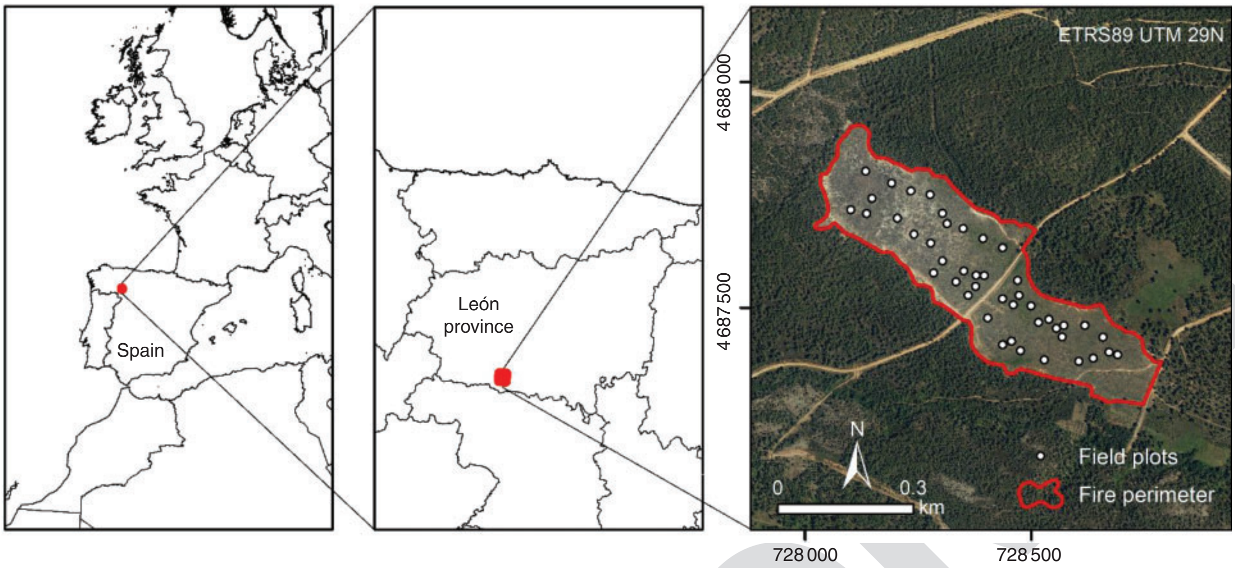

Fig. 1. Location of the study area on the Iberian Peninsula (left), and in León province (centre). The image on the right shows the fire perimeter and the spatial distribution of the field plots in an orthophotography taken in 2017.

Table 1. Factors and scores used as reference to quantify soil burn severity (substrates stratum of the Composite Burn Index) according to Fernández-García *et al.* (2018*a*)

Scores shown in the table were assigned to each rating factor, as well as mean values when the situation was considered intermediate (i.e. a value of 0.75 was assigned for litter and light fuel consumed when it was 10%). UB, unburned; *n* indicates the number of plots distributed in each severity category. Note that both UB and low-severity categories correspond to no apparent changes in mineral soil

Rating factors	Burn severity scale						
	UB ($n = 0$)	Low ($n = 2$)		Moderate ($n = 24$)		High ($n = 18$)	
	0	0.5	1	1.5	2	2.5	3
Litter and light fuel consumed	No changes	0–10%	10–20%	20–40%	40–80%	80–98%	98%
Char and colour	No changes	Blackened litter, no changes in soil		Charred remains, recognisable litter		Grey and white ash, grey soil	White ash, reddened soil

dry, as there was no precipitation for 34 days prior to field sampling.

We established a total of 44 (1 × 1-m) plots randomly distributed within the burned area (Fig. 1). In each plot, we estimated soil burn severity using the substrate stratum of the Composite Burn Index-based protocol (CBI) proposed by Fernández-García *et al.* (2018*a*) for *P. pinaster* ecosystems in the Iberian Peninsula (Table 1; Fig. 2). Visual evidence used in this index was: (1) the proportion of litter and light fuel (leaves, needles and woody material less than 2 cm in diameter located on the ground surface) consumed by fire, and (2) char depth (considering litter and mineral soil), and colour of ash and mineral soil. Char depth and colour of ash and mineral soil were closely linked, and therefore, considered together (see characterisation in Table 1).

To analyse the relationships between soil burn severity and soil properties, we collected a soil sample in each field plot. Each sample was composed of four subsamples collected along two perpendicular transects using an auger (5-cm diameter × 3-cm depth), after removing the litter and post-burn residues (ash and scorched debris). The soil samples were air-dried, sieved (<2 mm) and stored until laboratory analysis (20°C).

Soil analyses

From each soil sample, we analysed soil physical (mean weight diameter (MWD)), chemical (pH, organic C, total N and available P) and biological (microbial biomass C, β-glucosidase, urease and acid phosphatase) properties.

Aggregate size distribution was determined by dry-sieving the soil samples through 1-, 0.25-, 0.1- and 0.05-mm sieves for 120 s in an electromechanical shaker (Kemper and Rosenau 1986). The results were expressed as MWD, which reflects the average size of the stable aggregates (Cerdà and Jordán 2010; Mataix-Solera *et al.* 2011). MWD was calculated using the following equation (Eqn 1):

$$\mathrm{MWD} = \sum\nolimits_{i=1}^{n} X_i W_i \tag{1}$$

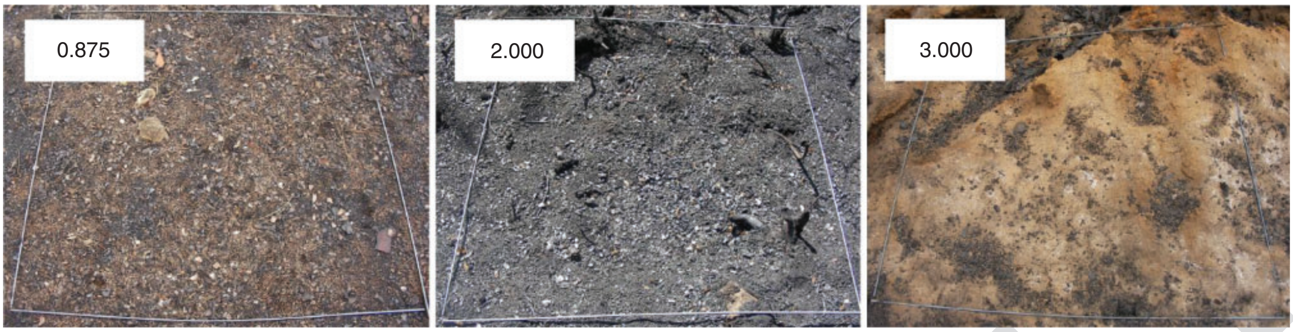

Fig. 2. Examples of plots with different soil burn severity values in Composite Burn Index (CBI) units. The panel on the left (CBI = 0.875) shows a field plot burned at low severity (10% of litter and light fuel was consumed and litter blackened with, in general, no changes in mineral soil). The panel in the middle (CBI = 2) shows a field plot burned at moderate severity (60% of the litter and light fuel was consumed and there were charred remains with recognisable litter in some areas). The panel on the right (CBI = 3) shows soil burned at high severity (98% of litter and light fuel was consumed, all ash was white and the soil was completely reddened). See Table 1 for further information.

where X is the mean particle size in millimetres, and W the percentage weight of each soil fraction.

Soil pH was determined at 25°C in a suspension of soil : deionised water (1 : 2.5 w/v). To analyse the soil organic C, we ground the soils to <0.15-mm particle size using a pestle and mortar, and we applied Walkley–Black dichromate digestion (Nelson and Sommers 1982). As the soil in the study site is non-calcareous, values obtained for soil organic C and total C are very similar (Nelson and Sommers 1982; Vega *et al.* 2013). Total N (sum of organic N, NO_3^-, NO_2^- and NH_4^+) was determined by the Kjeldahl method (Bremner and Mulvaney 1982) using a DK 20 digestion unit (VELP Scientifica). Available P was analysed following the procedure of Olsen *et al.* (1954), at 882-nm wavelength on an UV Mini 1240 spectrophotometer (Shimadzu Corporation).

We analysed microbial biomass C (MBC) by the fumigation–extraction method (Vance *et al.* 1987). This procedure uses the difference (E_C) in organic C (analysed by Walkley–Black dichromate oxidation) between filtered soil extracts of chloroform fumigated ($CHCl_3$, 24 h) and non-fumigated samples. Then, we used an extraction efficiency coefficient (K_{EC}) of 0.38 (Vance *et al.* 1987; Joergensen 1996) to calculate microbial biomass C using the following equation (Eqn 2):

$$\mathrm{MBC} = \mathrm{E_C}/\mathrm{K_{EC}} \tag{2}$$

Three soil enzymatic activities corresponding to the biogeochemical cycles of C, N and P were determined. Specifically, we selected β-glucosidase (enzyme nomenclature: EC 3.2.1.21; β-D-glucoside glucohydrolase), urease (EC 3.5.1.5; urea amidohydrolase) and acid phosphatase (EC 3.1.3.2; phosphate-monoester phosphohydrolase). To analyse β-glucosidase and acid phosphatase, we followed the procedure described by Tabatabai (1994), whereas urease activity was analysed according to Kandeler and Gerber (1988). For the analysis of the three enzymatic activities, the soils were incubated with their corresponding enzyme substrates (*p*-nitrophenyl-β-D-glucopyranoside, urea and *p*-nitrophenyl phosphate respectively). The products released by the enzymatic activity were determined colorimetrically with a UV-1700 PharmaSpec spectrophotometer (Shimadzu Corporation). We measured the absorbance of the *p*-nitrophenol (*p*NP) produced by β-glucosidase and acid phosphatase activities at 400 nm, whereas absorbance of the NH_4^+ released by urease activity was measured at 690 nm.

From the analysed soil properties, we calculated several soil quotients: (1) C : N quotient calculated as micrograms of soil organic C to micrograms of total N; (2) the microbial quotient (Q_{mic}) as micrograms of microbial biomass C to micrograms of soil organic C; and (3) the specific activity of soil enzymes (β-glucosidase, urease and acid phosphatase) expressed as the micrograms of product released (*p*NP for β-glucosidase and acid phosphatase, and NH_4^+ for urease) per micrograms of microbial biomass C.

Data analysis

We evaluated the relationship between burn severity (explanatory variable) and each single soil property and each quotient (response variables) by fitting linear regression models (LMs). Models including single soil properties and quotients were calibrated using linear and quadratic terms to account for potential non-linear relationships, and the most parsimonious model was selected following Akaike's information criterion (AIC). The normality and homogeneity of the model residuals were checked using diagnostic plots. The goodness-of-fit of the models to the data was assessed from the coefficient of determination (R^2) and statistical significance of the relationships (P) obtained from the model summary outputs.

All data analyses were carried out with *R* (R Core Team 2017).

Results

Soil properties

The relationships between most soil properties (MWD, pH, organic C, available P, microbial biomass C and β-glucosidase) and field-estimated soil burn severity were quadratic (Table 2; Fig. 3). All soil properties, except total N and urease activity, were significantly affected by soil burn severity immediately after fire ($P < 0.05$) (Fig. 3).

Table 2. Akaike's information criterion (AIC) values of models calculations performed between soil properties (response variables) and soil burn severity measured as the substrate stratum of the Composite Burn Index (CBI) (explanatory variable) using a linear ('CBI') and a quadratic function ('poly(CBI,2)')

The lowest AIC values for each soil property, which indicate the most adequate model, are in bold face. MWD, mean weight diameter; MBC, microbial biomass C

Soil property	CBI	AIC
MWD	Linear	−50.226
	Quadratic	**−59.248**
pH	Linear	114.122
	Quadratic	**104.907**
Organic C	Linear	382.682
	Quadratic	**381.924**
Total N	Linear	**140.792**
	Quadratic	142.847
Available P	Linear	302.148
	Quadratic	**299.502**
MBC	Linear	650.972
	Quadratic	**649.480**
β-Glucosidase	Linear	33.336
	Quadratic	**14.853**
Urease	Linear	**120.225**
	Quadratic	122.219
Phosphatase	Linear	**121.942**
	Quadratic	122.725

MWD showed a non-linear relationship with burn severity ($R^2 = 0.284$; $P < 0.001$) (Fig. 3), increasing at low to moderate severities (≤1.75 CBI units) and decreasing at high severities (>2.25 CBI units).

Soil pH had a strong significant relationship with burn severity (Fig. 3), and was the soil property with the highest proportion of variance explained by burn severity ($R^2 = 0.802$). pH values ranged from 3–5 in the areas burned at low severity to 7–9 in the most severely burned areas. The most pronounced change in pH was observed at moderate and high severities (CBI > 1.25 CBI units).

Focusing on soil major nutrients, we found a significant relationship between organic C ($R^2 = 0.594$; $P < 0.001$) and available P ($R^2 = 0.666$; $P < 0.001$) with burn severity (Fig. 3). Nevertheless, the patterns of change in both nutrients were opposed. Organic C decreased with burn severity, whereas available P increased with burn severity, mainly at moderate and high severities (CBI > 1.25 CBI units). We did not find significant effects of burn severity on total N ($R^2 = 0.059$; $P > 0.05$).

Microbial biomass C showed an inverse relationship with burn severity ($R^2 = 0.504$; $P < 0.001$) (Fig. 3) following the same pattern of change as organic C. The highest microbial biomass C contents (>1000 µg g^{-1} dry soil) were obtained in soils that did not burn with high severity (≤2.25 CBI units).

Similarly, the activities of soil enzymes β-glucosidase and acid phosphatase were inversely related to burn severity. β-Glucosidase had a quadratic response to burn severity ($R^2 = 0.623$; $P < 0.001$), stronger than the linear relationship exhibited by acid phosphatase activity ($R^2 = 0.398$; $P < 0.001$). Both activities decreased from the low-severity scenario and showed values corresponding to no activity in the severely burned areas, thus being the studied soil properties most sensitive to burn severity. We did not find significant effects of burn severity on urease activity ($R^2 = 0.005$; $P > 0.05$).

Soil quotients

Several soil quotients showed significant linear (C : N) and quadratic (Q_{mic} and β-glucosidase : microbial biomass C) inverse relationships with burn severity (Table 3; Fig. 4).

Burn severity had a significant effect on the C : N ratio, explaining a high percentage of variance on that quotient ($R^2 = 0.740$; $P < 0.001$) (Fig. 4). The C : N ratio strongly decreased from values of 30 µg organic C µg^{-1} total N in the lowest severities to values ~5 µg organic C µg^{-1} total N in the highest severities.

The Q_{mic} showed a significant decrease with burn severity ($R^2 = 0.443$; $P < 0.001$) (Fig. 4). The quadratic relationship between Q_{mic} and burn severity suggested that changes in Q_{mic} appeared at moderate to high severities (CBI > 1.75).

Among the specific activities of soil enzymes, only the β-glucosidase : microbial biomass C quotient significantly decreased with burn severity ($R^2 = 0.497$; $P < 0.001$). As occurred with β-glucosidase activity, the β-glucosidase : microbial biomass C quotient was very sensitive to fire, showing notable decreases even at low severities with the same pattern in relation with burn severity as β-glucosidase (Figs 3, 4).

Discussion

We developed a study to analyse fire effects on acidic soils along a burn severity gradient in a *Pinus pinaster* ecosystem immediately after fire (7–9 days). Our results revealed significant relationships between burn severity and physical, chemical and biological soil properties (MWD, pH, organic C, available P, microbial biomass C, β-glucosidase activity and acid phosphatase activity).

In the present study, we observed that the physical property MWD increased at low to moderate severities and decreased mainly at high severities. The increase in MWD may be related to the enhancement of aggregate stability that typically occurs at moderate severities in hydrophilic soils with organic matter as the main binding agent (Mataix-Solera *et al.* 2011). Laboratory heating experiments have clarified that aggregate stability can be enhanced at low temperatures (75–200°C) (Santín and Doerr 2016) owing to the volatilisation of some organic matter, which then condenses on the aggregates, contributing to their stability (Mataix-Solera *et al.* 2011). However, at higher temperatures, stable aggregates can be dispersed (Benito *et al.* 2009; Varela *et al.* 2015; Santín and Doerr 2016) owing to major alterations in the main binding agents: the organic matter, which is depleted at high severities, and clay minerals, which are modified at extreme severities (Neary *et al.* 1999; Santín and Doerr 2016). The decrease in MWD at high severities involves other negative effects on ecosystems, such as loss of structure (Cerdá and Jordan 2010), and consequently a decrease in infiltration capacity and an increase in erosion rates (Vieira *et al.* 2015). Therefore, the burn severity impact on MWD has significant

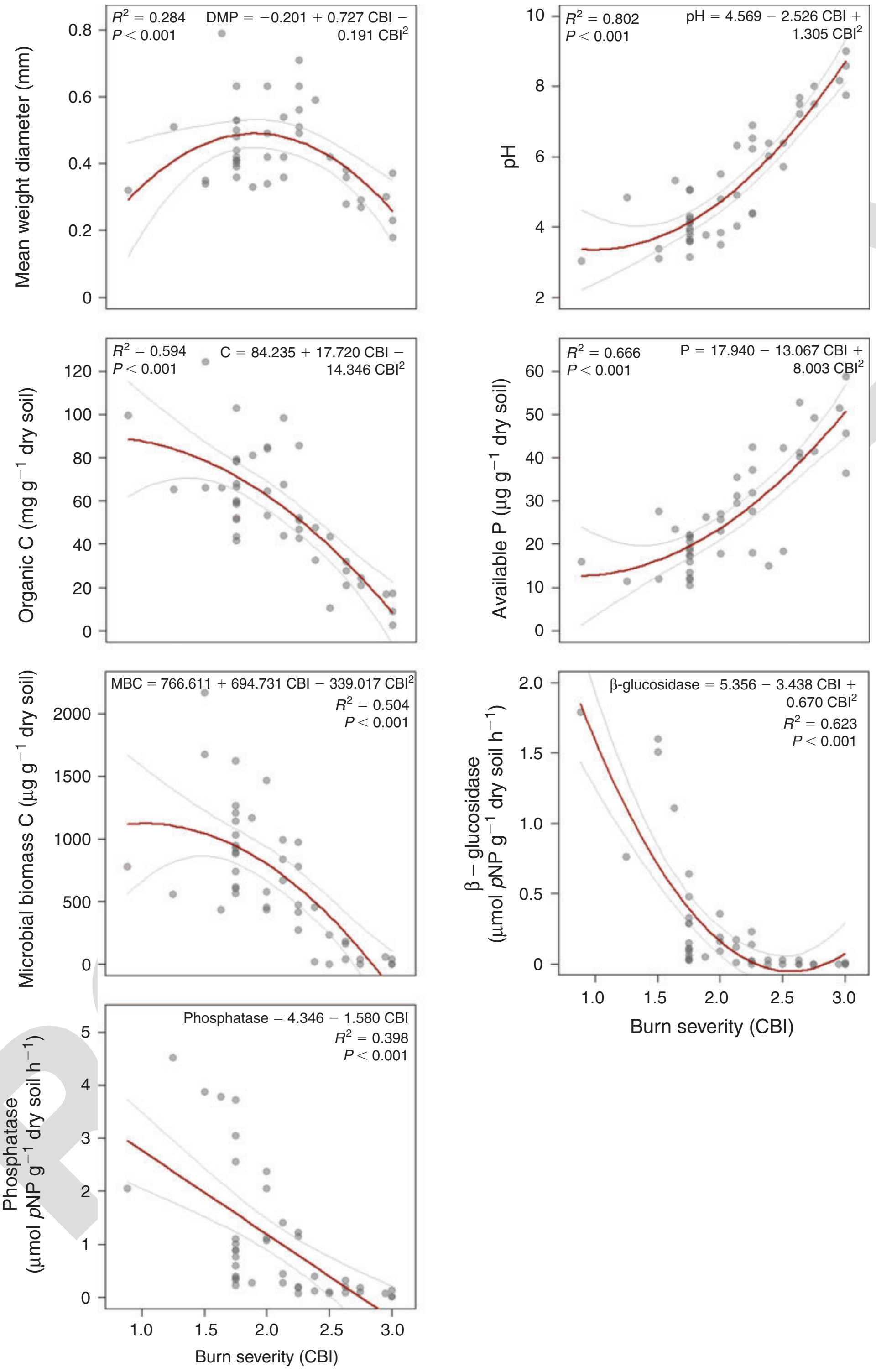

Fig. 3. Relationships between soil properties (response variables) and soil burn severity measured as the substrate stratum of the Composite Burn Index (CBI) (explanatory variable). The lines represent model-predicted values (mean ± 95% confidence intervals) for each soil property along the burn severity gradient. MWD = mean weight diameter; MBC = microbial biomass C.

Table 3. Akaike's information criterion (AIC) values of models calculations performed between soil quotients (response variables) and soil burn severity measured as the substrate stratum of the Composite Burn Index (CBI) (explanatory variable) using a linear ('CBI') and a quadratic function ('poly(CBI, 2)')

The lowest AIC values for each soil property, which indicate the most adequate model, are in bold face. Q_{mic}, microbial quotient; MBC, microbial biomass C

Soil quotient	CBI	AIC
C : N	Linear	**246.773**
	Quadratic	247.696
Q_{mic}	Linear	76.414
	Quadratic	**71.997**
β-Glucosidase : MBC	Linear	−101.162
	Quadratic	**−111.828**
Urease : MBC	Linear	**47.694**
	Quadratic	49.612
Phosphatase : MBC	Linear	**52.281**
	Quadratic	54.105

ecological consequences, especially in areas where the potential erosion risk is very high, such as southern Europe (Van der Knijff *et al.* 2000).

The soil chemical properties pH, soil organic C and available P were also affected by burn severity immediately (7–9 days) after fire. We found proportional decreases in organic C concentration at moderate and high severity, our results being consistent with those found in previous research on fire ecology (Vega *et al.* 2013). Loss of organic C in the mineral soil starts at moderate severities (Key and Benson 2006; Keeley 2009) and is a consequence of organic matter mineralisation and volatilisation caused by fire (Caon *et al.* 2014). Laboratory heating experiments indicated that organic C concentration in Mediterranean *P. pinaster* ecosystems with acidic soils decreases at temperatures higher than 220°C (Varela *et al.* 2015). The opposite trend was found for pH and available P. pH showed a large increase (almost 6 pH units) with burn severity, exceeding figures reported in other field studies (Certini 2005; Marcos *et al.* 2007; Vega *et al.* 2013) and is similar to increases found in acidic soils from *P. pinaster* ecosystems heated to 800°C under laboratory conditions (Fuertes 2015), suggesting that temperatures reached in the study site were fairly high. Increases in pH are due to denaturation of organic acids and the release of bases from organic matter mineralisation (Neary *et al.* 2008; Alcañiz *et al.* 2018). Similarly, the mineralisation of organic matter converts organic P into available P (Certini 2005; Marcos *et al.* 2007), which is frequently limiting in forest soils (Neary *et al.* 2008), especially in those developed from highly siliceous lithologies (Binkler and Fisher 2013), which is the case in our study area. Additionally, burn severity may have a significant effect on the concentration of available P in acidic soils through the increase in pH, as maximum P availability is between pH 6 and 7.5 (Neary *et al.* 2008). Therefore, changes caused by burn severity in both soil properties, pH and available P content, are particularly relevant in acidic soils in *P. pinaster* ecosystems, because they have notable implications in soil fertility. In this study, we did not find significant effects of burn severity on total N concentration, which can be attributed to low N losses by volatilisation (Caon *et al.* 2014). Other studies have not found changes in total N in Mediterranean soils after fire (Caon *et al.*

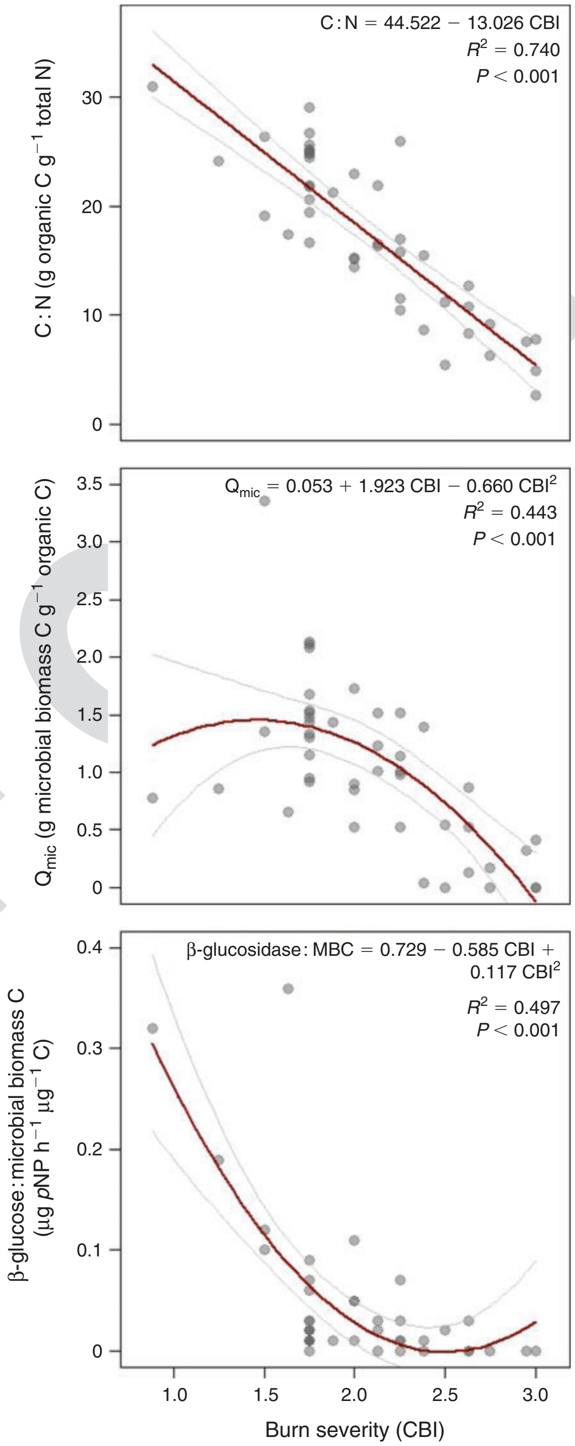

Fig. 4. Relationships between soil quotients (response variables) and soil burn severity measured as the substrate stratum of the Composite Burn Index (CBI) (explanatory variable). The lines represent model-predicted values (mean ± 95% confidence intervals) for each soil property along the burn severity gradient. MBC = microbial biomass C.

2014), or in soils heated in laboratory at 350°C for 1 h (Tecimen and Sevgi 2011).

The biological soil properties significantly affected by burn severity (microbial biomass C, β-glucosidase activity and acid phosphatase activity) strongly decreased (to zero) in the highest-severity scenario. Declines in microbial biomass C with burn severity were found by Vega *et al.* (2013) in *P. pinaster* ecosystems. The decrease in microbial biomass C is attributed to the mortality of microorganisms occurring between 50 and 160°C (Neary *et al.* 2008; Vega *et al.* 2013). Additionally, changes caused by fire in soil nutrients, moisture and temperature may affect the abundance of microorganisms (Dooley and Treseder 2012). Similarly, temperatures above 60–70°C bring about the inactivation and denaturation of soil enzymes (Tabatabai 1994). These temperatures are lower than necessary to produce significant effects on the other soil properties analysed in the present study (Neary *et al.* 2008). Therefore, the soil enzymes β-glucosidase and acid phosphatase are the soil properties most sensitive to burn severity in the present study, understood to be the first properties affected, decreasing from severities of ~1.25 CBI units (considered the severity threshold between visible changes and no visible changes in the mineral soil; see Table 1; Key and Benson 2006) to 2.5 CBI units, where no activity was found. In contrast, we did not find significant effects of burn severity on urease activity. We hypothesise that the high NH_4^+ concentration, which is expected in soils immediately after fire (Certini 2005; Caon *et al.* 2014), may inhibit the enzyme reaction (Goberna *et al.* 2012), as well as mask the enzyme activity determination, as it is quantified by the NH_4^+ released (Kandeler and Gerber 1988).

Among the soil quotients, we found a significant relationship between burn severity and the C : N ratio, Q_{mic} and β-glucosidase : microbial biomass C quotient. The decrease in C : N ratio with fire (González-Pérez *et al.* 2004; Santín *et al.* 2008) and, particularly, the inverse relationship between C : N ratio and burn severity have been noted in previous studies (Vega *et al.* 2013). Thus, burn severity can transform oligotrophic soils into eutrophic soils (from C : N >20 to <12; Porta *et al.* 1999), conditioning the composition of the future plant community (Pöyry *et al.* 2017). This result can be attributed to higher losses caused by fire in soil organic C than in N, which is volatilised at higher temperatures (Neary *et al.* 2008), and to the increase in recalcitrant organic N forms in charred remains (Santín *et al.* 2008). Apart from the sensitivity to fire, the quite constant values of the C : N ratio among undisturbed forest soils (Wild 1992) suggest that this quotient may be a generalisable tool to analyse fire impact on soils. Similarly, we found decreases in Q_{mic} with burn severity, which can be attributed to a higher impact on microbial biomass C than on organic C (Bastida *et al.* 2008). Several authors have noted that Q_{mic} values of 2.2 reflect a good equilibrium between both C fractions (Bastida *et al.* 2008), whereas Q_{mic} values lower than 2 are indicative of organic matter depletion (Lagomarsino *et al.* 2009; Paz-Ferreiro and Fu 2016). This suggests a large decrease in organic matter in the highest-severity scenarios (>2.5 CBI units) of our study, where Q_{mic} values were always lower than 1. Among the specific activities of soil enzymes, β-glucosidase : microbial biomass C decreased with burn severity. Studies carried out by Lagomarsino *et al.* (2009) in agrarian ecosystems have pointed out the β-glucosidase : microbial biomass ratio as the most sensitive specific enzyme activity to indicate land-use impacts. The inverse relationship between this ratio and burn severity could be explained by (1) a decrease in β-glucosidase activity per microbial biomass unit (Waldrop *et al.* 2000); (2) β-glucosidase immobilisation in clays or humic colloids; and (3) a decrease in the concentration of glucopyranosides (Bastida *et al.* 2008).

The present study contributes to advancing knowledge of what soil burn severity means for the soil status and functioning immediately after fire in *P. pinaster* ecosystems with acidic soils. Additionally, our results revealed several soil properties that could be considered key for monitoring fire impacts on soils, owing to their high sensitivity to burn severity and their relationship with relevant soil ecological processes. Among them, (1) pH showed the strongest relationship with burn severity, with a large increase at moderate and high severities; (2) the activity of soil enzymes acid phosphatase and particularly β-glucosidase (both the single soil property and the soil quotient as specific enzyme activity) showed the highest sensitivity to fire, decreasing from the low-severity scenario; and (3) the C : N quotient, whose results could be more generalisable, showing a progressive decrease with burn severity. However, it has been recognised that not all changes caused by fire in soil properties persist for a long time, depending on the magnitude of the fire impact and the recovery of vegetation, among other factors (Certini 2005; Alcañiz *et al.* 2016; Muñoz-Rojas *et al.* 2016). Therefore, we recommend monitoring the evolution of soil properties in relation to burn severity over the medium and long term after a wildfire in fire-prone pine ecosystems.

Research on how burn severity affects soil properties is particularly relevant in the European countries of the Mediterranean Basin, because land-use changes occurring during recent decades have led to an increase in fuel amount and continuity (Pausas *et al.* 2008; Doblas-Miranda *et al.* 2017), thus constituting landscapes prone to large high-severity fires (San-Miguel-Ayanz *et al.* 2016; García-Llamas *et al.* 2019). This new fire-regime pattern could be enhanced in the current context of climate change owing to climate-driven increases in burn severity and the area affected by fire because of the drier and warmer climate expected in many regions of the world (Azpeleta *et al.* 2014; San-Miguel-Ayanz *et al.* 2016). Consequently, the present study not only contributes to a better understanding of changes occurring in a particular situation, but can also inform on fire impacts in *P. pinaster* forests under in future scenarios of increasing burn severity.

Conclusions

This study adds to the knowledge on how field-estimated burn severity is related to changes in single soil properties (physical, chemical and biological) as well as in soil quotients indicative of soil status and processes in *Pinus pinaster* ecosystems with acidic soils immediately (7–9 days) after fire. Specifically, our results showed that decreases in the size of stable aggregates (MWD) occurred mainly at high burn severities, whereas changes in chemical properties (pH, organic C and available P) were associated with moderate and high severities. We also demonstrated that biological properties, and particularly the activity of soil enzymes (β-glucosidase and acid phosphatase) are one of the most sensitive properties to burn severity as they largely decrease from the low-severity scenarios, becoming depleted at the highest severities. Furthermore, burn severity affected the C : N ratio, microbial quotient and the specific activity of β-glucosidase. In view of our results, we propose pH, β-glucosidase activity and C : N ratio as key properties in surveying fire impacts on soils immediately after fire in *P. pinaster* ecosystems, because of their sensitivity and potential generalisation.

This research constitutes a benchmark for monitoring longer-term relationships between burn severity and soil properties in *P. pinaster* ecosystems, as well as forecasting the effects of fire in the future, considering the predictions of more severe wildfires in the Mediterranean region.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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