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Wildfire effects on soil properties in fire-prone pine ecosystems: indicators of burn severity legacy over the medium term after fire

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ABSTRACT

The aim of this study was to determine the effects of burn severity on soil properties (chemical, biochemical and microbiological) in fire-prone pine ecosystems three years after fire. To achieve these goals, we selected two large wildfires that occurred in summer 2012 within the Iberian Peninsula: the Sierra del Teleno wildfire, which burned 119 km^2 dominated by *Pinus pinaster* forests developed over acidic soils, and the Cortes de Pallás wildfire, which burned 297 km^2, part of them dominated by *Pinus halepensis* ecosystems with calcareous soils. We classified the burned areas into low or high burn severity categories using spectral indices. Three years after the wildfires, we distributed 56 field plots proportionally to the extent of each severity category. In each field plot, we collected samples of mineral soil from a depth of 0-3 cm. We analysed soil chemical (pH, electrical conductivity, organic carbon, total nitrogen, available phosphorus) biochemical (β-glucosidase, urease and acid phosphatase enzymatic activities) and microbiological (microbial biomass carbon) properties in each soil sample. The relationship between burn severity and soil properties was analysed by a Permutational Multivariate Analysis of Variance and Generalized Linear Models. The results showed a significant influence of the original ecosystem and of burn severity on the overall soil status over the medium term after fire. Available P content increased with burn severity in the acidic soils of the *P. pinaster* ecosystem. However, the three enzymatic activities and microbial biomass carbon decreased with burn severity in both types of pine ecosystems. β-glucosidase, urease and microbial biomass carbon showed common patterns in relation to burn severity in the two different *Pinus* ecosystems (acidic and calcareous soils), and therefore we suggest that they could be potential indicators of the burn severity legacy on soils over the medium term after fire in fire-prone pine Mediterranean forests. Available P and acid phosphatase could be potential indicators in the *P. pinaster* ecosystem. This study provides useful knowledge for developing hazard reduction and restoration strategies after large wildfires.

KEYWORDS

Fire severity, Large wildfire, Mediterranean Basin, *Pinus pinaster*, *Pinus halepensis*

1 INTRODUCTION

Wildfires are one of the recurrent ecological disturbances in forest ecosystems (Fultz et al., 2016; Heydari et al., 2017; Taboada et al., 2017). During recent decades, wildfires in the Mediterranean Basin can be perceived as disasters due to increased numbers of large fires and area burned (Pausas et al., 2008). Besides, wildfire-related problems are more pronounced in Southern Europe, where there is an increase in burn severity associated with land use change and climate change (Hinojosa et al., 2016; Catalanotti et al., 2017). For these reasons, the effects of burn severity on the recovery of Mediterranean ecosystems is one of the main current issues in scientific research into fire ecology (e.g. Fernández-Manso et al., 2016; Francos et al., 2016; Fernández-García et al., 2017).

Burn severity is defined as the loss of or change in ecosystem biomass, caused by fire (Keeley, 2009). It is related to fire intensity, which denotes the energy released from fire. Both parameters, burn severity and fire intensity, may determine the impacts of fire on ecosystems, and therefore, may help predict post-fire recovery (Keeley, 2009; Dzwonko et al., 2015; Pereira et al., 2017). However, most studies use burn severity instead of fire intensity, because it can be measured after fire (Zavala et al., 2014) over extended time frames ranging from days to decades (Heward et al., 2013). There are two different approximations to assessing burn severity: using remote sensing methods (Fernández-Manso et al., 2016; Fernández-García et al., 2018a) or field data (Fernández-García et al., 2017). Among field methods to estimate burn severity, one of the most straightforward and widespread procedures in Mediterranean ecosystems is to measure the minimum diameter of remaining twigs (Keeley, 2009), as this indicates the magnitude of impacts caused by fire aboveground (Fernández-García et al., 2017) and belowground (Keeley et al., 2008; Maia et al., 2012). Fire, and hence burn severity, plays an essential role in the mineral soil status of forest ecosystems (Certini, 2005; Zavala et al., 2014; Knelman et al., 2015) by modifying soil properties, chiefly in the uppermost 2-3 cm (Badía et al., 2014; Caon et al., 2014). Thus, to assess the influence of burn severity on overall soil status after fire, some authors have used a combination of fire-sensitive soil properties, such as chemical, biochemical and microbiological properties (Vega et al., 2013; Pourreza et al., 2014; Hedo et al., 2015; Muñoz-Rojas et al., 2016).

In general, soil chemical properties show significant changes after fire, such as increased pH and electrical conductivity (EC) (Certini, 2005; Notario et al., 2008; Fontúrbel et al., 2016; Pereira et al., 2017). The modification of soil pH, and high temperatures reached during a fire may induce relevant changes in major soil nutrients such as organic carbon (C), nitrogen (N) and phosphorus (P), essential for the post-fire recovery of soil microbiota and vegetation (Serrasoles et al., 2008; Caon et al., 2014; Otero et al., 2015; Ferreira et al., 2016). Nutrient concentrations and bioavailability are also controlled by the activity of soil enzymes (Tabatabai, 1994; Fultz et al., 2016; Hinojosa et al., 2016). Due to their relevance in the cycles of major nutrients and high sensitivity to disturbances, enzyme activities such as glucosidase, urease and phosphatase have been considered as indicators of the degree of impact on soils (Pourreza et al., 2014; Hedo et al., 2015; Hinojosa et al., 2016). Soil enzymes can originate from plant and animal residues, but mainly from microbial biomass (Tabatabai, 1994). Consequently, both soil enzyme activities and microbial biomass contentusually show similar patterns after fire (Vega et al., 2013; Pourreza et al., 2014), and decrease with burn severity (Lombao et al., 2015; Fontúrbel et al., 2016; Holden et al., 2016). There are many examples of short-term fire effects on soil properties (e.g. Vega et al., 2013; Badía et al., 2014; Fultz et al., 2016; Heydari et al., 2017; Prendergast-Miller et al., 2017), but data on how fire affects soils over the medium term (2-5 years after fire) are scarce (Muñoz-Rojas et al., 2016), and most studies do not consider burn severity (Certini, 2005; Caon et al., 2014), highlighting the importance of further research to better understand soil resilience across gradients of burn severity. Therefore, identifying appropriate indicators of ecosystem resilience in relation to burn severity remains an important challenge for distinguishing recovered soils from those that are still affected by fire.

However, the impacts of burn severity on soil can also vary depending on plant community characteristics and soil type (Certini, 2005; Knicker, 2007; Badía et al., 2014; Keesstra et al., 2017; Prendergast-Miller et al., 2017). In the Mediterranean Basin, *Pinus pinaster* Ait. and *Pinus halepensis*

Mill. ecosystems are two of the fire-prone forests most frequently affected by fire (Pausas et al., 2008). Both plant communities are fire-sensitive and have common structural characteristics (De las Heras et al., 2012), since the dominant tree species in both is a highly flammable obligate seeder, the post-fire regeneration of which relies mainly on seeds stored in serotinous cones (Pausas et al., 2008). However, the two communities have preference for different types of soils. *P. pinaster* usually grows on sandy-acidic soils, whereas *P. halepensis* communities prefer basic soils developed from lithologies such as marls, limestones or dolomites (Richardson, 2000; De las Heras et al., 2012). This niche preference can influence the magnitude and direction of fire impacts on soil properties (Terefe et al., 2008; Martin et al., 2012; Caon et al., 2014; Ferreira et al., 2016).

In this study we aimed to characterize the medium-term effects of burn severity on soils affected by fire in two fire-prone, pine-dominated Mediterranean forest types. Specifically, we addressed the following questions: (I) Are soil properties (chemical, biochemical and microbiological) affected by burn severity over the medium term after fire in the same way in *P. pinaster* and *P. halepensis* ecosystems? (II) Can we identify potential indicators of burn severity impact on soils over the medium term after fire in Mediterranean fire-prone pine ecosystems? We hypothesise that burn severity effects on soil chemical properties will be unnoticeable in both ecosystems over the medium term after the fire, since fire impacts on soil pH, EC and nutrients are, in general, ephemeral (Certini, 2005; Zavala et al., 2014). Conversely, we expect that the effect of burn severity will be noticeable on soil properties that are largely modified by high severities (Martin et al., 2012) and that need long periods to recover from the burn severity impact. This may be the case of biochemical and microbiological properties (Dumonet et al., 1996; Dooley and Treseder, 2012; Hedo et al., 2015), whose response to burn severity can be modulated by the different edaphic conditions in the studied ecosystems (Terefe et al., 2008; Martin et al., 2012; Ferrerira et al., 2016). Therefore, we predict that soil biochemical and microbiological properties will be potential indicators of burn severity over the medium term after fire.

2 MATERIAL AND METHODS

2.1 Study sites

The study was conducted on two large wildfires that occurred in the Iberian Peninsula: the Sierra del Teleno wildfire and the Cortes de Pallás wildfire (Fig. 1).

The Sierra del Teleno wildfire occurred in León province (NW Iberian Peninsula). It burned 119 km^2 in August 2012 (Table 1), 103 km^2 being occupied by *P. pinaster* forests, with the understorey community dominated by *Pterospartum tridentatum* (L.) Willk., *Halimium lasianthum* (Lam.) Spach and *Erica australis* L. In this site, the climate is temperate with dry temperate summers (AEMET-IM, 2011). The orography is heterogeneous, ranging from flat to mountainous areas. Soils are developed over siliceous lithologies, predominantly Haplic Umbrisol and Dystric Regosol, according to the World Reference Base for Soil Resources (WRB) classification (Jones et al., 2005).

The Cortes de Pallás wildfire occurred in Valencia province (Eastern Iberian Peninsula) in June 2012. In this fire, an area of 297 km^2 was affected, burning 66 km^2 of *P. halepensis* ecosystems (Table 1) with presence of *P. pinaster*. The understory of these ecosystems was dominated by *Ulex parviflorus* Pourr., *Quercus coccifera* L. and *Rosmarinus officinalis* L. Its climate is temperate, with hot dry summers (AEMET-IM, 2011). This study site is mountainous with calcareous lithologies. In general, its soils are classified as Haplic Calcisol and Calcari-lithic Leptosol (Jones et al., 2005).

Table 1. Characteristics of the study sites.

	Sierra del Teleno wildfire	Cortes de Pallás wildfire
Fire alarm date	August 19th, 2012	June 28th, 2012
Wildfire size (km^2)	118.91	297.52
Dominant pine species	*P. pinaster*	*P. halepensis*
Pine ecosystem burned (km^2)	102.65	65.69
Elevation (m)	836 - 1,493	120 - 942
Aspect	N, S, W, E	N, S, W, E
[1] Mean annual precipitation (mm)	600 - 800	400 - 600
[1] Mean annual temperature (K)	281 - 284	286 - 290
[2] Lithology	Quartzite, conglomerate, sandstone, sand, slate, silt	Limestone, dolomite, sandstone, marl
[3] Soil WRB classification	Haplic Umbrisol, Dystric Regosol	Haplic Calcisol, Calcari-lithic Leptosol
[4] Soil textural class	sandy loam	loamy sand, sandy loam
[5] Soil $CaCO_3$ (mg/g)	-	193.2 ± 116.9
[6] Soil pH	4.86 ± 0.14	8.14 ± 0.06
[6] Soil electrical conductivity (dS m^{-1})	0.04 ± 0.01	0.15 ± 0.02
[7] Soil organic matter (mg/g)	75.5 ± 1.26	70.2 ± 12.8

[1] Precipitation and temperature were obtained from Ninyerola et al. (2005).
[2] Lithologies were determined according to the geological map of Spain (GEODE, 2017).
[3] World Reference Base for Soil Resources classification according to Jones et al., (2005).
[4] Soil textures are USDA classes. Particle-sizes were obtained according to Bouyoucos (1936).
[5] $CaCO_3$ was determined using a Bernard calcimeter (M.A.P.A., 1986).
[6] A suspension of soil:deionized water was used to determine pH (1:2.5, w/v) and conductivity (1:5, w/v).
[7] Organic matter was quantified according to Nelson and Sommers (1982).

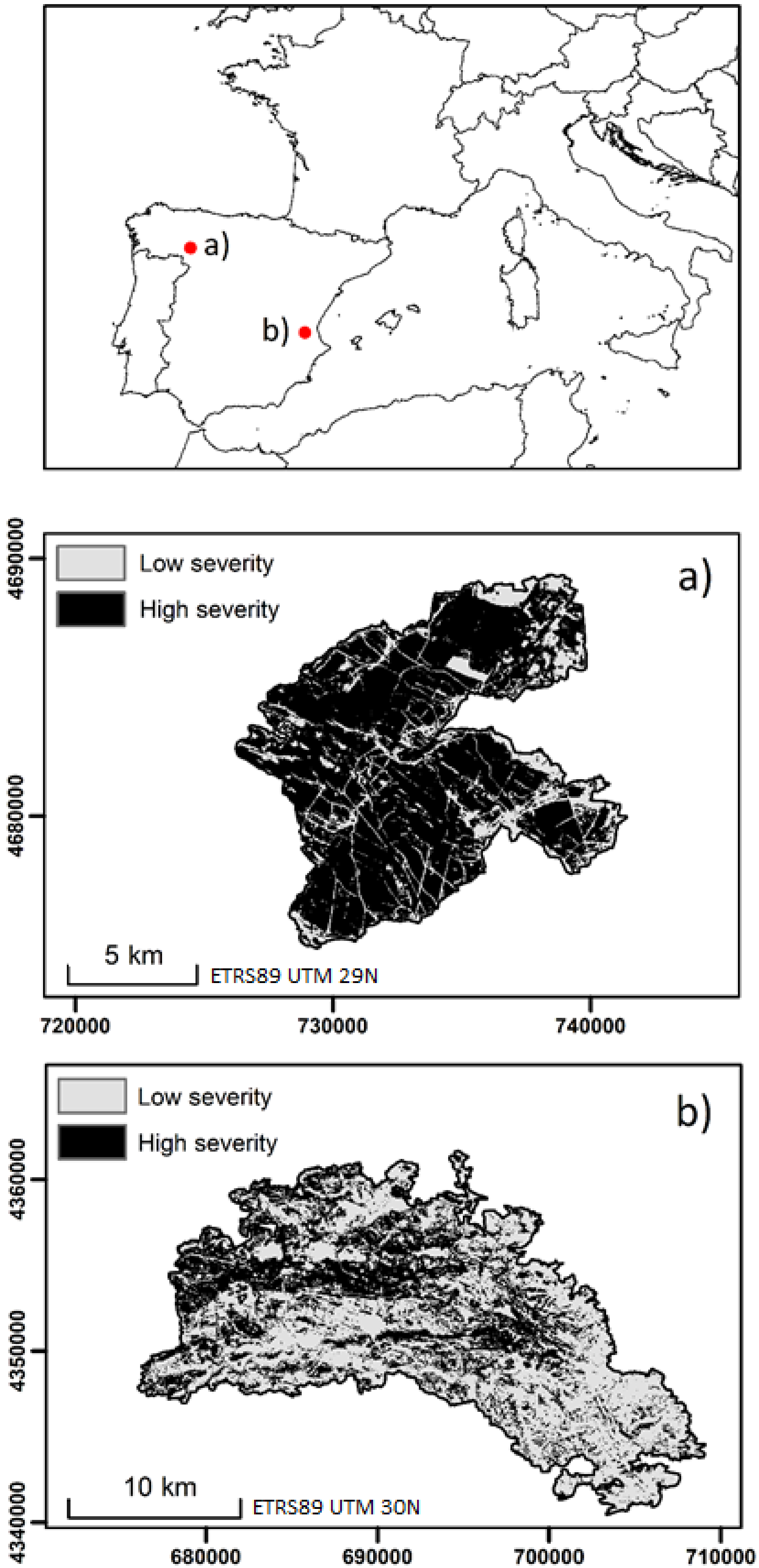

Fig. 1. Location of the Sierra del Teleno wildfire (a) and the Cortes de Pallás wildfire (b) in SW Europe, and burn severity maps (a and b study sites) differentiating low and burn severity areas through the dNBR index.

2.2 Field sampling

In each study site we mapped burn severity using the spectral index differenced Normalized Burn Ratio (dNBR) (Key, 2006) in order to design the field sampling. The dNBR, which is usually calculated from Landsat imagery, is considered to be a reference for burn severity mapping (Fernández-García et al., 2018a; Fernández-García et al., 2018b). This index uses the difference between the pre- and post-fire reflectance of Near Infrarred and Short Wave Infrarred regions to estimate the degree of change caused by fire in ecosystems (see Key, 2006). The dNBR maps of the study sites (30 m spatial resolution) were classified into low and high severity, using the value of 550 as threshold (Fernández-Manso et al., 2015). Sierra del Teleno dNBR was obtained using the Landsat 7 ETM+ scenes from September 20th, 2011 (pre-fire) and from September 6th, 2012 (post-fire); Cortes de Pallás dNBR was obtained using the Landsat 7 ETM+ scenes from August 22nd, 2011 (pre-fire) and from August 25th, 2012 (post-fire). Three years after the wildfires, a total of 56 field plots (30 m x 30 m) were established in the study sites following a stratified random design with proportionate allocation in the severity categories defined by the dNBR: 26 plots in the *P. pinaster* ecosystem in Sierra del Teleno (5 at low severity, 21 at high severity) and 30 in the *P. halepensis* ecosystem in Cortes de Pallás (12 at low severity, 18 at high severity).

In each plot we calculated field burn severity by measuring the minimum twig diameter remaining of characteristic shrub species in each community (Keeley et al., 2008; Keeley, 2009; Maia *et al*., 2012). Shrub skeletons of *Erica australis* L. were used in the *P. pinaster* ecosystem, whereas *Quercus coccifera* L. was used in the *P. halepensis* ecosystem. Within each 30 m x 30 m plot, four shrub skeletons were randomly selected, and four of the thinnest burned terminal branches were measured in each skeleton. Values were averaged obtaining a twig diameter remaining value per plot (d). We then calculated the Twig Diameter Index of burn severity (TDI) for each plot according to the model proposed by Maia *et al.* (2012): TDI = d / dmax, where dmax is the maximum diameter measured in the study site. TDI values ranged from near zero (low burn severity) to one (maximum burn severity).

To analyse the effects of burn severity on soil properties, in Spring 2015 we collected two soil samples from each 30 m x 30 m plot (Fig. 2). Each sample was composed of four subsamples. Each subsample corresponded to the volume of an auger of 5 cm diameter x 3 cm depth. Herbs, woody debris and litter were removed before collecting the soil subsamples. The soil samples were air-dried, sieved (< 2 mm) and stored at 20 °C for 2-3 months until laboratory analysis.

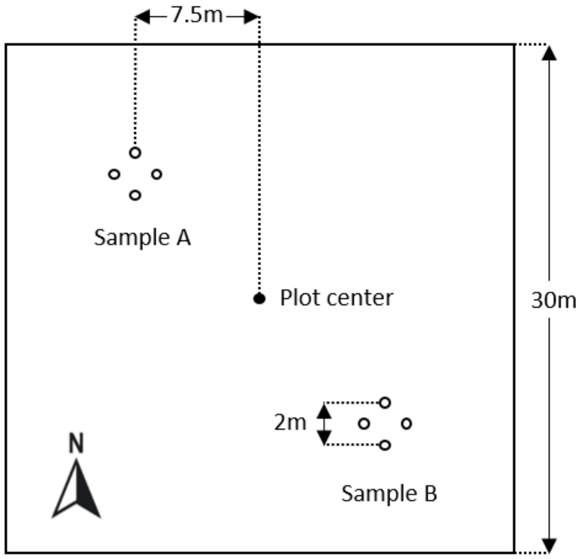

Fig. 2. Soil sampling design within each 30 m x 30 m plot. Hollow circles represent the subsamples with which each sample (sample A and sample B) was composed.

2.3 Soil analysis

We analysed soil chemical [pH, electrical conductivity (EC), organic C, total N and available P], biochemical (β-glucosidase, urease and acid phosphatase) and microbiological (microbial biomass C) properties of the soil. The two samples taken in each plot were analysed independently for all soil properties. For each soil sample, two laboratory replicates were analysed. Average values were calculated to obtain a single value per 30 x 30 m plot, for each measured property.

Soil pH was determined in a suspension of soil:deionized water (1:2.5, w/v) and EC was determined in a suspension of soil:deionized water (1:5, w/v) at 25 °C. Soil organic C was obtained by Walkley-Black dichromate oxidation (Nelson and Sommers, 1982) after grinding the soils to < 0.15 mm particle size. Total N was determined by the Kjeldahl method (Bremner and Mulvaney, 1982) using a

DK 20 digestion unit (VELP Scientifica, Italy) and available P was analysed following the Olsen *et al*., (1954) procedure, at 882 nm wavelength on a UV Mini 1240 spectrophotometer (Shimadzu Corporation, Japan).

We analysed three soil extracellular enzymatic activities corresponding to the biogeochemical cycles of C, N and P. Specifically, we selected β-glucosidase (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 enzymatic activities, we followed the procedure described by Tabatabai (1994). Thus, soils were incubated with correspondent enzyme substrates and the product released was determined colorimetrically. Two sample blanks were used for each soil sample. The *p*-nitrophenol (*p*NP) produced by the activities of β-glucosidase and acid phosphatase was measured at 400 nm wavelength, and the NH_4^+ released by urease activity was measured at 690 nm with a UV-1700 PharmaSpec spectrophotometer (Shimadzu Corporation, Japan).

Microbial biomass C was determined by the fumigation-extraction method (Vance *et al*., 1987). This procedure is based on Walkley-Black dichromate digestion to calculate the difference (E_C) in organic C between filtered extracts of chloroform fumigated ($CHCl_3$, 24 h) and non-fumigated soil samples. We then used an extraction efficiency coefficient (k_{EC}) of 0.38 (Vance *et al*., 1987; Joergensen, 1996) to calculate microbial biomass C following the formula: microbial biomass C = E_C/ k_{EC}.

2.4 Statistical analysis

A Permutational Multivariate Analysis of Variance (PERMANOVA) using the *adonis* function implemented with 1000 permutations was carried out in order to identify the effects of the ecosystem type and burn severity on soil properties considered together. We included in the analysis all the soil properties as response variables, and as predictors (1) the type of ecosystem (*P. pinaster* and *P. halepensis*) and (2) field burn severity (continuous TDI values).

To display overall similarity among soil samples for the full dataset, we performed a non-metric multidimensional scaling (NMDS) using the Bray-Curtis dissimilarity among the analysed soil properties, using values relativized (from 0 to 1) within variables. To facilitate visualization of the associations between soil samples and burn severity, the NMDS solution was rotated, matching the first axis to the external variable burn severity (continuous TDI values). Vectors of soil properties were fitted in the NMDS ordination using the *envfit* function implemented with 1000 random permutations, obtaining the directions of the vectors, the strength of the gradients (R^2) and their significances (*P*).

In order to identify which soil properties are affected by burn severity (potential indicators), and to investigate whether the effects are similar between *P. pinaster* and *P. halepensis* ecosystems, we performed an ANOVA of the Generalised Linear Models (GLMs). GLMs were fitted using Gamma error distribution with the "log" link function to predict the EC, available P, acid phosphatase and soil microbial C. We used Gaussian error distribution with the "identity" link function to model the other analysed soil properties (pH, organic C, total N, β-glucosidase and urease). The goodness of fit of the models was assessed by visual analysis of homoscedasticity and normality of residuals.

All data analyses were carried out with R (R Core Team, 2016), using the *vegan* package (Oksanen *et al*., 2016).

3 RESULTS

The results of the PERMANOVA (Table 2) showed that the type of ecosystem had a significant effect on the overall soil status of fire-prone pine forests three years after fire ($P < 0.01$). Furthermore, the analysis revealed a significant influence of burn severity on soil properties ($P < 0.05$), but no significant interaction was found between ecosystem type and burn severity.

Table 2. Results of the Permutational Multivariate Analysis of Variance (PERMANOVA) [‘adonis()’ outputs], showing the effects of the factor pine ecosystem (*P. pinaster* and *P. halepensis*), and the effects of the variable burn severity (Twig Diameter Index), and the interaction (Pine ecosystem * Burn severity), on soil properties (pH, EC, organic C, total N, available P, β-glucosidase, urease, acid phosphatase and microbial biomass C). Df are degrees of freedom. Significant P-values are in bold face.

Model term	Df	Sums of Squares	Mean of Squares	Pseudo-F	*P*
Pine ecosystem	1	0.58	0.58	7.98	**<0.01**
Burn severity	1	0.31	0.31	4.26	**0.03**
Pine ecosystem * Burn severity	1	0.04	0.04	0.49	0.59
Residuals	52	3.79	0.07		
Total	55	4.71			

The final NMDS ordination resulted in a two-dimensional solution with low stress (stress = 0.14; Fig. 3). The external parameters type of pine ecosystem (*P. pinaster* and *P. halepensis*) and burn severity (continuous TDI values) showed significant correlations with the NMDS ordination (Table 3). All the analysed soil properties had a significant role in the ordination (Table 3). Soil samples formed clearly separated clusters by ecosystem type along NMDS axis 2 (Fig. 3). In general, soils of the *P. halepensis* ecosystem were characterized by higher pH, electrical conductivity (EC), total N, and available P content, and higher β-glucosidase and urease activity than the *P. pinaster* ecosystem soils. Furthermore, NMDS significantly ordinated soil samples according to burn severity, which increased with the axis 1 (Fig 3; Table 3). Burn severity was directly related to available P, and inversely related to organic C, microbial biomass C, and the activity of enzymes, especially acid phosphatase and urease which showed a strong gradient.

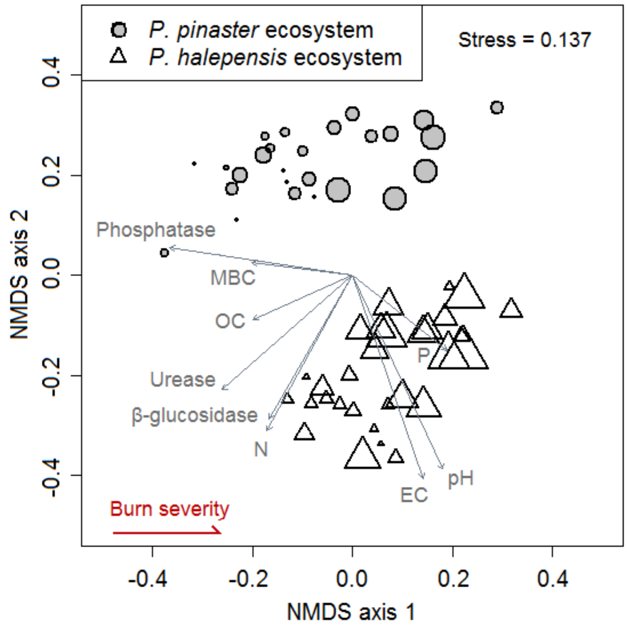

Fig. 3. NMDS ordination of soil samples from the two studied pine ecosystems (*P. pinaster* and *P. halepensis*). NMDS was performed using 9 soil properties: pH, EC (electrical conductivity), OC (organic C), N (total N), P (available P), β-glucosidase, urease, acid phosphatase and MBC (microbial biomass C). Vectors of each soil property were included to represent the direction and strength of the gradients. Shape sizes are directly proportional to burn severity (Twig Diameter Index).

Table 3. Determination coefficients (R^2) and significance (*P*) of vectors determined by the NMDS ordination (nine soil parameters in a two-dimensional ordination space). The table includes the relation of the NMDS ordination with the external parameters burn severity (Twig Diameter Index) and type of ecosystem (*P. pinaster* and *P. halepensis*). R^2 and *P* were obtained using 1000 random permutations.

NMDS term	R^2	*P*
Ordination vectors		
pH	0.92	<0.01
EC	0.93	<0.01
Organic C	0.24	<0.01
Total N	0.63	<0.01
Available P	0.29	<0.01
β-glucosidase	0.55	<0.01
Urease	0.61	<0.01
Acid phosphatase	0.69	<0.01
Microbial biomass C	0.21	<0.01
Pine ecosystem	0.66	<0.01
Burn severity	0.34	<0.01

The GLMs (Table 4) showed that all the analysed soil properties were affected by the type of pine ecosystem (P < 0.01) except organic C and microbial biomass C. Soil pH, EC, total N, available P, glucosidase and urease were higher in the *P. halepensis* ecosystem, whereas acid phosphatase was higher in the *P. pinaster* forest soils (Fig. 4).

We found that burn severity (continuous TDI values) had no effects on most chemical properties, such as pH, EC (marginally significant), total N and organic C (Table 4). However, the EC showed a different response in the two ecosystems ($P < 0.01$), increasing with burn severity in the *P. pinaster* ecosystem and with no changes in the *P. halepensis* ecosystem (Fig. 4). Available P content was significantly affected by burn severity (P < 0.05) (Table 4), with different behaviour in the two ecosystems, since it only increased with severity in the *P. pinaster* ecosystem (Fig. 4).

In relation to soil biochemical properties, we observed that burn severity significantly decreased the activity of the three enzymes (Table 4; Fig. 4). Among them, soil urease activity showed the greatest decrease with burn severity, with an analogous response in both ecosystems. In fact, burn severity explained much more of variance in urease activity (21.88 %) than in the other analysed soil properties (≤ 8.59 %). β-glucosidase activity decreased with burn severity in both ecosystems. However, we found a difference between the two ecosystems with regard to the decrease in acid phosphatase activity caused by burn severity, with a greater effect on soils in the *P. pinaster* forest.

Microbial biomass C showed a significant reduction with burn severity in both pine ecosystems without any interaction between them.

Table 4. Results of the Generalized Linear Models (GLMs) [‘anova()’ outputs] showing the effects of the factor Pine ecosystem (*P. pinaster* and *P. halepensis*), the effects of the variable Burn severity (Twig Diameter Index), and interaction (Pine ecosystem * Burn severity), on each soil property. Df are degrees of freedom. Significant *P*-values are in bold face.

Response variable	Model term	Df	Deviance explained	Residual deviance	F	*P*
pH	Null			165.04		
	Pine ecosystem	1	162.95	2.08	4286.52	**<0.01**
	Burn severity	1	0.02	2.07	0.45	0.51
	Pine ecosystem * Burn severity	1	0.09	1.98	2.36	0.13
Electrical conductivity	Null			37.10		
	Pine ecosystem	1	34.47	2.64	814.12	**<0.01**
	Burn severity	1	0.16	2.48	3.81	0.06
	Pine ecosystem * Burn severity	1	0.41	2.06	9.75	**<0.01**
Organic C	Null			59.51		
	Pine ecosystem	1	0.45	59.06	0.43	0.52
	Burn severity	1	3.36	55.70	3.21	0.08
	Pine ecosystem * Burn severity	1	1.22	54.49	1.16	0.29
Total N	Null			0.36		
	Pine ecosystem	1	0.11	0.25	23.62	**<0.01**
	Burn severity	1	0.00	0.24	0.69	0.41
	Pine ecosystem * Burn severity	1	0.00	0.24	0.39	0.54
Available P	Null			12.31		
	Pine ecosystem	1	1.48	10.83	9.46	**<0.01**
	Burn severity	1	0.66	10.17	4.25	**0.04**
	Pine ecosystem * Burn severity	1	2.19	7.98	14.01	**<0.01**
β-glucosidase	Null			21.14		
	Pine ecosystem	1	5.80	15.35	23.02	**<0.01**
	Burn severity	1	1.82	13.53	7.22	**<0.01**
	Pine ecosystem * Burn severity	1	0.43	13.10	1.72	0.20
Urease	Null			234.61		
	Pine ecosystem	1	36.18	198.42	12.86	**<0.01**
	Burn severity	1	51.33	147.10	18.24	**<0.01**
	Pine ecosystem * Burn severity	1	0.78	146.32	0.28	0.60
Acid phosphatase	Null			28.109		
	Pine ecosystem	1	10.32	17.79	40.13	**<0.01**
	Burn severity	1	2.22	15.57	8.62	**<0.01**
	Pine ecosystem * Burn severity	1	1.34	14.23	5.20	**0.03**
Soil microbial C	Null			27.09		
	Pine ecosystem	1	1.34	25.76	2.98	0.09
	Burn severity	1	1.76	24.00	3.91	**0.05**
	Pine ecosystem * Burn severity	1	0.78	23.22	1.73	0.19

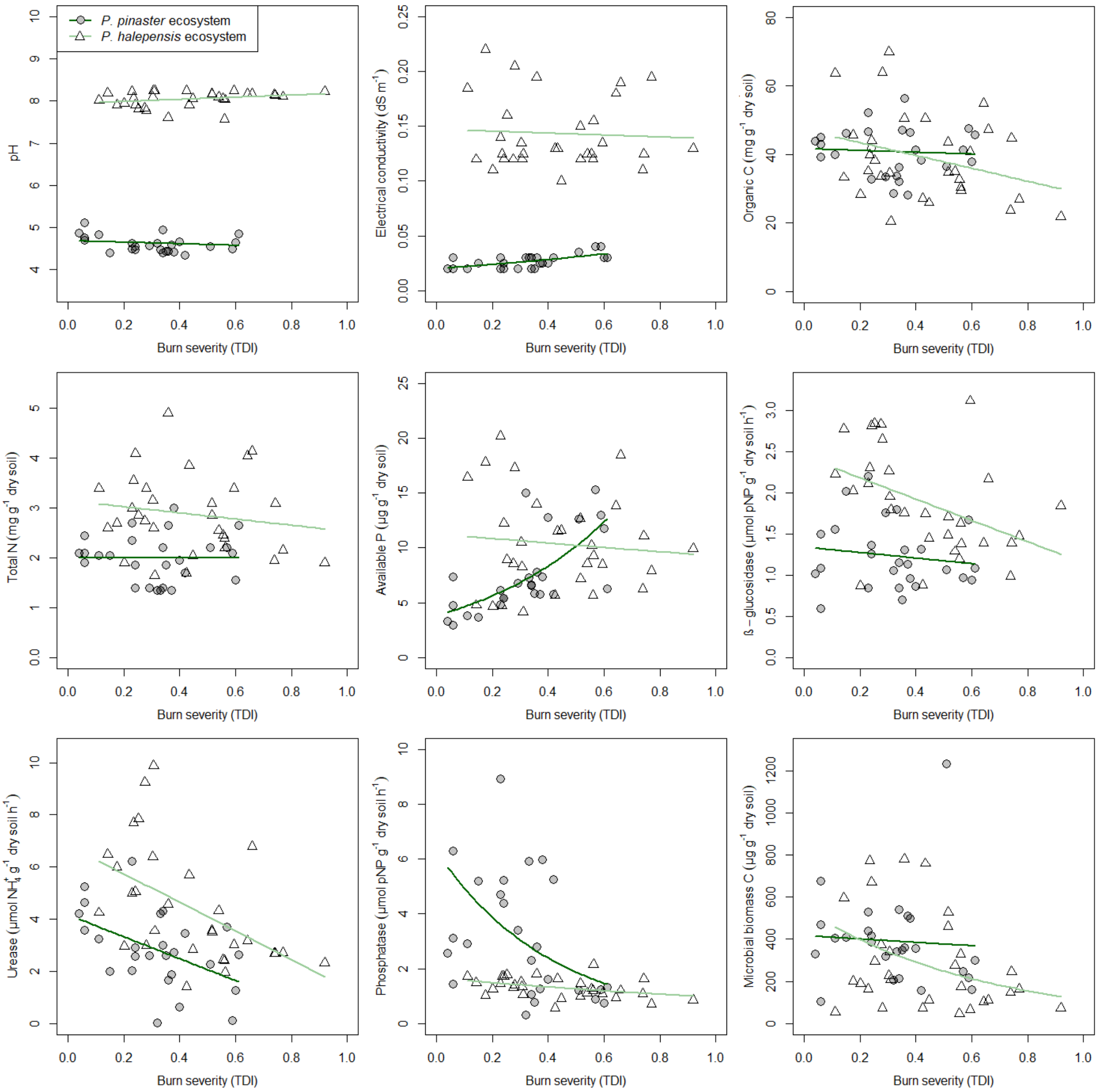

Fig. 4. Relationship between each soil property and burn severity (measured as Twig Diameter Index) over the medium term after fire in the two studied ecosystems (*P. pinaster* and *P. halepensis*). The lowest TDI values correspond to the lowest burn severities whereas the highest TDI values correspond to the highest burn severities.

4 DISCUSSION

Our results demonstrate that the type of ecosystem and burn severity determined the overall soil status over the medium term (three years after fire) in two contrasting Mediterranean fire-prone pine forest types. Burn severity effects on soils were exerted on all the biochemical and microbiological properties and available P. Conversely, burn severity did not alter other soil parameters three years after the fire, such as pH, electrical conductivity (EC), organic C or total N.

Different studies have shown a clear increase in soil alkalinity and EC for short-term post-fire measurement events (Notario et al., 2008; Knelman et al., 2015; Heydari et al., 2017), and some of them have related this effect to burn severity, in both *P. pinaster* (Vega et al., 2013; Martin et al., 2012) and *P. halepensis* (Henig-Sever et al., 2001; Bárcenas-Moreno and Bååth, 2009) ecosystems. However, it has been noted that these changes in pH and in EC are not persistent for a long time (Certini, 2005; Zavala et al., 2014; Pereira et al., 2017), coinciding with our results over the medium term after fire, where no effect of burn severity was found. The lack of burn severity effects over the medium term after the fire on soil pH may be associated with the removal of ash bases, which are expected to be higher in the severely burned areas, by water and wind (Certini, 2005; Notario et al., 2008), and the formation of new humus at longer term (Zavala et al., 2014). Similar processes may result in the uniformity of EC values within the burned area over the medium term after fire, since soluble salts are quickly leached or transported by runoff (Zavala et al., 2014). However, we found different trends for EC between *P. pinaster* and *P. halepensis* ecosystems that can be attributed to the different behaviour of available P in the studied ecosystems, since a higher available P content contributes to increases in EC (Bolan et al., 1996).

Among the soil major nutrients, available P content was affected by burn severity over the medium term after fire. We found a large increase in available P in the *P. pinaster* ecosystem with burn severity. These results agree with those obtained by Dzwonko et al. (2015) in acidic soils in a *P. sylvestris* forest three years after fire, and with other shorter-term (0-12 months post-fire) studies in

P. pinaster ecosystems (Martin et al., 2012; Vega et al., 2013). Available P in soil can increase after fire proportionally to burn severity (Vega et al., 2013; Pourreza et al., 2014; Dzwonko et al., 2015; Heydari et al., 2017) because burning transforms organic P from litter, soil organisms and vegetation into orthophosphate (Knicker, 2007; Serrasoles et al., 2008). In the longer term, available P content can continue to increase through sorption-desorption processes (Serrasoles et al., 2008). In this way, Romanyà et al. (1994) revealed that large ash inputs, typical in high-severity fires, facilitate P sorption to the solid phase. This sorption process hinders P losses by percolation or runoff over the short term, and consequently P can be released over the medium term after fire, thereby increasing the available P content in soils (Serrasoles et al., 2008; Otero et al., 2015). However, fire effects on available P are highly dependent on the type of ecosystem (Certini, 2005; Ferreira et al., 2016) mainly due to differences in soil type (Martin et al., 2012). For example, in calcareous soils, P retention is dominated by precipitation reactions, which forms apatite – a long-term P sequestration form – several months after fire, thereby keeping P unavailable for use by biota (Caon et al., 2014; Otero et al., 2015). This effect may explain the different response obtained in the *P. halepensis* ecosystem, with calcareous soils, where we did not find a positive effect of burn severity on available P.

Soil organic C and total N were not significantly affected by burn severity three years after fire. Contrasting results can be found in the literature about the effects of wildfire on soil C and N concentration on mineral soils (Johnson & Curtis, 2001; Certini, 2005; Neary et al., 2008; Badía et al., 2014), indicating a high dependence on factors that are variable among and within fires, such as the depth of burning, litter inputs, post-fire vegetation or the modification of decomposition rates (Johnson & Curtis, 2001; Caon et al., 2014). Some specific studies focused on burn severity effects on Mediterranean soils have shown significant decreases in soil organic C concentration with burn severity (Vega et al., 2013), whereas others have found increases (Maestrini et al., 2017) or no effects (Mataix-Solera and Doerr, 2004), which is in in agreement with the results obtained in this study. In the case of soil total N, several studies have suggested that the effect of burn severity on this soil property is not important (Caon et al., 2014). The meta-analysis carried out by Wan et al. (2001) indicates that fire has no significant effects on total N content. Furthermore, Tecimen and

Sevgi (2011) confirmed that fire intensity is not a relevant factor on total N in Mediterranean soils, even at temperatures of 350 °C sustained for a four-hour time period.

In relation to soil biochemical properties, we found that soil extracellular enzyme activity rates (for β-glucosidase, urease and acid phosphatase) decreased with burn severity over the medium term after fire. In general, these results are in agreement with those obtained by most studies analysing fire effects on enzymatic activities over the short (Fontúrbel et al., 2012; Vega et al., 2013; Pourreza et al., 2014; Knelman et al., 2015) and medium term post-fire (Gutknecht et al., 2010; Miesel et al., 2011). The negative effects of burn severity on soil extracellular enzyme activities over the short and medium term after fire could be explained by (1) direct enzyme denaturation (Knicker, 2007; Vega et al., 2013; Fultz et al., 2016) occurring when the temperature reached during fire exceeds 60-70°C, and the complete destruction of soil enzymes occurring at 180°C (Mataix-Solera et al., 2009); (2) the removal of vegetation – which increases with burn severity (Keeley, 2009) – and consequent changes in the composition of soil microbiota (Knicker, 2007; Mataix-Solera et al., 2009), because they are the main sources of soil enzymes (Tabatabai, 1994); and (3) the increase in nutrients after burning, such as available N and available P, which often persist over the medium term after fire (Lezberg et al., 2008; Dzwonko et al., 2015). Several authors have indicated the influence of soil nutrients on soil extracellular enzyme activity (Mataix-Solera et al., 2009; Miesel et al., 2011; Pourreza et al., 2014), because organisms generate enzymes to catalyse the release of nutrients. When concentrations of nutrients are high, organisms do not need to produce these extracellular enzymes (Bünemann, 2008), which are highly energetically costly for biota (Pourreza et al., 2014). Additionally, the release of elevated concentrations of the end reaction products caused by fire may inhibit enzyme activities (Schmidt et al., 1983; Goberna et al., 2012). These reasons explain the different response of acid phosphatase activity in the two studied ecosystems, which was inversely related to the concentration of available P in both.

The decreases we found in soil enzyme activities can also be related to the loss of microbial biomass C (Knelman et al., 2015). Although some studies have shown transient increases in microbial biomass

C immediately after low severe fires, attributed to increases in the concentration of oxidisable C and nutrients (Bárcenas-Moreno and Bååth, 2009; Goberna et al., 2012), decreases in soil microbial C have been largely reported in the literature over the short term after fire (e.g. Miesel et al., 2012; Vega et al., 2013; Lombao et al., 2015; Muñoz-Rojas et al., 2016; Prendergast-Miller et al., 2017), and even up to 11 (Dumonet et al., 1996) or 15 years post-fire (Dooley and Treseder, 2012). The decrease in microbial biomass C content with burn severity can be explained by the direct mortality of microorganisms due to lethal temperatures (50-160 °C according to Neary et al., 2008) reached during fire (Holden and Treseder, 2013; Muñoz-Rojas et al., 2016), as well as by indirect effects due to changes in the soil environment and vegetation abundance and composition (Hedo et al., 2015). For example, decreases in the availability of organic resources in soils (Pérez-Varela et al., 2015), or the incorporation of organic pollutants and heavy metals during combustion can limit post-fire development of microorganisms (Certini, 2005; Vega et al., 2013). Additionally, decreases in soil microbial C have been related to modifications in substrates such as soil drying or depletion and recovery of litter following fire, depending on burn severity (Dooley and Treseder, 2012).

Our results indicated that burn severity left an important legacy on soil biochemical and microbiological properties over the medium term after fire. We identified that enzymatic activities β-glucosidase and urease, and microbial biomass C may be informative as indicators of burn severity legacy on soils over the medium term after fire in both *P. pinaster* and *P. halepensis* ecosystems. Furthermore, available P content and acid phosphatase activity were identified as potential indicators in the *P. pinaster* ecosystem, which has acidic soils. Biochemical and microbiological properties have been proposed as indicators of soil status after wildfires by other authors (Hedo et al., 2015; Lombao et al., 2015; Muñoz-Rojas et al., 2016), not only because they are affected by fire, but also because of their relevance in the functioning of the ecosystem, since they are involved in processes related to soil conservation through stabilization of soil structure, nutrient cycling and many other physico-chemical properties (Pourreza et al., 2014; Hinojosa et al., 2016).

5 CONCLUSIONS

Soil chemical (available P), biochemical (β-glucosidase, urease and acid phosphatase) and microbiological (microbial biomass C) properties were affected by burn severity over the medium term after fire in fire-prone pine ecosystems.

In general, soil biochemical (β-glucosidase, urease) and microbiological (microbial biomass C) properties were negatively affected by burn severity, showing similar patterns in the *P. pinaster* and *P. halepensis* ecosystems. Soil available P increased with burn severity in the *P. pinaster* ecosystem (acidic soils), the only ecosystem where acid phosphatase activity was reduced.

We identified β-glucosidase, urease and microbial biomass C as potential indicators of the burn severity legacy on soils in both type of ecosystems (*P. pinaster* and *P. halepensis*) over the medium term after fire. Available P content and acid phosphatase activity were potential indicators in the *P. pinaster* ecosystem.

This study provides a reference for monitoring fire effects in fire-prone pine ecosystems in the Mediterranean Basin. We encourage managers to take into account burn severity when developing hazard reduction and restoration strategies over the medium term after large wildfires.

APPENDIX A. SUPPLEMENTARY DATA

Table A1. Location, environmental description (lithology, elevation, slope, aspect) and burn severity measurements (differenced Normalized Burn Ratio, dNBR; and Twig Diameter Index, TDI) of the studied plots. Reference system for coordinates is ETRS89, Zone 29N for plots of the *Pinus pinaster* ecosystem and Zone 30N for plots of the *Pinus halepensis* ecosystem. Lithological information was obtained from GEODE (2017).

Plot ID	Ecosystem	X UTM	Y UTM	Lithology	Elevation (m)	Slope (°)	Aspect (°)	dNBR	TDI
L002	*P. pinaster*	732437	4682073	Quartzite, sandstone and slate	1104	17	104	254	0.51
L005	*P. pinaster*	729862	4679334	Alluvium, slate, quartzite and silt	1086	11	183	865	0.06
L011	*P. pinaster*	732845	4682626	Alluvium, slate, quartzite and silt	1028	19	195	383	0.57
L013	*P. pinaster*	733489	4683203	Quartzite, sandstone and slate	1029	13	209	453	0.40
L015	*P. pinaster*	729538	4679480	Alluvium, slate, quartzite and silt	1113	12	177	682	0.35
L024	*P. pinaster*	730644	4678809	Conglomerate, sandstone and silt	1004	3	129	901	0.34
L043	*P. pinaster*	730750	4684346	Conglomerate, sandstone and silt	1002	5	97	1039	0.60
L045	*P. pinaster*	732374	4682926	Quartzite, sandstone and slate	1065	16	192	898	0.59
L049	*P. pinaster*	733121	4683445	Quartzite, sandstone and slate	990	6	15	915	0.32
L060	*P. pinaster*	730747	4682832	Quartzite, sandstone and slate	1111	8	211	780	0.06
L061	*P. pinaster*	732123	4682403	Quartzite, sandstone and slate	1103	18	71	691	0.29
L065	*P. pinaster*	731963	4682227	Quartzite, sandstone and slate	1114	10	190	870	0.24
L067	*P. pinaster*	731619	4681427	Conglomerate, sandstone and silt	1067	8	240	864	0.33
L075	*P. pinaster*	732433	4680308	Quartzite, sandstone and slate	1072	12	270	816	0.61
L080	*P. pinaster*	730176	4683983	Quartzite, sandstone and slate	1069	13	49	713	0.42
L082	*P. pinaster*	729524	4683232	Quartzite, sandstone and slate	1204	12	157	794	0.23
L084	*P. pinaster*	730506	4683178	Quartzite, sandstone and slate	1184	15	207	514	0.15
L09	*P. pinaster*	732542	4682393	Quartzite, sandstone and slate	1045	14	31	535	0.34
L091	*P. pinaster*	729557	4683719	Quartzite, sandstone and slate	1122	13	22	699	0.24
L092	*P. pinaster*	730141	4683813	Quartzite, sandstone and slate	1091	10	44	1016	0.36

L093	*P. pinaster*	730453	4683678	Quartzite, sandstone and slate	1082	13	26	876	0.23
L094	*P. pinaster*	732089	4682535	Quartzite, sandstone and slate	1097	17	84	815	0.37
L095	*P. pinaster*	730970	4683617	Quartzite, sandstone and slate	1079	16	3	1113	0.38
L130	*P. pinaster*	732189	4681005	Quartzite, sandstone and slate	1098	17	197	619	0.11
L131	*P. pinaster*	730403	4682268	Alluvium, slate, quartzite and silt	1119	17	185	749	0.04
L133	*P. pinaster*	732601	4681549	Quartzite, sandstone and slate	1031	7	77	828	0.06
V001	*P. halepensis*	682735	4356504	Clay, conglomerate, sand and calcarenite	401	6	287	385	0.45
V002	*P. halepensis*	678772	4351460	Dolomite	491	16	226	516	0.17
V005	*P. halepensis*	682031	4353228	Undifferentiated alluvial	621	2	15	778	0.28
V006	*P. halepensis*	678648	4351972	Sand, sandstone, loam and red clay	586	18	223	546	0.23
V007	*P. halepensis*	682507	4356301	Clay, conglomerate, sand and calcarenite	397	14	292	298	0.11
V008	*P. halepensis*	683026	4356323	Clay, conglomerate, sand and calcarenite	396	5	85	811	0.92
V009	*P. halepensis*	678891	4352043	Red calcareous conglomerate with clay	567	18	184	768	0.77
V011	*P. halepensis*	679739	4353245	Dolomite	708	12	245	806	0.64
V012	*P. halepensis*	680012	4353036	Dolomite	696	7	168	693	0.66
V016	*P. halepensis*	680663	4353374	Limestone and marl	663	7	88	788	0.59
V017	*P. halepensis*	678883	4353931	Sand, sandstone, marls and limestone	759	21	178	394	0.30
V018	*P. halepensis*	681667	4351666	Limestone and marl	651	4	347	634	0.36
V019	*P. halepensis*	679125	4350611	Red calcareous conglomerate with clay	443	18	349	464	0.20
V021	*P. halepensis*	680651	4350852	Versicolor gypsum	378	14	294	787	0.31
V024	*P. halepensis*	678904	4351030	Red calcareous conglomerate with clay	452	15	54	272	0.31
V027	*P. halepensis*	682274	4351895	Limestone and marl	577	19	47	812	0.52
V028	*P. halepensis*	682560	4354588	Undifferentiated alluvial	601	4	32	895	0.74
V029	*P. halepensis*	681820	4355223	Clay, conglomerate, sand and calcarenite	433	14	315	778	0.42
V035	*P. halepensis*	682995	4352953	Sand, sandstone, marl and limestone	615	11	229	702	0.51
V038	*P. halepensis*	681691	4354068	Undifferentiated alluvial	610	15	317	781	0.56
V039	*P. halepensis*	683294	4352553	Limestone, marl and calcarenite	550	7	23	376	0.23
V040	*P. halepensis*	682994	4352339	Sand, sandstone, marl and limestone	515	17	164	398	0.14
V041	*P. halepensis*	680944	4355204	Clay, conglomerate, sand and calcarenite	521	11	95	461	0.25
V042	*P. halepensis*	679589	4354259	Dolomite	713	13	125	450	0.27
V044	*P. halepensis*	678706	4354681	Dolomite	843	12	194	404	0.24

V048	*P. halepensis*	681091	4355938	Clay, conglomerate, sand and calcarenite	442	14	18	697	0.56
V050	*P. halepensis*	681167	4355048	Sand, sandstone, marl and limestone	461	13	157	644	0.54
V051	*P. halepensis*	683047	4352416	Sand, sandstone, marl and limestone	504	23	51	659	0.43
V052	*P. halepensis*	682958	4355088	Sand, sandstone, marl and limestone	463	12	50	751	0.74
V058	*P. halepensis*	681235	4353660	Undifferentiated alluvial	626	3	95	580	0.56

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