Do Fire Regime Attributes Affect Soil Biochemical Properties in the Same Way under Different Environmental Conditions?

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Abstract: Global change is altering fire frequency and severity in many regions across the world. In this work, we studied the impact of different frequency and severity regimes on the soil biochemical properties in burned areas with different environmental conditions. We selected three sites dominated by pine ecosystems along a Mediterranean-Transition-Oceanic climatic gradient, where we determined the fire frequency, and severity of the last wildfire. Four years after the last wildfire, we established 184 4 m^2 plots. In each plot, we collected a composed soil sample from a 3 cm depth, and measured several ecological variables potentially affected by the fire frequency and severity (cover of bare soil, cover of fine and coarse plant debris, cover of vegetation, and vegetation height). From each soil sample, we analyzed the enzymatic activities corresponding to the biogeochemical cycles of carbon, nitrogen, and phosphorus (β-glucosidase, urease, and acid-phosphatase, respectively), and the microbial biomass carbon. The results indicated that fire frequency only played a significant role in soil biochemical properties at the Mediterranean and Transition sites. Specifically, we found that increases in frequency contributed to increased urease and phosphatase activities (at the Transition site), as well as microbial biomass carbon (at the Mediterranean and Transition sites). In relation to burn severity, we found opposite patterns when comparing the Mediterranean and Oceanic sites. Specifically, increased severity significantly decreased β-glucosidase, urease, and microbial biomass carbon at the Mediterranean site, whereas at the Oceanic one, severity significantly increased them. Burn severity also decreased microbial biomass carbon at the Transition site. Our results also indicated that, overall, fire frequency determined the studied ecological variables at the Mediterranean and Transition sites, but clear indirect effects on biochemical properties due to changes in ecological variables were not found. This study adds to the knowledge on the impact of shifts in fire regimes on soils in the current context of change.

Keywords: burn severity; fire frequency; enzymatic activities; microbial biomass; environmental conditions

1. Introduction

More than half of the Earth's surface is vulnerable to forest fires [1,2]. In general, ecosystems are adapted to natural fire regimes, recovering under a relatively wide range of fire frequencies and severities [3]. However, fire regimes are changing in many regions across the globe [4–6]. In view of vegetation encroachment due to farmland abandonment that has occurred in the last decades [7,8] and

climate change projections [9], Southern Europe is one of the regions where fire regimes may change to a greater extent [10,11].

The term fire regime refers to the spatial, magnitude, and temporal attributes of fire in a given ecosystem [12–14]. Nowadays, much research investigates the importance of changes in fire regimes, most of which focuses on the fire frequency and severity [15–19]. Fire frequency is a temporal fire regime attribute defined as the number of fires in a given period [12]. Burn severity is a magnitude fire regime attribute defined as the loss of or change in ecosystem biomass that can be measured by remote sensing methods [20]. Both attributes have been shown to shape the ecosystem structure, composition, and resilience [12,20] and determine the fire impacts in relation to soil properties [16,17]. However, it is not clear whether the fire frequency and burn severity effects on soils are consistent across ecosystems with different environmental conditions, such as different climates and soil types [17].

Fire impacts on soils are particularly intense in fire-prone pine forests [7]. In these forests, which are the most affected by wildfires in Southern Europe [21–23], fire causes immediate changes in soils [16] that may persist for several years because of the direct impact and changes in the ecological variables, such as vegetation cover [17–25]. In the long term (>5 years), the effects of fire on soil properties are less evident [26,27]. Many works have studied the impact of fire on soil physical properties, reporting decreases in the surface water repellency after frequent fires [19], and decreases in the size of soil aggregates at high severities [16,28]. Other studies have focused on soil chemical properties. For instance, it has been demonstrated that frequent fires can decrease some nutrients, such as the total nitrogen (N) [29,30], whereas severe fires usually decrease organic carbon (C) and increase available phosphorus (P) concentrations [16,17]. However, although some studies have addressed fire frequency [31,32] and burn severity [15,33–35] impacts on soil biochemical properties, to our knowledge, none of them have analyzed potential interaction effects between both attributes on soil enzymatic activities and microbial biomass.

Soil enzymatic activities catalyze all biochemical reactions in soils [36]. There are multiple soil extracellular enzymes, and each one catalyzes a specific chemical reaction. However, those involved in the cycles of major soil nutrients, such as glycosidases (involved in the C cycle), amidohydrolases (involved in the N cycle), and phosphatases (involved in P mineralization), are particularly relevant [10,26,37]. β-glucosidases are the predominant glycosidases in soils [36]. β-glucosidases hydrolyze glycosidic bonds of glucosides and oligosaccharides releasing glucose, being key elements in cellobiose degradation [38]. Urease is one of the most important amidohydrolases in soils [36], as it hydrolyzes urea into ammonia [38]. Among phosphatases, acid phosphatase has been the most extensively studied [36] because it hydrolyzes phosphate monoesters into phosphate [38], contributing to P mineralization and plant nutrition. The main source of soil extracellular enzymes is the microbial biomass (bacteria plus fungi) [10,36], which can also act as a nutrient source due to fast turnover, and contributes to stabilizing soil organic matter [39,40]. Consequently, soil enzyme activities and microbial biomass usually show similar patterns in undisturbed soils [34,37]. However, fire frequency and severity may have different impacts on the activity of soil enzymes and microbial biomass [17,18,34].

Fire regimes might affect enzymatic activities and the microbial biomass because of direct impacts [15,16,31,32], but may also modify ecological variables, such as the vegetation cover or plant debris, which are expected to be lower after frequent and severe fires [18,20,41]. In fact, densely vegetated areas usually have higher enzymatic activity and microbial biomass C [42,43], because roots and plant residues are, along with the microbial biomass, important sources of soil extracellular enzymes [36,42]. However, potential indirect effects of fire frequency and severity on soil biochemical properties through changes in ecological variables have not been studied in depth. Further knowledge is important to elucidate how changes in fire regimes modify the enzymatic activity of soils and microbial biomass in the current scenario of global change.

The aim of this work is to study the effects of fire frequency and burn severity on soil biochemical properties four years after a fire. Specifically, we aim to answer the following questions: (i)

Are fire frequency and severity related to the activity of soil enzymes (β-glucosidase, urease, and acid-phosphatase) and microbial biomass C in the same way under different environmental conditions? (ii) Are fire frequency and severity impacts on soil biochemical properties a consequence of changes in vegetation and plant residues covering soil?

2. Materials and Methods

2.1. Study Sites

We selected three study sites (Figure 1) within the Iberian Peninsula along the Mediterranean-Oceanic climatic gradient: the Mediterranean site, the area affected by a wildfire in Cortes de Pallás in summer 2012 (297 km^2); the Transition site, the area affected by a wildfire in Sierra del Teleno in summer 2012 (119 km^2); and the Oceanic site, the area affected by a wildfire in Monte Pindo in summer 2013 (25 km^2). The three wildfires were largely stand-replacing fires affecting serotinous pine forests.

The Mediterranean site is located in the eastern Iberian Peninsula (39°18′ N, 0°54′ W; province of Valencia). The climate in this region is characterized by hot dry summers that give rise to four months of summer drought, annual precipitation of 400–600 mm, and a mean annual temperature of 13–17 °C [44]. The orography is mountainous, with an altitude between 120 and 942 m. The lithology is calcareous, with basic soils (8.14 ± 0.06; mean pH ± standard error) predominantly classified as Haplic Calcisol and Calcari-lithic Leptosol [45]. Soil texture ranges from loamy sand to sandy loam. Much of the vegetation affected by the wildfires were *Pinus halepensis* Mill. forests with a Mediterranean scrub undergrowth comprised of *Ulex parviflorus* Pourr., *Quercus coccifera* L., and *Rosmarinus officinalis* L., among other species. The Mediterranean site was affected by wildfires in 1978, 1991, 1994, and 2012.
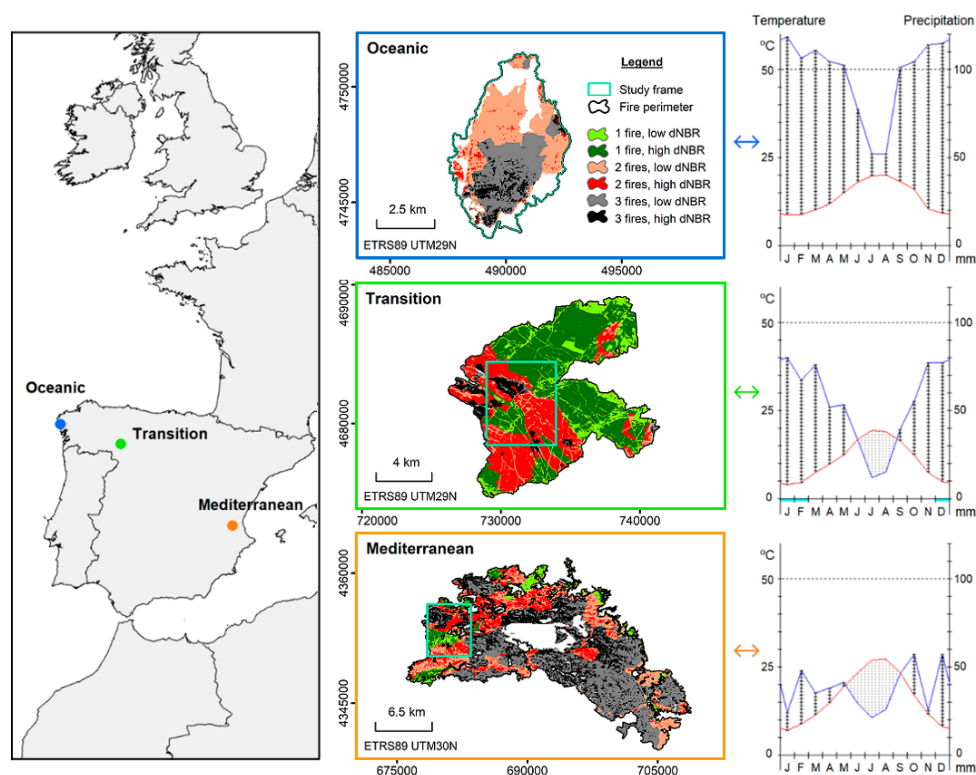

Figure 1. Location of the study sites in the Iberian Peninsula (panel on the left) and perimeters of the last wildfire in each study site with the different fire frequencies of 1, 2, and 3 fires and severities of the last wildfire of low and high differenced Normalized Burn Ratio (dNBR), using 0.55 as the threshold (panel in the centre). Panels on the right show the climate diagrams of the closest meteorological stations for a period ≥ 13 years [46]. Maps based on [47].

The Transition site is located in the northwestern Iberian Peninsula (42°15′ N, 6°11′ W, province of León). The climate at this site is characterized by temperate dry summers with two months of summer drought, annual precipitation of 600–800 mm, and a mean annual temperature of 8–11 °C [44]. The orography is mountainous, with altitudes ranging between 836 and 1493 m. The lithology is siliceous, with sandy loam acidic soils (pH 4.86 ± 0.14), predominantly Haplic Umbrisol and Dystric Regosol [45]. The Transition site is occupied by *Pinus pinaster* Ait. ecosystems, with a shrub community dominated by *Pterospartum tridentatum* (L.) Willk., *Halimium lasianthum* (Lam.) Spach, and *Erica australis* L. The Transition site was affected by wildfires in 1978, 1991, 1998, and 2012.

The Oceanic site is located in the northwestern Iberian Peninsula (42°53′ N, 9°7′ W; province of La Coruña). The climate in this region is characterized by temperate summers with no drought, annual precipitation of 1700–1800 mm, and a mean annual temperature of 12–15 °C [44]. The orography is mountainous, with an altitude rising from sea level to 929 m. Soils are siliceous (pH 5.08 ± 0.10) with frequently exposed bedrock (biotite granite), predominantly classified as Umbrisols [45]. Vegetation comprises *P. pinaster* forests highly variable in terms of tree density, with some of them having developed over abandoned crop fields and plantations. The presence of *Eucalyptus globulus* Labill is also common. The understorey is mostly comprised of *Ulex europaeus* L., *Rubus* sp., *Cytisus scoparius* (L.) Link, and *Erica umbellata* Loefl. ex L. The Oceanic site was affected by fires in 1995, 1999, 2000, 2001, 2004, 2005, and 2013.

2.2. Fire Regime Attributes: Frequency and Severity

In order to calculate fire frequency, we obtained the fire perimeters for 1978–2012 at the Mediterranean and Transition sites and for 1990–2013 at the Oceanic one. For the Oceanic site, we selected a shorter period because of major changes in land use prior to that date and the lack of reliable sources of information to quantify burn severity for longer (low availability of cloud-free Landsat scenes). For the Mediterranean site, official cartography of fires was available for 1978 onwards. For the Transition and Oceanic sites, we digitized the fire perimeters using false color composites from Landsat imagery (MSS, TM, ETM+, and OLI sensors), according to Fernández-García [48]. Finally, we spatially characterized the fire frequency by geo-processing the fire perimeters, calculated as the number of wildfires.

We calculated the burn severity as a continuous variable, using the differenced Normalized Burn Ratio (dNBR) of the last wildfire. This index is a standard measurement of burn severity and its performance has been largely validated in many ecosystems worldwide. In pine forests of the Iberian Peninsula, models relating dNBR and field measurements of burn severity (Composite Burn Index) have shown R^2 values ranging from 0.68 to 0.88 [49]. Landsat 7 scenes from 22 August 2011 (pre-fire) and from 25 August 2012 (post-fire) were used to calculate the dNBR at the Mediterranean site; Landsat 7 scenes from 20 September 2011 (pre-fire) and 6 September 2012 (post-fire) were used to calculate the dNBR at the Transition site; and Landsat 8 scenes from 30 August 2013 (pre-fire) and 15 September 2013 (post-fire) were used to calculate the dNBR at the Oceanic site. Images were atmospherically and topographically corrected before applying the dNBR algorithm. See [48] for a detailed description of the imagery pre-processing and dNBR calculation. We used the dNBR index with values ranging from −2 to 2.

2.3. Field Sampling: Soil and Ecological Variables

In order to focus field sampling in areas with a heterogeneous fire frequency and severity, we developed maps for each study area (Figure 1) that combined frequency (one to three fires) and severity (low and high severity; using the dNBR value 0.55 as the threshold, with this value representing moderate severity, according to [17,18,48,49]) categories. At the Mediterranean and Transition sites, we focused on a study frame of 3000 ha, and at the Oceanic site, we selected the entire wildfire (2500 ha) (Figure 1). Then, we distributed 30 m × 30 m field plots throughout all fire frequency and severity situations that were extensively represented (six categories at the Mediterranean and Transition sites,

and four categories at the Oceanic site). The size of 30 m × 30 m corresponds to the spatial resolution of the fire regime maps. The minimum distance between each 30 m × 30 m plot was fixed at 200 m, and the minimum number of plots per study site was fixed at 18. In each 30 m × 30 m plot, we established two 2 m × 2 m field plots (one at NW, and the other at SE, separated by 20 m). A total of 184 2 m × 2 m field plots were established: 60 plots at the Mediterranean site, 88 plots at the Transition one, and 36 plots at the Oceanic one. Plot centers were georeferenced with high-precision GPS (RMSE X, Y < 0.5 m).

Four years after the last wildfires (May-June 2016 at the Mediterranean and Transition sites, and May-June 2017 at the Oceanic site), in each 2 m × 2 m plot, we collected a soil sample composed of four subsamples collected at the cardinal points of the plot. Soils were collected after removing herbs, woody debris, and litter, and using an auger of a 5 cm diameter × 3 cm depth. This depth is commonly used in fire ecology studies [16,17] because, in general, fire effects are confined to the uppermost 3 cm of soil [1]. Soils were air-dried immediately after sampling, sieved (<2 mm), and stored at 20 °C for 2–3 months until laboratory analyses. It is important to note that the method of storing soil samples may influence the absolute values of biochemical properties, such as enzymatic activities [50,51].

Likewise, in each 2 m × 2 m plot, we sampled several ecological variables. Specifically, we visually estimated the percentage cover of (i) bare soil, (ii) fine plant debris, (iii) coarse plant debris, and (iv) vegetation, and we measured the (v) maximum height of living vegetation. To accurately estimate these ecological variables in each 2 m × 2 m plot, we used four 1 m^2 quadrats as the sampling unit.

2.4. Analyses of Soil Biochemical Properties: Enzymatic Activities and Microbial Biomass Carbon

From each soil sample, we analyzed the following extracellular enzymatic activities: 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). β-glucosidase and acid phosphatase activities were analyzed according to Tabatabai [36], and urease activity was analyzed following the procedure described by Kandeler and Gerber [52], using two sample blanks for each soil sample. The analytical procedure for the three enzymes consisted of (i) adding excess enzyme substrate to soil samples (p-nitrophenyl-β-D-glucopyranoside for β-glucosidase, p-nitrophenyl-phosphate for phosphatase, and urea for urease), (ii) incubating soil samples with the enzyme substrate, and (iii) colorimetrically determining the enzyme products released (p-nitrophenol -pNP- for β-glucosidase and acid phosphatase, and NH_4^+ for urease) in the incubation period. The p-nitrophenol (pNP) was measured at a 400 nm wavelength, and the NH_4^+ at 690 nm, with a UV-1700 PharmaSpec spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

Microbial biomass C was analyzed following the fumigation-extraction method [53]. This procedure calculates the microbial biomass using the difference in organic C (EC) between filtered extracts of chloroform-fumigated (CHCl$_3$, 24 h) and non-fumigated soil samples. Organic C was analyzed with Walkley-Black dichromate digestion. Then, we used an extraction efficiency coefficient (k$_{EC}$) of 0.38 [53,54] to calculate microbial biomass C according to the following formula: microbial biomass C = EC/ k$_{EC}$.

2.5. Data Analyses

To analyze the effects of fire frequency (number of fires) and burn severity (continuous dNBR spectral index of the last fire) (explanatory variables) on the soil biochemical properties (β-glucosidase, urease, acid-phosphatase, and microbial biomass C; response variables), we developed generalized linear mixed models (GLMMs). GLMMs were developed independently for each study site (*n* = 60 in the Mediterranean; *n* = 88 in the Transition; *n* = 36 in the Oceanic) via penalized quasi-likelihood (*glmmPQL* function) to account for overdispersion. The identity of each 30 m x 30 m plot was included in the models as a random factor. According to Zuur [55], we used a Gamma error distribution in the GLMMs to fit the response variables, which are continuous and positive. Then, to select the most appropriate link function, we compared the identity and log link functions, and we selected

the one with a better performance, according to the generalized R^2 of the GLMMs, calculated via the standardized generalized variance approach (*r2beta* function). The interaction term (Frequency × Severity) was only retained in the models when it was significant ($P < 0.05$)

We used Permutational Multivariate Analysis of Variance (PERMANOVA) to determine the effects of fire frequency and severity on ecological variables (percentage cover of bare soil, fine plant debris, coarse plant debris, vegetation, and maximum height of living vegetation). PERMANOVAs were performed for each study site using the Adonis function (1000 random permutations). Then, we investigated the potential effects of fire frequency and severity on soil biochemical properties (enzyme activities and microbial biomass C) through changes in ecological variables by performing a non-metric multidimensional scaling ordination (NMDS) for each study site. MMDSs were executed with the *metaMDS* function, using the ecological variables as ordination factors. A Wisconsin double standardization was applied to the data and Bray–Curtis dissimilarity was used to ordinate the samples. We then used the *envfit* function (1000 random permutations) to calculate the correlation of the ordination with the external variables of fire frequency, burn severity, and soil biochemical properties, as well as with the ordination factors, obtaining the strength (R^2) and significance (P) of the correlations. Fire frequency, severity, and vectors of biochemical properties significantly related to the ordination were represented by the NMDSs.

All data analyses were carried out with R [56], using *vegan* [57] *nlme* [58], *MASS* [59], and *r2glmm* [60] packages.

3. Results

3.1. Relationships between Fire Regime Attributes and Soil Biochemical Properties

GLMMs indicated that, overall, the microbial biomass C was the property most strongly related to fire regime attributes (0.41 ± 0.13; mean generalized $R^2 \pm$ standard error), and β-glucosidase activity was the property most weakly related (0.13 ± 0.06) (Table 1). However, the strength of the relationships between fire regime attributes and soil biochemical properties varied, depending on the study site (Table 1; Figure 2).

Table 1. Mean generalized R^2 for the Generalized Linear Mixed Models (GLMMs), semi-partial R^2 for each model predictor (fire frequency and burn severity), and 95% confidence levels for the R^2 values (UC: upper confidence level; LC: lower confidence level) ['*r2beta* ()' outputs] at each study site (Mediterranean, Transition, and Oceanic) and average values of the three study sites. Significant interactions between frequency and severity were not found. SE = standard error.

Response Variable	Predictor	Mediterranean			Transition			Oceanic			Average
		R^2	UC	LC	R^2	UC	LC	R^2	UC	LC	$R^2 \pm$ SE
β-glucosidase	Model	0.15	0.35	0.03	0.02	0.15	0.00	0.23	0.50	0.05	0.13 ± 0.06
	Frequency	0.05	0.22	0.00	0.02	0.13	0.00	0.02	0.22	0.00	0.03 ± 0.01
	Severity	0.12	0.31	0.01	0.00	0.07	0.00	0.22	0.48	0.03	0.11 ± 0.06
Urease	Model	0.16	0.36	0.04	0.13	0.30	0.04	0.47	0.68	0.26	0.25 ± 0.11
	Frequency	0.01	0.11	0.00	0.12	0.27	0.02	0.04	0.26	0.00	0.06 ± 0.03
	Severity	0.14	0.34	0.02	0.04	0.15	0.00	0.46	0.67	0.23	0.21 ± 0.13
Phosphatase	Model	0.04	0.21	0.00	0.34	0.50	0.20	0.28	0.54	0.08	0.22 ± 0.09
	Frequency	0.03	0.18	0.00	0.34	0.49	0.19	0.09	0.34	0.00	0.15 ± 0.09
	Severity	0.01	0.12	0.00	0.03	0.13	0.00	0.11	0.36	0.00	0.05 ± 0.03
Microbial biomass C	Model	0.30	0.50	0.14	0.26	0.42	0.12	0.67	0.81	0.51	0.41 ± 0.13
	Frequency	0.20	0.40	0.05	0.19	0.35	0.07	0.00	0.15	0.00	0.13 ± 0.07
	Severity	0.10	0.28	0.04	0.14	0.30	0.04	0.63	0.78	0.45	0.29 ± 0.17

GLMMs also showed that fire frequency affected the soil biochemical properties in different ways than burn severity, and significant interaction effects between frequency and severity were not detected (Figure 2; Table S1), suggesting that a separate analysis of the effects of these fire regime attributes is appropriate.

Therefore, in relation to fire frequency, we found significant effects on soil biochemical properties at the Mediterranean and Transition sites, whereas no effects at the Oceanic site (Figure 2, Table S1). In particular, our results showed that frequency significantly increased urease and phosphatase activities at the Transition site, and significantly increased microbial biomass C at the Mediterranean and Transition sites.

Focusing on burn severity, we found significant decreases in β-glucosidase and urease activities with severity at the Mediterranean site, whereas the activity of both enzymes significantly increased at the Oceanic site (Figure 2). Likewise, burn severity decreased microbial biomass C at the Mediterranean and Transition sites, and increased it at the Oceanic one.
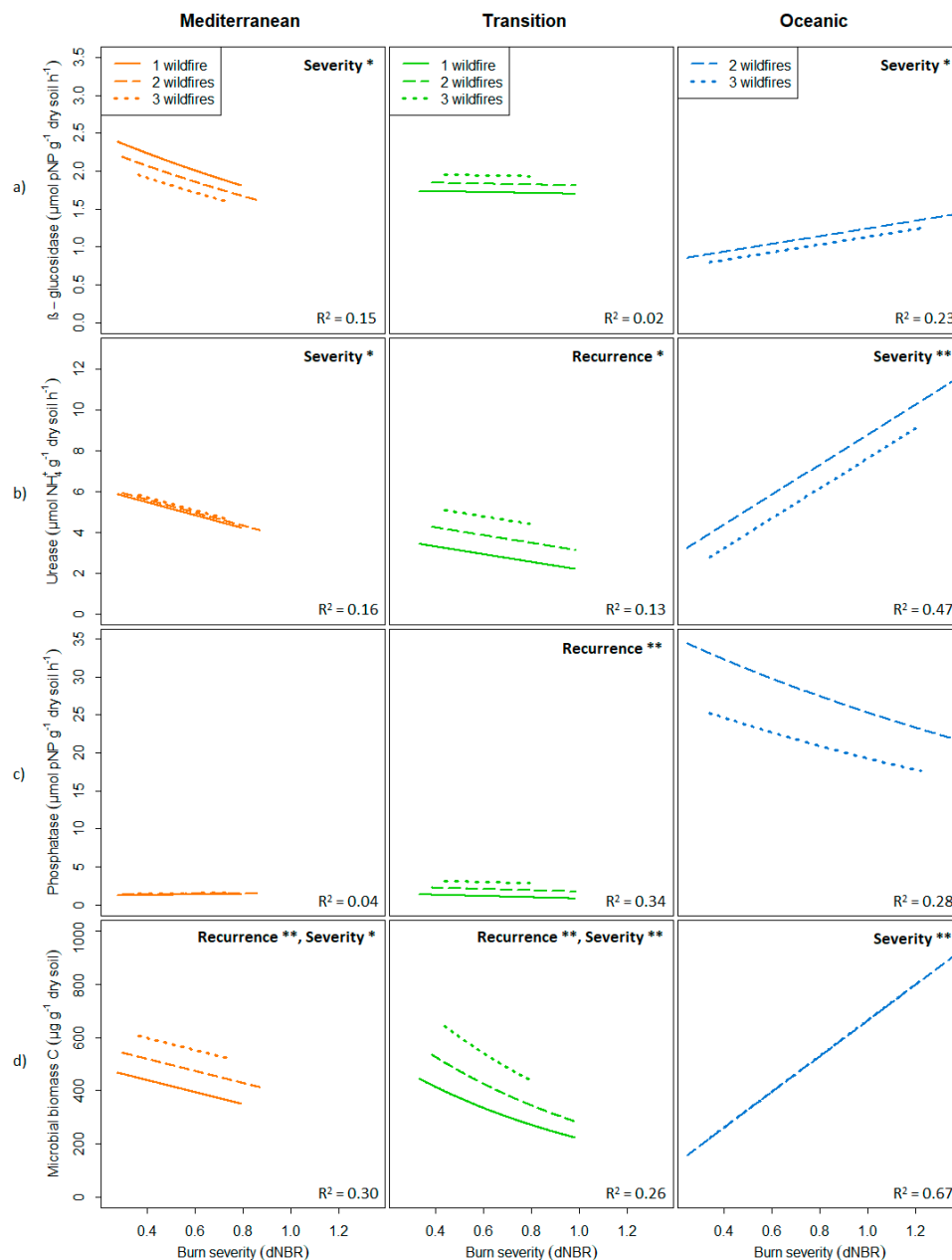

Figure 2. Mean predicted (**a**) β-glucosidase, (**b**) urease, (**c**) and phosphatase activities, and (**d**) microbial biomass C in relation to fire frequency (number of fires) and severity (dNBR of the last fire) at the Mediterranean (panels on the left, orange), Transition (panels in the centre, green), and Oceanic (panels on the right, blue) sites. R^2 values indicate the mean generalized variance explained by the GLMMs. Significance of model predictors (frequency and severity) is represented by * ($P < 0.05$) and ** ($P < 0.01$).

3.2. Impacts of Fire Regime Attributes on Soil Biochemical Properties Through Changes in Ecological Variables

The PERMANOVA (Table 2) showed significant effects of fire frequency on the studied ecological variables considered (percentage cover of bare soil, of fine plant debris, and of coarse plant debris; vegetation; and maximum height of living vegetation) at the Mediterranean and Transition sites. The results also showed significant effects of burn severity at the Transition site. For the Oceanic site, the statistical model showed no evidence of an effect.

Table 2. Results of the Permutational Multivariate Analysis of Variance (PERMANOVA) ['*adonis*()' outputs], showing the effects of the variables fire frequency and burn severity on ecological variables (percentage cover of bare soil, of fine plant debris, and of coarse plant debris; vegetation; and maximum height of living vegetation).

Response Variable	Predictor	Mediterranean		Transition		Oceanic	
		F	***P***	**F**	***P***	**F**	***P***
Ecological variables	Frequency	4.37	**0.02**	5.28	**<0.01**	0.53	0.59
	Severity	1.86	0.16	3.20	**0.04**	0.14	0.86

Significant *P*-values are shown in a bold face.

The NMDS at the Mediterranean site (Figure 3a) was able to ordinate plots in relation to fire frequency ($R^2 = 0.17$; Table 3). At this site, phosphatase activity was the only biochemical property related to the ordination ($R^2 = 0.18$). Phosphatase activity was higher in plots with high vegetation cover and low coarse debris. In this sense, increases in fire frequency could positively affect phosphatase activity through decreases in coarse debris (as it decreased with frequency), but we have not found a clear relationship between vegetation cover and fire frequency.

Table 3. Relationship between the NMDS (Figure 3) and the external variables fire frequency; burn severity; β-glucosidase, urease, and phosphatase activities; microbial biomass C; and the ordination vectors (percentage cover of bare soil, fine, and coarse plant debris; vegetation; and vegetation maximum height). Determination coefficients (R^2) and significance (P) were obtained using 1000 random permutations.

Variable	Mediterranean		Transition		Oceanic	
	R^2	***P***	R^2	***P***	R^2	***P***
Frequency	0.17	**0.02**	0.25	**<0.01**	0.12	0.13
Severity	0.04	0.42	0.08	0.05	0.02	0.71
β-glucosidase	0.02	0.68	0.11	**0.02**	0.20	**0.02**
Urease	0.04	0.41	0.03	0.29	0.23	**0.02**
Phosphatase	0.18	**0.02**	0.06	0.11	0.05	0.48
Microbial biomass C	0.10	0.08	0.06	0.10	0.03	0.63
Bare soil	0.93	**<0.01**	0.82	**<0.01**	0.63	**<0.01**
Fine plant debris	0.88	**<0.01**	0.81	**<0.01**	0.79	**<0.01**
Coarse plant debris	0.85	**<0.01**	0.37	**<0.01**	0.47	**<0.01**
Vegetation cover	0.83	**<0.01**	0.85	**<0.01**	0.71	**<0.01**
Vegetation height	0.20	**<0.01**	0.41	**<0.01**	0.26	**<0.01**

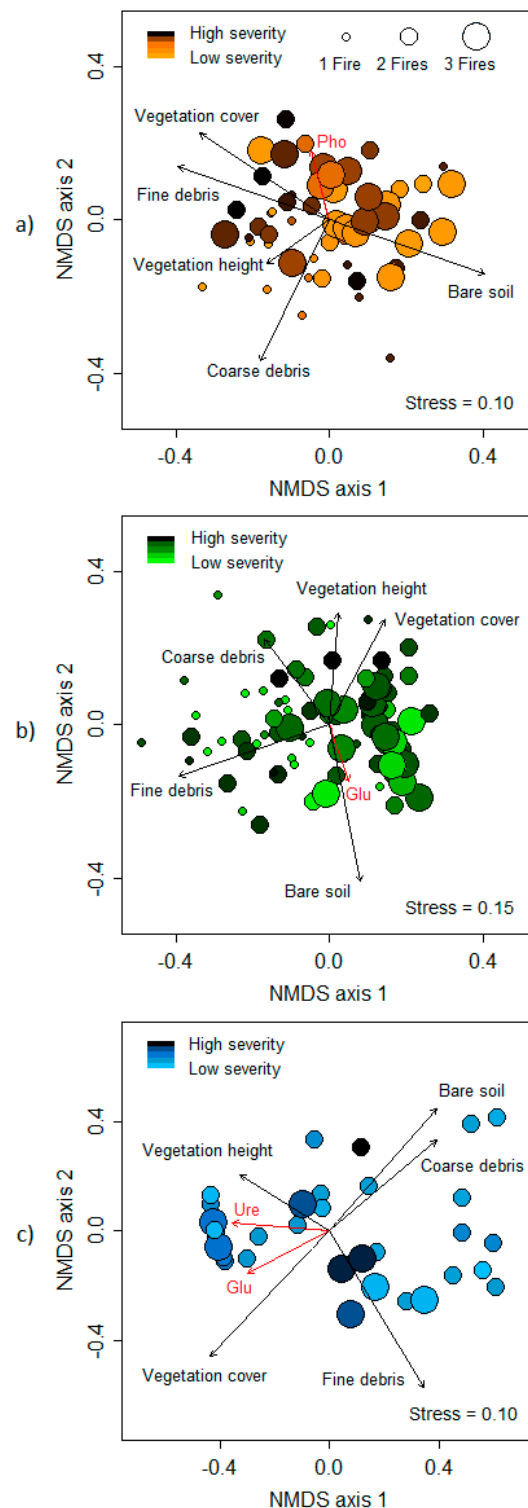Significant *P*-values are shown in a bold face.

Figure 3. Non-metric multidimensional scaling ordination (NMDS) ordination of the plots from the three study sites ((**a**) Mediterranean in orange, (**b**) Transition in green, and (**c**) Oceanic in blue) performed with the ecological variables represented by black vectors: percentage cover of (i) bare soil, (ii) fine, and (iii) coarse plant debris; (iv) vegetation; and (v) vegetation maximum height. The external variables β-glucosidase (Glu), urease (Ure), and phosphatase (Pho) activities, and microbial biomass C, were fitted to the NMDS (red vectors) and shown when significant. Shape size represents fire frequency (number of fires) and color ramp represents severity (dNBR of the last fire). See Table 3 for further information.

The NMDS at the Transition site (Figure 3a) significantly ordinated plots according to fire frequency ($R^2 = 0.25$; Table 3). At this site, we found that β-glucosidase activity was related to the ordination ($R^2 = 0.11$), increasing with the cover of bare soil, and decreasing with vegetation height, vegetation cover, and coarse debris.

The ordination at the Oceanic site (Figure 3c) showed that urease and β-glucosidase activities were positively related to vegetation height. However, we did not find a significant relationship between this ordination and the fire regime attributes.

4. Discussion

Our results demonstrate that fire regime attributes (fire frequency and severity) measured through remote sensing methods are related to soil biochemical properties (microbial biomass and soil enzyme activities) four years after a fire. Previous works have shown that soil biochemical properties are highly sensitive to environmental disturbances such as wildfires [16,17,61]. However, the effects of wildfires seem to depend on the environmental conditions at each site. In fact, the effect of fire regime attributes could be reduced at the Oceanic site because of fast regeneration and favorable conditions of humidity and temperature, which increase biological activity.

Burn severity increased soil microbial biomass, and β-glucosidase and urease activities at the Oceanic site, whereas the opposite effect was detected at the Mediterranean and Transition sites. Soil microbial biomass is a good indicator of changes in soil because it is very sensitive to stress conditions produced by disturbances such as fires [62]. Immediately after a fire, soil heating is lethal for many microorganisms, and microbial biomass can decrease to undetectable levels [27,63]. In soils affected by a high burn severity, negative effects on microbial biomass have been detected for more than 10 years after fires because of the slow recovery of fungi [33], whose contribution to soil microbial biomass is greater than that of bacteria. Microorganisms are sensitive to soil temperature (threshold for microbial mortality of around 60 °C, according to DeBano [64]) and to changes in pH and toxic substances after a fire [34]. The recovery of microbial biomass after a fire may be slow and depends on key factors, such as climatic conditions, land use patterns, and vegetation cover [65]. The increase of microbial biomass with burn severity at the Oceanic site can be related to the spatial heterogeneity and the diverse land use history at this site. Whereas the Mediterranean and Transition sites presented a more homogeneous *Pinus* forest, the Oceanic site was a diverse mosaic of *pine* forests, plantations, and abandoned crop fields colonized by pines. Therefore, the highest values of microbial biomass and enzymatic activity in our study could be linked to forests, which usually present more biomass and activity than plantations and abandoned crop fields [66]. In addition, the post-fire regeneration of the dominant shrub at the Oceanic site (*Ulex europaeus*) was faster than the post-fire recovery of shrub species in the other two areas. The regeneration of vegetation improves the soil structure and detoxifies toxic substances that developed during combustion [67], and these factors are decisive for soil microbial recovery.

Soil enzymes can be inactivated after intense wildfires due to heat denaturation. Four years after fires, we found that the soil extracellular enzyme activity rates (β-glucosidase, urease, and acid phosphatase) were also affected by burn severity in different ways, depending on the study site. Burn severity decreases β-glucosidase and urease activities at the Mediterranean site, according to Miesel et al. [68], but increases them at Oceanic sites. This pattern shows a strong relationship between soil microbial biota and soil enzymatic activity, since the further enzymatic extracellular activities are produced by microorganisms due to their high biomass, metabolic activity, and short life cycle [69]. The low microbial activity associated with a high severity level reflects the poor conditions for the development of microorganisms, such as the lack of labile C, N, and P [34,70,71] and the presence of organic pollutants [72].

Fire frequency had no influence on soil biochemical properties at the Oceanic site, but at the Transition site, fire frequency tended to counteract the negative effect of burn severity. This effect could be related to two factors: (i) the level of nutrients and (ii) the fast recovery of vegetation.

Fire frequency increased urease and phosphatase activity at the Transition site, exhibiting a greater reliance of the soil nutrient concentrations [37], because organisms generate enzymes to catalyze the release of nutrients [17]. In fact, at our study sites, available phosphorous levels were not recovered in the highest fire frequency situations (unpublished data from the GESFIRE project). Furthermore, the areas with a high frequency are characterized by the dominance of resprouter shrubs with fast regeneration [48] and phosphatase activity increased with the vegetation due to a higher influence of roots, the main source of acid phosphatase [42]. On the other hand, fire frequency increased soil microbial biomass at the Mediterranean and Transition sites, which could also be related to the increase in woody species cover favoring the recovery of organic matter and microbial biomass.

The statistical model also showed significant effects of fire frequency on the analyzed ecological variables considered (cover of bare soil, fine plant debris, and coarse plant debris; vegetation; and vegetation height) at the Transition and Mediterranean sites, but not at the Oceanic one. The NMDS was able to find a significant relationship between the analyzed ecological variables and phosphatase activity at the Mediterranean site, which increased in the areas with a high vegetation cover. This finding was in agreement with López-Poma and Bautista [73] and Mayor et al. [42], who observed that in Mediterranean areas, there is a close relationship between phosphatase activity and woody resprouters cover after a fire. A similar pattern was detected at the Oceanic site, with higher values of β-glucosidase and urease in those plots with higher vegetation cover and height. These relationships can be attributed to increases in enzyme substrates by litter accumulation and to increases in the abundance of microorganisms, as they are the main source of β-glucosidase and urease [42]. Further studies are necessary to better understand the underlying factors responsible for the different effects of fire frequency and severity on soil biochemical properties along climatic gradients. We also encourage future research to characterize fire regimes using field-based methods, as they could be suitable (i) for characterizing fire frequency for longer periods, (ii) and obtaining accurate burn severity measurements, as remote sensing methods have a limited capacity to quantify soil burn severity [49].

5. Conclusions

Changes in climate and land-use are modifying fire regimes across the globe [5,6,8]. Therefore, it is interesting to know how shifts in fire frequency and severity affect soil biochemical properties such as enzymatic activities and microbial biomass C.

Our study indicated that fire frequency affects soil biochemical properties in different ways than burn severity, with the effects varying among the study sites. Therefore, increases in fire frequency increase microbial biomass C in Mediterranean conditions, and increase urease, phosphatase, and microbial biomass C in Transition conditions, whereas the effects of fire frequency were insignificant under Oceanic conditions. Moreover, increases in burn severity had opposite effects at the Mediterranean (decreasing β-glucosidase, urease, and microbial biomass C) and Oceanic sites (increasing β-glucosidase, urease, and microbial biomass C). Mediterranean and Transition sites showed the same pattern in relation to microbial biomass C.

Overall, fire frequency determines ecological variables (cover of bare soil, of fine plant debris, and of coarse plant debris; vegetation; and maximum height of living vegetation) in Mediterranean and Transition conditions, but indirect effects of fire frequency on soil biochemical properties due to changes in these ecological variables were not clearly identified.

Therefore, we conclude that fire frequency and severity continue to influence soil biochemical properties even four years after a fire, but the strength and direction of the effects largely vary among sites with different environmental conditions. Future research could focus on clarifying the importance of each environmental condition, particularly the soil type and climate, in determining the effects of fire frequency and severity on soil biochemical properties.

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