CASCADE
Catastrophic shifts in drylands:
How can we prevent ecosystem degradation?

CASCADE REPORT series

Identification of critical changes preceding catastrophic shifts: ecosystems affected by increasing wildfire recurrence

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CASCADE
Catastropic shifts in drylands

Deliverable 3.1a

Identification of critical changes preceding catastrophic shifts: ecosystems affected by increasing wildfire recurrence

Project: CASCADE
CAtastrophic Shifts in drylands:
how CAn we prevent ecosystem DEgradation?

Coordinator: Prof. Dr. Coen J Ritsema.
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This deliverable has been prepared in the framework of WP3, coordinated by WUR. Contributors to the present deliverable are the WP3 coordinator (WUR), the partners leading the Várzea and Valencia CASCADE study sites (UAVR and CEAM, respectively), V Ramón Vallejo (UB) and Susana Bautista (UA).

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Preface

WP3 focuses on observational and manipulative field experiments to investigate changes in plant-soil ecosystems in response to external stress (i.e., increasing fire frequency and increasing grazing intensity, individually and each of them combined with severe drought), and to identify the most sensitive indicators to these changes. For this purpose, two WP3-experiments have been conducted in the 6 CASCADE study sites, where measurements of soil quality and plant performance have been taken in three levels of stress determined by fire recurrence or grazing intensity depending on the site (*stress-gradient* experiment), and in a rainfall-exclusion experiment in one of the former stress levels, the most likely to experience a tipping point (*drought-stress* experiment).

The present deliverable (WP3-3.1a) includes the assessment of changes in soils in response to increasing fire frequency in the two study sites affected by this degradation driver (Várzea in NC Portugal and Valencia in E Spain). A future WP3 deliverable (WP3-3.1b, to be delivered in the coming months) will include the assessment of changes in plant-soil ecosystems in response to grazing intensity, and also in response to the combination of high fire frequency or grazing intensity with severe drought. The reasons for separating WP3 results into two deliverables are of different nature. First, as reported during the CASCADE 2nd periodic report, the *drought-stress* experiment was extended beyond the original planned time (namely until plants would show stress signs). Therefore, databases for this experiment are being completed at present. Second, but not least important, we have been experiencing that the observational *stress-gradient* and the manipulative *drought-stress* experiments nicely feed each other, particularly in the grazing sites. Therefore, providing the most solid and better supported conclusions on the effects of grazing pressure and drought will only be possible once the *drought-stress* experiment is completed.

Contents

1 Introduction

1.1 Increasing fire recurrence in Europe and impact on soil quality

Since the mid of the last century fire recurrence –number of fire events that occur at a site in a given period of time– has increased in the Iberian peninsula and the overall Mediterranean basin (Pausas and Fernández-Muñoz, 2012). This occurs due to fuel accumulation from land abandonment and extensive reforestation (Koutsias et al. 2012) and to extreme weather events (Camia and Amatulli 2009, Carvalho et al. 2011, Hoinka et al. 2009, Koutsias et al. 2012). The future warmer and drier climate projected for this region will further increase the risk of wildfire occurrence and of increasing fire recurrence (Giorgi and Lionello 2008). Future wildfire risk is projected to increase in Southern Europe (Lindner et al. 2010, Carvalho et al. 2011, Dury et al. 2011). The annual burned area is projected to increase by a factor of 3 to 5 in Southern Europe compared to the present under the A2 scenario by 2100 (Dury et al. 2011).

There is ample literature on the effects of fire recurrence on vegetation. Recurrent fires can lead to long-term cumulative effects at plant community level such as changes in plant composition and structure (Lloret et al 2003, Eugenio et al 2006, Santana et al 2010), losses in plant productivity (Díaz-Delgado et al 2002, Eugenio and Lloret 2004 Delitti et al 2005), and delays in post-fire plant regeneration. Such changes in vegetation are likely to be associated to changes in soil quality. For instance, it has been suggested that loss of plant productivity with subsequent fires is associated with a cumulative reduced availability of nutrients in mineral soils (Ferran et al 2005). However, although the impact of wildfires on soil nutrient content in Southern Europe has been extensively studied, only a few studies have assessed this impact on the basis of fire recurrence (Caon et al 2014).

One of the most common changes in plant communities driven by high fire recurrences in Southern Europe is the replacement of pine woodlands by shrublands. Despite the high post-fire resilience of the most common pines in the Mediterranean (*Pinus halepensis*, *P. brutia* and *P. pinaster*), these trees fail to regenerate when time interval between fires is shorter than the time needed to accumulate a sufficient seed bank, i.e. around 15 years (Eugenio et al. 2006, Santana et al 2010). Surprisingly, it has been poorly studied whether a shift from pine woodlands to shrublands is associated with a shift in soil fertility. Most of the available research assessing the impact of fire recurrence on soil fertility is performed in ecosystems dominated by species with resprouting ability after fire (i.e., *Quercus suber* woodlands or *Q. coccifera* shrublands), and thus, with no major shifts in plant community (e.g., Trabaud 1991, Carreira et al 1994, Guenon et al 2001, Ferran et al 2005). One of the few works studying effects of repeated burning on soils in Mediterranean pine woodlands found that, nine years after the last fire, sites burned twice in an interval of 18 years had less developed organic horizons but similar mineral soils than sites only burned once in that period (Eugenio and Lloret, 2005). The authors attributed this response to the lower vegetation development in twice- than in one-burnt areas. The lack of cumulative effects of recurrent fires in mineral soils could however not be concluded as pooled soils up to 20 cm depth were sampled, whereas maximum depth commonly affected by fires is around 5 cm (Giovannini, 1994).

1.2 Early warning indicators of soil functioning

A significant decline in soil quality has occurred throughout the entire world as a result of adverse changes in its physical, chemical, and biological properties, caused by human activity and climate change (Van Camp et al. 2004). Soil degradation processes in drylands are particularly acute due to the fragility imposed in these areas by water scarcity in combination with large human and climatic pressures. Thus, soil degradation in drylands is one of the main environmental problems worldwide, including Europe (32% of the land mass are drylands, home to 25% of the population). Moreover, this problem is expected to get worse in the face of current global change (Millennium Ecosystem Assessment, 2005; Reynolds et al., 2007), where Europe is forecasted to be one of the world's regions most impacted. Further, both theoretical developments and empirical data provide evidence that healthy drylands can shift to a degraded state in response to small increases in human and climatic pressure once a threshold has been surpassed (Scheffer and Carpenter 2003, Rietkerk et al 2004, Schroder et al 2005, Gao et al 2011). This implies the possibility for sudden major and difficult-to-recover ecological and economic losses, what explains the research emphasis on identifying early warning indicators (Dakos et al. 2012, Kéfi et al. 2014). In this context, the identification of early warnings of changes in ecosystem functioning, including changes in soils, is prioritary for identifying areas with higher risk of degradation in response to specific pressures, being recurrent wildfires one of the most common pressures in European forest and shrublands.

1.3 Objectives

The work reported in this deliverable assesses the changes in organic soil carbon and nutrient status of two forest ecosystems in the Iberian Peninsula (and CASCADE study sites), Várzea (N Portugal) and Valencia (E Spain), affected by different levels of fire recurrence (1, 2, 3 or 4 fires in 37 years) and where short inter-fire periods have promoted a transition from pine woodlands to shrublands.

The main objectives of this work were (1) to determine whether fire recurrence levels promoting shifts from pine woodlands to shrubland communities in Southern Europe are associated with shifts in soil fertility, (2) to assess if different types of soil surface cover (i.e., that below shrub patches and that in the openings between shrubs) have different sensitivity to fire recurrence effects, and (3) to identify the most sensitive indicators of changes in soil functioning in response to repeated fires.

2 Material and methods

2.1 Study sites

The two study sites, Várzea (North-Central Portugal) and Valencia (Eastern Spain), are affected by increased wildfire recurrence where high fire frequencies have promoted a transition from pine woodlands to shrublands. The two sites are representative of fire-prone regions in Southern Europe, Valencia under fully Mediterranean climate and Várzea under Atlantic climate with some Mediterranean influence. A general description of these sites and the other CASCADE study sites was given in CASCADE Deliverable 2.1 and 2.2, including information on physiography, soils, climate, land use/land cover, degradation drivers, socioeconomic status, historical evolution of vegetation health and climate. These two study sites include plots characterized by different fire histories that are summarized in Table 1 and described next. We combine a diachronic approach (Várzea) for assessing short-term fire effects with a synchronic approach (Valencia) for long-term effects of fire recurrence. In Várzea, two fire recurrence areas (1 and 4 fires), both last burned by a wildfire in the summer of 2012, and a reference fire-free area for the last 35 years were selected. Three plots (30 x 30 m) were set up in each of the three areas (total of 9 plots). The study site in Valencia has plots affected by natural and experimental fires from previous studies and represents a chronosequence in fire recurrence and time since the last burn. It includes three fire recurrence levels (1, 2, and 3 fires) and a reference fire-free for the last 30 years in three different areas (Ayora, Alcoy, and Onil). Three plots (30 x 30 m) were set up in each fire level and area (total of 12 plots). In each site, the plots were selected to have physiographic and edaphic properties as comparable as possible (Table 1).

Table 1. Fire history and physiographic characteristics of the experimental sites

Site name	Fire regime	Fire year	Altitude (m)	Slope (°)	Aspect
Várzea	Unburned	No fire	460	24	SSW
	1 fire	2012	468	22	SSW
	4 fires	1978, 1985, 2005, 2012	530	25	SSW
Valencia-Ayora	Unburned	No fire	763	10	SSW
	1 fire	1979	1041	31	NNE
	2 fires	1979, 1996	1041	23	NE
	3 fires	1979, 1996, 2006[1]	1041	23	NE
Valencia-Pardines	Unburned	No fire	900	4	N
	1 fire	1984	900	4	N
	2 fires	1984, 1994[1]	900	4	N
	3 fires	1984, 1994[1], 2006[1]	900	4	N
Valencia-Onil	Unburned	No fire	940	47	ENE
	1 fire	1984	940	35	NW
	2 fires	1984, 1994[1]	940	35	NW
	3 fires	1984, 1994[1], 2006[1]	940	35	NW

[1] Experimental fire

2.2 Sampling and chemical analysis

In both study sites, microsites beneath and between shrubs (hereafter, shrub and intershrub) of the most representative species were identified and three 1-m^2 subplots per plot were randomly located in each microsite. Shrub microsites were *Pterospartum* *tridentatum* (resprouter) in Várzea, and *Quercus coccifera* (resprouter) and *Rosmarinus officinalis* (obligate seeder) in Valencia. One sample of mineral soil at 0-5 cm was taken in each subplot in spring 2013. For both study sites, soil samples were analysed for total organic carbon (SOC), total nitrogen (N), NH_4, NO_3, Potentially Mineralizable Nitrogen (PMN), and available phosphorus (P_{ava}). Additionally, hot-water extractable carbon (HWC) and dissolved organic carbon (DOC) were analysed in Várzea and Valencia, respectively. HWC was determined as the amount of dissolved organic carbon that is released during incubation of a soil sample in hot water during 16 hours at 80°C (Ghani et al, 2003). This is a measure of easily decomposable (labile) organic carbon. The HWC fraction of organic matter is rich in amorphous polysaccharides (mucigel) which originate mainly from microbial exudates and to a lesser extent from plant exudates. This fraction is highly available to microorganisms and is also regarded as one of the key labile components of organic matter responsible for soil micro-aggregation, which is an important soil physical parameter to consider in terms of soil quality (Ghani et al. 2003, Haynes 2005). Total organic C was determined using the potassium dichromate oxidation (Walkley-Black) method (Nelson and Sommers, 1982). Total N was determined by the Kjeldahl method (Bremmer and Mulvaney, 1982), and available P by the NaHCO3-extractable Pi (Olsen-Pi) as described by Watanabe and Olsen (1965). Potentially mineralizable N was determined by anaerobic incubation of a soil sample under water for 1 week at 40°C (Keeny and Nelson, 1982; Canali and Benedetti, 2006) These warm and anoxic conditions are optimal for a quick mineralization of organic matter by anaerobic bacteria. The lack of oxygen prevents conversion of released $NH4^+$ to $NO3^-$ (nitrification) and uncontrolled N losses by denitrification cannot occur. The amount of mineral nitrogen (NH^4-N) released is a measure of the quality (N-content and decomposability) of the organic matter, and thus for biological soil fertility.

In addition to the total values, we calculated the next ratios expressing the values of different nutrients relative to their source: HWC:SOC, PMN:N, NH_4:N, NO_3:N, and P_{ava}:SOC. These ratios are used as indicators of the quality of the soil organic matter and allow comparisons between sites with contrasting amounts of soil organic matter, as it is the case for the two study sites.

2.3 Statistical analysis

An analysis of variance was performed for each of the soil variables measured, using *recurrence* and *microsite* as fixed factors. Additionally, *plot* (nested in *recurrence*), and *area* and *plot* (this latter nested in the interaction *recurrence* X *area*) were used as random factors in the Várzea and Valencia datasets, respectively.

Principal component analysis (PCA) was used to reduce the original set of variables of the soil organic matter *quantity* matrix (SOC, N, NH_4, NO_3, PMN, P_{ava}, and HWC or DOC) and *quality* matrix (HWC:SOC, PMN:N, NH_4:N, NO_3:N, and P_{ava}:SOC) into a smaller set of uncorrelated components that represent most of the information found in the original variables. Variables were transformed if needed to fit normal distributions. Statistical analyses were performed with SPSS vs. 20.

3 Results

3.1 Várzea study site

Values of soil organic C and nutrients in relation to fire recurrence, and ANOVA results for each soil variable of Várzea study site are shown in Fig. 1 and Table 2, respectively. Although not significantly different, soil organic C showed higher values in burned than in unburned soils eight months after the occurrence of the last fire. Hot-water extractable C was however similar for burned and unburned soils but decreased from 1fire to 4 fires. HWC:SOC ratio was significantly lower in 4 fires than in 1 fire or unburned sites (Fig. 2). Similar to soil organic C, total N was higher in burned than in unburned soils, but values significantly decreased with increasing fire recurrence. However, the potentially mineralizable N decreased from unburned to 4 fires, and thus, its value relative to total N was lower in burned than in unburned soils, without differences between areas burned once or four times (Fig. 2). Both NH_4 and available P showed a trend with lower values for unburned than for burned soils, and without differences between soils burned one or four times. Microsite did not have any significant effect on any of the soil variables (Table 2).

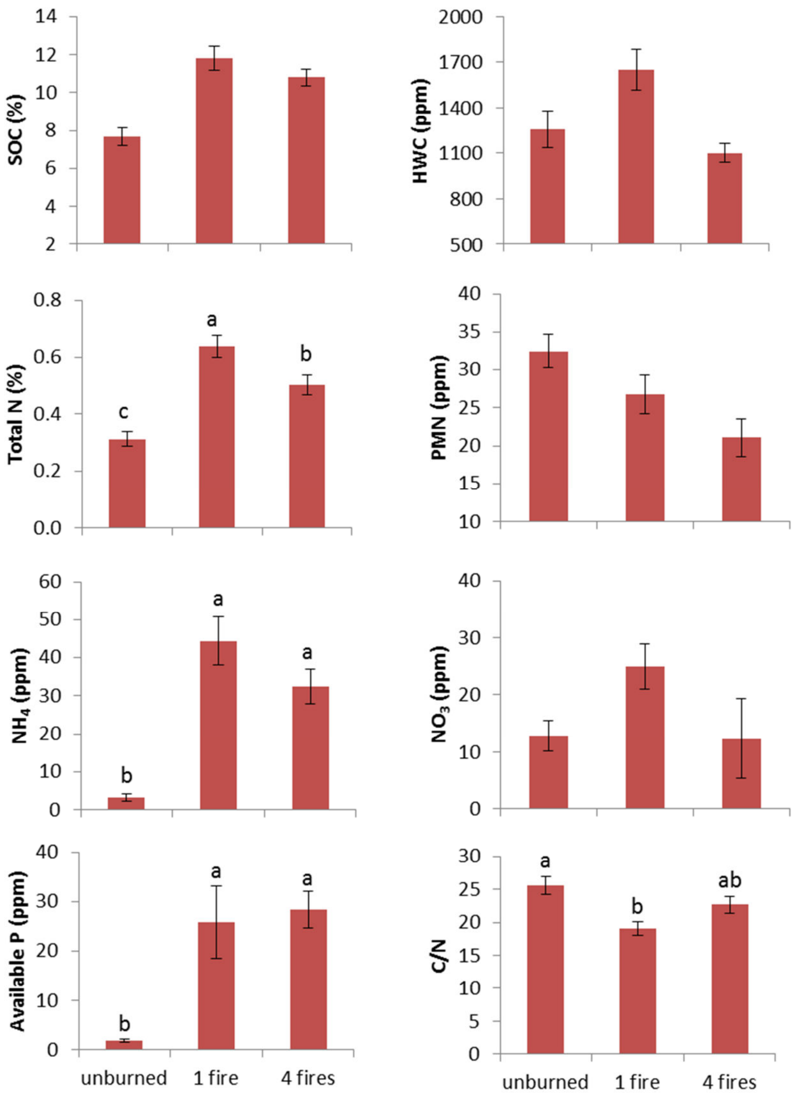

Figure 1. Soil organic C and nutrients in the upper 0-5 cm (mean ± SE) for different fire recurrence in the Várzea study site. The letters indicate significant differences between fire recurrence levels.

Table 2. Results from ANOVA on soil organic C and nutrients for the two study sites

	VÁRZEA			VALENCIA		
	Recurrence	Microsite	R X M	Recurrence	Microsite	R X M
SOC	F = 3.021	F = 0.027	**F = 4.020**	F = 0.915	**F = 21.53**	F = 0.223
	P = 0.124	*P* = 0.869	***P* = 0.025**	*P* = 0.488	***P* = 0.007**	*P* = 0.925
HWC/DOC[1]	F = 2.714	F = 0.330	F = 1.726	F = 2.678	F = 1.344	F = 1.045
	P = 0.145	*P* = 0.569	*P* = 0.191	*P* = 0.141	*P* = 0.358	*P* = 0.399
N	**F = 6.528**	F = 0.280	F = 0.135	F = 0.770	**F = 9.801**	**F = 2.359**
	***P* = 0.031**	*P* = 0.599	*P* = 0.874	*P* = 0.551	***P* = 0.029**	***P* = 0.033**
PMN	F = 1.729	F = 0.241	F = 0.831	F = 0.862	F = 6.265	F = 1.712
	P = 0.255	*P* = 0.626	*P* = 0.442	*P* = 0.510	*P* = 0.059	*P* = 0.122
NH4	**F = 5.862**	F = 0.101	F = 0.370	**F = 6.663**	F = 2.009	F = 0.944
	***P* = 0.039**	*P* = 0.752	*P* = 0.693	***P* = 0.024**	*P* = 0.249	*P* = 0.465
NO3	F = 1.461	F = 0.045	F = 0.323	F = 1.275	F = 0.345	**F = 2.380**
	P = 0.304	*P* = 0.832	*P* = 0.726	*P* = 0.365	*P* = 0.727	***P* = 0.032**
Pava	**F = 35.10**	F = 1.393	F = 0.779	F = 1.769	F = 2.420	**F = 2.518**
	***P* < 0.001**	*P* = 0.245	*P* = 0.466	*P* = 0.253	*P* = 0.205	***P* = 0.024**
C:N	**F = 6.146**	F = 1.031	F = 1.256	F = 1.321	F = 2.906	F = 0.360
	***P* = 0.035**	*P* = 0.316	*P* = 0.295	*P* = 0.352	*P* = 0.166	*P* = 0.903

[1]HWC in Várzea and DOC in Valencia. Highly ($P < 0.05$) and marginally significant ($P < 0.1$) results are highlighted in bold.

The first two components of the PCA performed with the soil organic matter *quantity* matrix had eigenvalues higher than one and together explained over 60% of the variance in the data (Fig. 3). Soil organic C, total N, and NH_4 were significantly correlated ($|\rho| \geq 0.6$) with the first component. Hot-water C and potentially mineralizable N were correlated with the second component. Also the two first components of the PCA performed with the soil organic matter *quality* matrix had an eigenvalue higher than one, explaining over 65% of the total variance (Fig. 3). The ratios PMN:N and HWC:SOC, and P_{ava}:SOC and NH_4:N, showed the strongest correlations with the first component (positively and negatively, respectively), while the NO_3:N ratio was most strongly related to the second component.

Fire recurrence had a significant effect on the first component of the PCA performed with the soil organic matter *quantity* matrix (Fig. 3, Table 3). This component was higher for burned than for unburned soils, but decreased with fire frequency so that values for one fire were higher than for four fires. On the contrary, the second component decreased from unburned to four fires, although differences were not significant in this case. Microsite had no significant effect on any of the two components. However, the interaction between the two factors (Recurrence X Microsite) was marginally significant for the second component. Indeed, when the analysis of variance was performed for both microsites separately (plant patches of *P. tridentatum* or intershrub spaces), the second component was significantly higher in unburned than in burned soils only for the intershrub microsite, without differences between 1 and 4 fires ($F = 5.082$, $p = 0.015$, Fig. 7). Fire recurrence also had a significant effect on the first component of the PCA performed with the soil organic matter *quality* matrix, being higher for unburned than for burned soils, without differences between 1 or 4 fires (Fig. 2). Microsite did not significantly affect any of the two components of the PCA performed with the *quality* matrix.

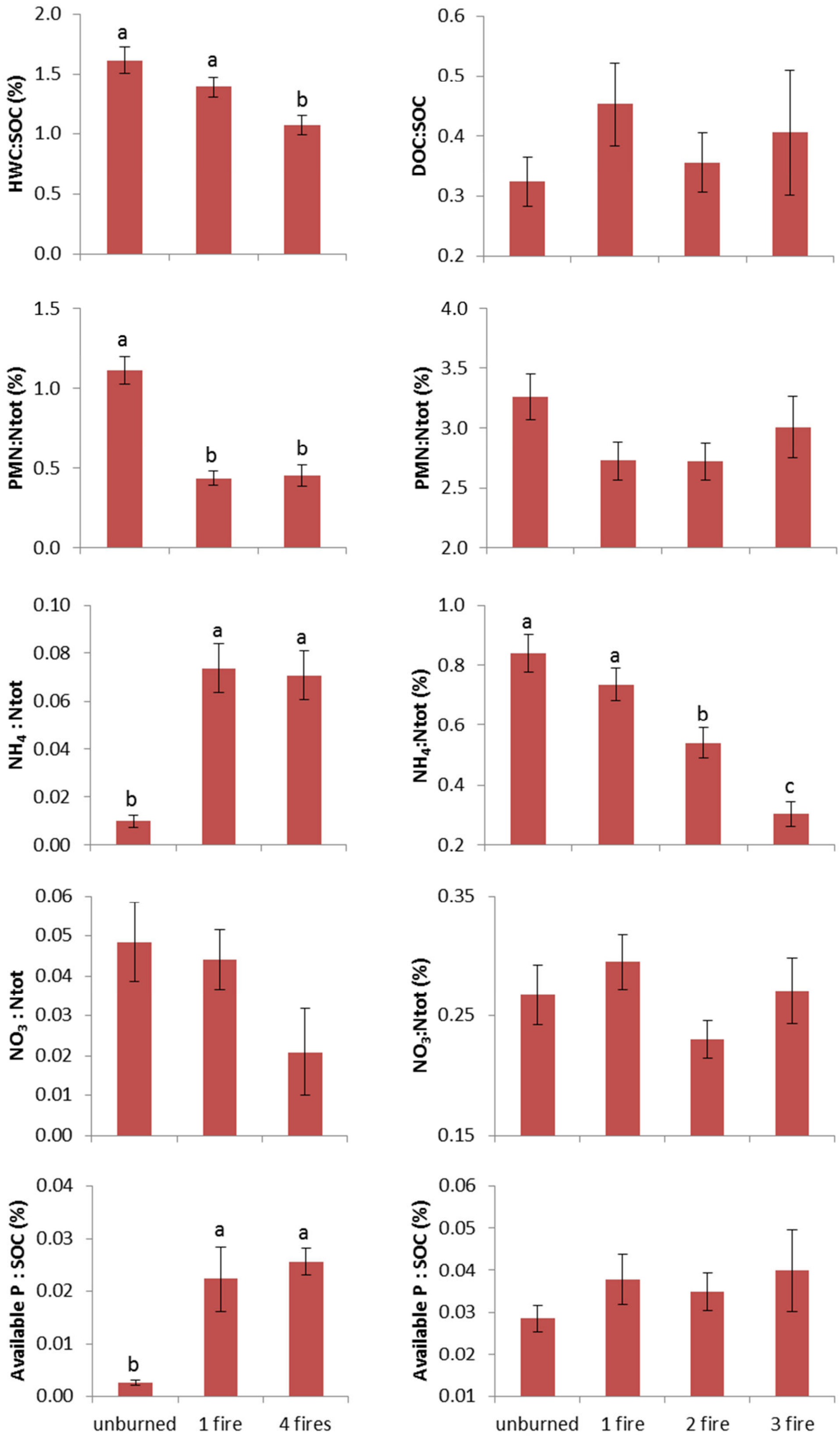

Figure 2. Indicators of soil organic matter quality in the upper 0-5 cm (mean ± SE) for different fire recurrence levels in Várzea (left) and Valencia (right). The letters indicate significant differences between fire recurrence levels.

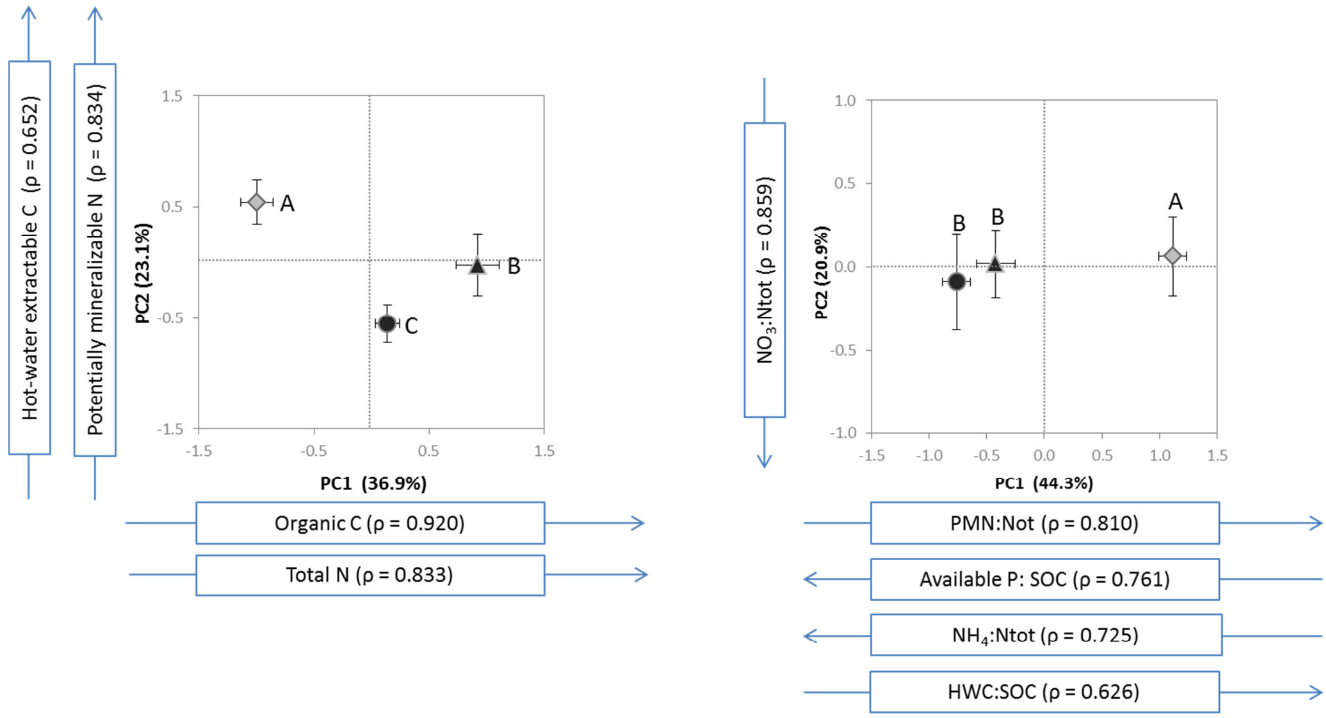

Figure 3. Principal component analysis (PCA) of soil organic matter *quantity* (left) and *quality* (right) characterising the different recurrence levels in the Várzea study site. Data represent means ± SE. Arrows represent soil nutrients significantly correlated (|*ρ*| ≥ 0.6) with the first two axes. Symbols: unburned plots (diamond), plots burned once (triangle), and plots burned four times (circle). The letters indicate significant differences between fire recurrence levels for the first component (PC1).

Table 3. Results from ANOVA on the components of the principal component analysis (PCA) of soil organic matter *quantity* and *quality* for the two study sites.

	VÁRZEA			VALENCIA		
	Recurrence	Microsite	R X M	Recurrence	Microsite	R X M
$PC1_{QT}$	**F = 9.086** ***P* = 0.015**	F = 0.265 *P* = 0.609	F = 1.020 *P* = 0.370	F = 1.284 *P* = 0.362	F = 1.319 *P* = 0.362	**F = 3.496** ***P* = 0.003**
$PC2_{QT}$	F = 1.843 *P* = 0.238	F = 0.763 *P* = 0.388	**F = 2.855** ***P* = 0.069**	F = 2.953 *P* = 0.120	F = 1.277 *P* = 0.372	**F = 2.289** ***P* = 0.063**
$PC1_{QL}$	**F = 41.88** ***P* < 0.001**	F = 0.003 *P* = 0.957	F = 1.307 *P* = 0.282	F = 0.992 *P* = 0.457	F = 0.828 *P* = 0.499	F = 0.904 *P* = 0.494
$PC2_{QL}$	F = 0.024 *P* = 0.976	F = 0.317 *P* = 0.577	F = 0.176 *P* = 0.839	F = 1.752 *P* = 0.256	F = 0.737 *P* = 0.534	F = 0.968 *P* = 0.450

QT: components of the PCA performed with the soil organic matter *quantity* matrix; QL: components of the PCA performed with the soil organic matter *quality* matrix. Highly (*P* < 0.05) and marginally significant (*P* < 0.1) results are highlighted in bold.

3.2 Valencia study site

Values of soil organic C and nutrients in relation to fire recurrence, and ANOVA results for the soil variables of the Valencia site are shown in Fig. 4 and Table 2. Most soil variables showed a decreasing trend from unburned soils to soils burned with the highest fire recurrence. However differences between recurrence levels were only significant for NH_4 and NH_4:N ratio, which had similar and highest values for long unburned areas and decreasing values with increasing fire recurrence. Microsite had a significant effect on soil organic C and total N. Thus, *Q. coccifera* microsites showed the highest value for both variables, while *R. officinalis* microsites were lowest in soil organic C and intershrub microsites were lowest in total N (data not shown). *Q. coccifera* microsites also showed the highest value for potentially mineralizable N but differences with the other microsites were only marginally significant.

Two components of the PCA performed with the soil organic matter *quantity* matrix had eigenvalues higher than one and captured 68% of the variance in the data (Fig. 5). Most variables were highly correlated to the first component, while the second component had the highest correlation with dissolved organic carbon. Also two components of the PCA performed with the soil organic matter *quality* matrix had an eigenvalue higher than one, explaining over 67% of its total variance. DOC:SOC and P_{ava}:SOC showed the highest correlations with the first component, while NH_4:N and NO_3:N were most strongly (and negatively) related to the second component (Fig. 5). The two components of the PCA performed with the *quantity* matrix, separated unburned and one fire from two and three fires, with higher values for the first group (Fig. 5). However, differences between these two groups were not significant due to the high variability between blocks (Fig. 6). Microsite also had no significant effect on any of the two components, but the interaction Recurrence X Microsite was highly significant for the first component. The independent ANOVAs performed with this component for the three microsites assessed (patches of *Q. coccifera*, patches of *R. officinalis* and intershrub) gave a significant effect of fire recurrence in the case of the intershrub, which decreased gradually from unburned to three fires (Fig. 7). Recurrence X Microsite was also marginally significant for the second component, and the independent ANOVAs performed with this component for the three microsites gave a significant effect of fire recurrence in the case of the *Q. coccifera* patch, which decreased gradually from long unburned to twice burnt plots (Fig. 7). The second component of the PCA performed with the *quality* matrix, also separated unburned and one fire from two and three fires. However, none of the factors nor their interaction had significant effects on any of the two components, again due to the high variability between the three areas analysed (data not shown).

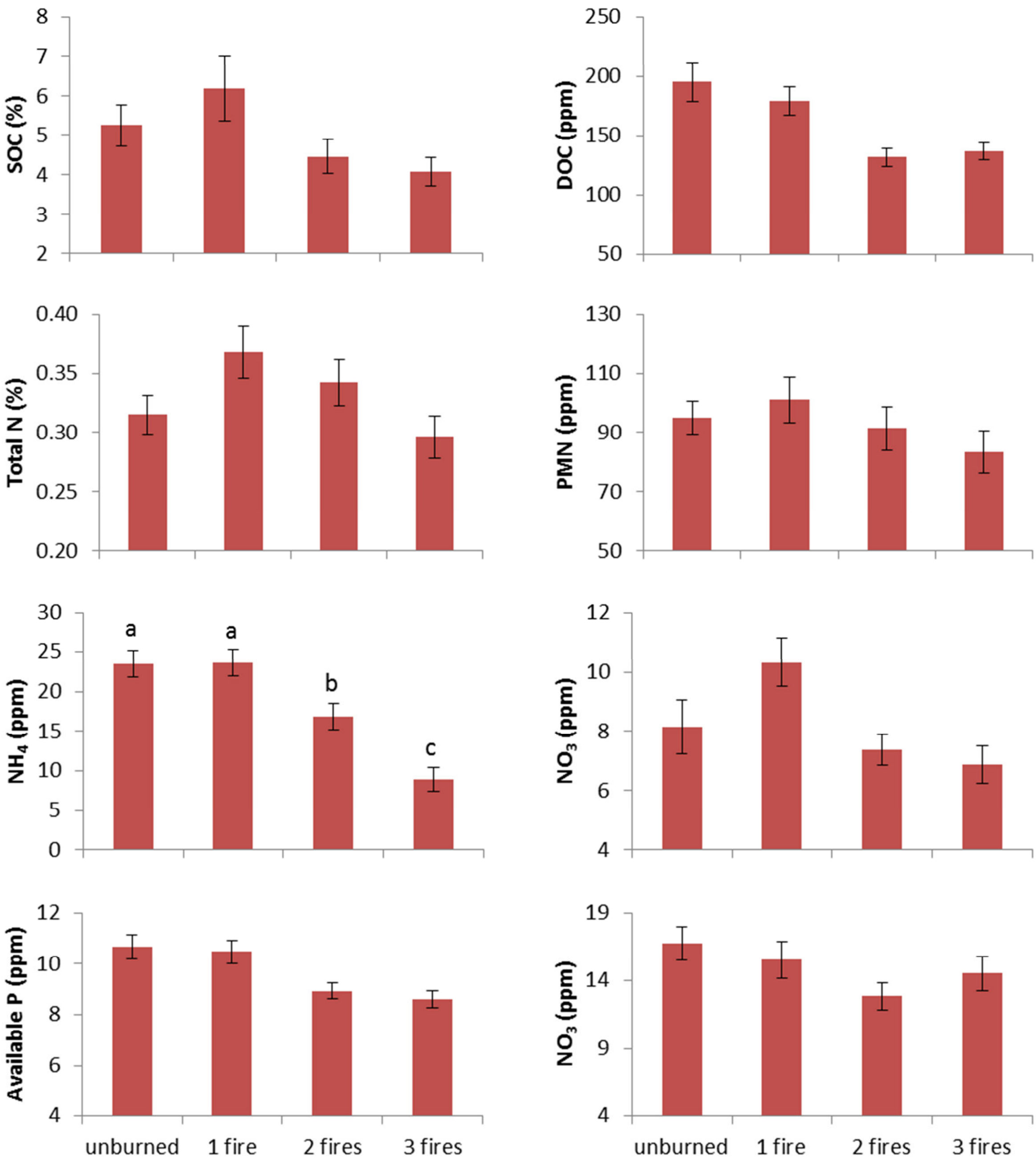

Figure 4. Soil organic C and nutrients in the upper 0-5 cm (mean ± SE) for different fire recurrence in the Valencia study site. The letters indicate significant differences between fire recurrence levels.

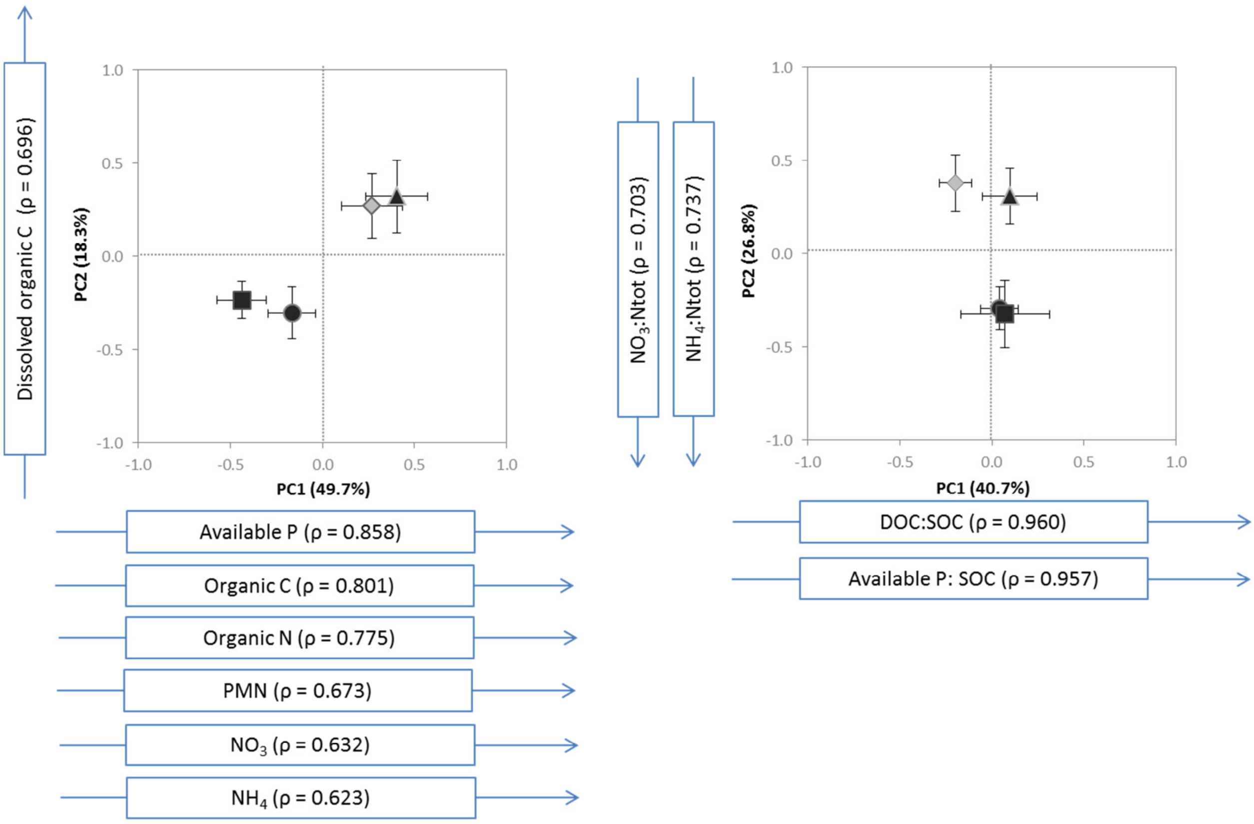

Figure 5. Principal component analysis (PCA) of soil organic matter *quantity* (left) and *quality* (right) characterising the different recurrence levels in the Valencia study site. Data represent means ± SE. Arrows represent soil nutrients significantly correlated ($|\rho| \geq 0.6$) with the first two axes. Symbols: unburned plots (diamond), plots burned once (triangle), plots burned twice (circle), and plots burned thrice (quadrat).

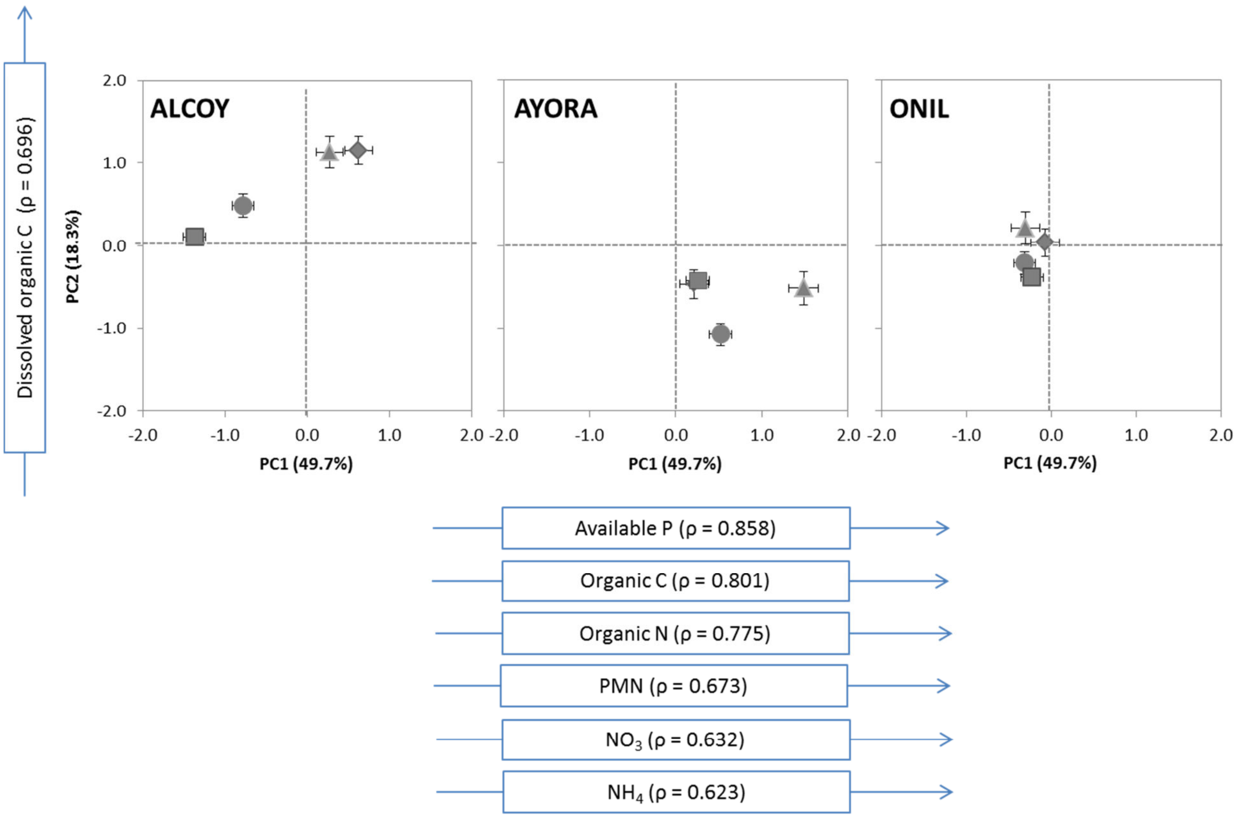

Figure 6. Principal component analysis (PCA) of soil organic matter quantity characterising the different recurrence levels in the three areas of the Valencia study site: Alcoy, Ayora and Onil. Data represent means ± SE. Arrows represent soil nutrients significantly correlated (|ρ| ≥ 0.6) with the first two axes. Symbols: unburned plots (diamond), plots burned once (triangle), plots burned twice (circle), and plots burned thrice (quadrat).

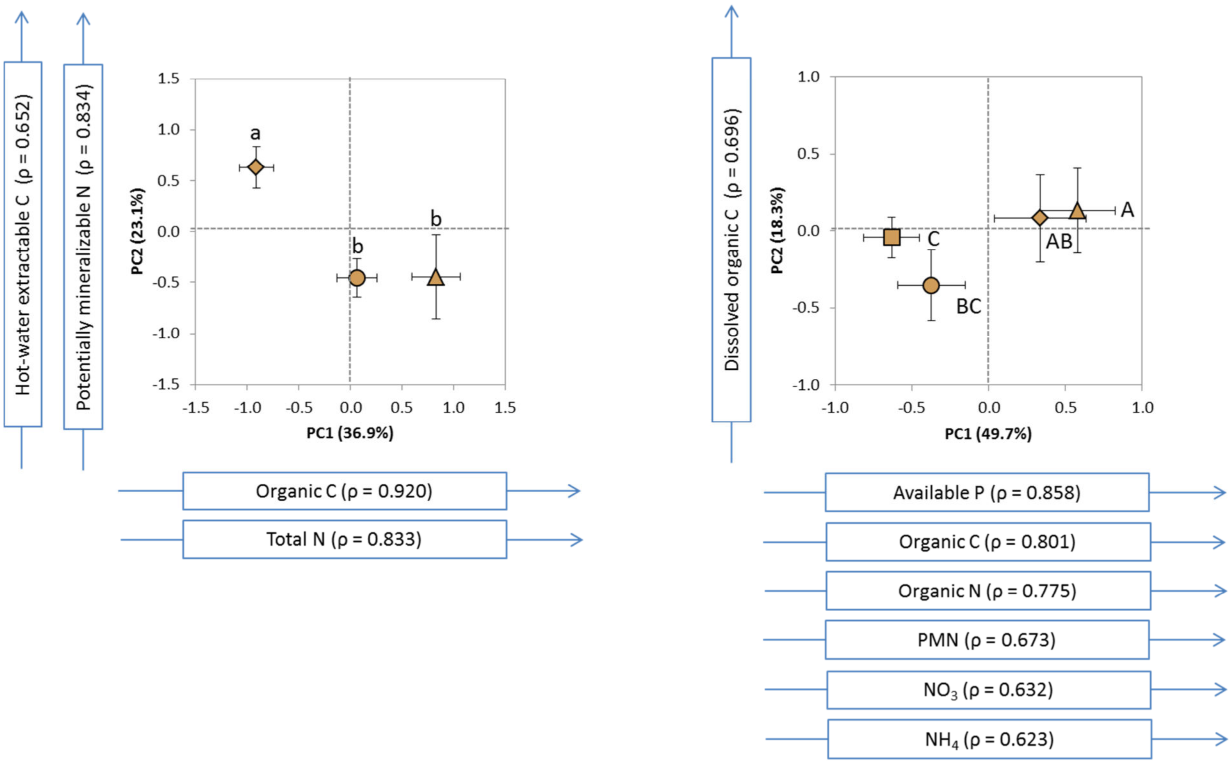

Figure 7. Principal component analysis (PCA) of soil organic matter quantity for the intershrub microsites characterising the different recurrence levels in Várzea (left) and Valencia (right). Data represent means ± SE. Arrows represent soil nutrients significantly correlated (|ρ| ≥ 0.6) with the first two axes. Symbols: unburned plots (diamond), plots burned once (triangle), plots burned 4 times in Várzea or twice in Valencia (circle), and plots burned thrice (quadrat). Capital letters indicate significant differences between fire recurrence levels for the first component (PC1) and small letters indicate significant differences between fire recurrence levels for the second component (PC2).

4 Discussion

4.1 Short-term effects of fire recurrence on soil nutrients

In general, the fertility of the top mineral soil (0-5 cm) one year after fire was more influenced by the occurrence of the last fire than by the number of previous fires (1 or 4 fires in the last 37 years). Still, there was a decreasing trend with increasing fire recurrence in soil organic C and total N, as well as with their labile fractions, although this latter trend was not significant (Fig. 3).

Thus, the occurrence of the last fire had a positive effect on soil organic C, total N, NH_4, and available P, which were higher in burned than in unburned soils eight months after the occurrence of one or even four fires. The short-term post-fire pulse in NH_4 and available P is commonly found in the literature and is associated with NH4 addition with ash deposition and soil heating, and with the pyro-mineralization of organic P during the fire, respectively (Serrasolsas and Khanna 1995, Certini 2005). Short-term (< 5 years) increases in soil organic matter following fire have also been reported in other woodland ecosystems in southern Europe (Rashid 1987, Dumontet et al 1996, Perez et al 2004, Knicker et al 2005) and are commonly attributed to the decomposition of partially burned woody fragments and charcoal formation (Almendros et al. 1990, Knicker et al 2006, Certini et al. 2011). However, the higher values of N in burned than in unburned soils does not support the idea of relevant quantities of fire-deposited charcoal as this component has a very low content in N. Despite the positive impact of fire occurrence on the total amount of soil nitrogen, its potentially mineralizable fraction did not increase resulting in a lower potentially mineralizable N to total N ratio in burned than in unburned soils (Fig. 2 and 3). Thus, fire increased the quantity of soil organic matter but decreased its quality, and to a similar degree for both soils burned once or four times (Fig. 3).

The fire-induced increase in soil organic C and total N, mainly represented by the first component performed with the quantity matrix, was mitigated by fire frequency as this component was lower in soils burned four times than in soils only burned once. These results may be attributed to a lower woody biomass before the 2012 fire in plots burned with higher fire frequency, relative to plots burned only once, and thus a lower increase in soil organic matter due to root death.

The fertility of the soils in the microsites assessed, shrub patches of *P. tridentatum* and interspaces in between these shrubs, generally showed the same short-term response to fire. The intershrub microsite was however more sensitive to the occurrence of fire, as it showed significant higher soil organic C and total N in burned than in unburned soils, but also significant lower values for C and N labile fractions in burned than in unburned soils (Fig 7).

4.2 Long-term effects of fire recurrence on soil nutrients

Soil organic C and most nutrients of the top mineral soil (0-5 cm) in Valencia showed higher values in long unburned soils (at least for 30 years) than in soils burned two or three times (20 and 8 years after the last fire, respectively). This trend was however not significant due to the large variation in the response of the blocks analysed (Fig. 6). Although two of the blocks, Alcoy and Ayora showed decreasing trends in soil fertility with increasing fire recurrence (1, 2, or 3 fires), the third block, Onil, only showed this trend for dissolved organic C.

In agreement with our results, most related studies in Mediterranean Europe measuring the long-term response following fire (> 5 years) found that shrublands or woodlands burned with high fire recurrence, that is three or four times in a period of up to 57 years, had significantly lower soil organic matter than sites only affected by one or no wildfire during that period (Carreira et al. 1994, Guénon et al. 2011, Tessler et al. 2013). A negative effect of fire recurrence in the quantity of mineral soil organic matter is associated with the cumulative effects of recurrent fires in reducing soil organic horizons (Eugenio et al. 2006). In our study site, long-term changes in soil organic C and nutrients related to fire impact on vegetation are also possibly related to changes in the composition of vegetation along post-fire succession since: unburned and 1-fire were pine forests, whereas 2- and 3-fires plots were shrublands. Litterfall quantity and quality, and decomposition rate, may be different for both groups of plots.

Long-term post-fire reductions in soil C may limit microbial activity and constrain gross production of NH_4 over time (Koyama et al 2010) as we observed in our study area (Figure 4). Furthermore, nitrogen availability may decline over secondary succession as plant and microbial competition for N increases and regenerating vegetation alters the soil microclimate. On the other hand, significant amounts of N can be volatilised or lost due to erosion. Thus, despite temporary post-fire enhanced mineral organic C and N availability, repeated burning in Mediterranean steep areas may lead to substantial depletion of the total N reserves, causing marked changes in the long-term pattern of N cycling (Carreira et al 1995, 1996).

As happened in the Várzea study site, the surface of the intershrubs in Valencia seemed to be more sensitive to fire impact than that of the shrub patches analysed in this site (*Q. coccifera* and *R. officinalis*), as intershrub microsites did show a significant decrease of soil organic matter with increasing fire recurrence (Fig. 7).

4.3 Indicators of changes in soil functioning in response to fire recurrence

Our results suggested a higher sensitivity of the soil surface between shrubs, relative to the surface below shrub patches, to the fire occurrence and recurrence. We attributed this result to differences between the two microsites in the recovery rate after fire. The canopies of shrub patches recover fast after the fire (e.g., Malanson and Trabaud, 1987; Pausas et al., 1999). This entails a higher accumulation of organic matter content in shrub patches, particularly for resprouter shrubs as supported by their higher soil organic C in both of ours study sites. The accumulation of organic matter in shrub patches is not only due to plant litter from the same patch but also through its sink role by trapping soil, seeds and litter from upslope areas during the first post-fire rainstorms (Cammeraat and Imeson, 1999; unpublished observations). Contrastingly, the openings between shrubs, either partially bare or covered by short herbaceous species, have a lower capacity to trap resources and function more like source areas.

Soil organic C and total N gave a similar response to fire recurrence at both the short- and long-term following fire, that is, a decreasing trend with increasing fire recurrence (1, 2, 3 or 4 fires in 37 years). However, the comparison with reference areas at the short term may give biased information due to the transient fire-induced pulse of soil fertility. Thus, relative to long unburned areas, a single fire may increase the quantity of soil organic matter in areas burnt with low or even high fire recurrence the first years following the fire. It, however, may still not increase the bioavailability of soil C and nutrients. In this regard, labile fractions of soil organic matter may be more robust indicators of changes in soil functioning in response to fire recurrence.

Our results also suggest a shift in mineral soil fertility associated with a fire-induced shift of pine woodlands to shrublands. Drops in soil fertility quantity and quality were generally higher between areas burned once and twice, corresponding with the replacement of woodlands by shrublands, than between areas burned twice and thrice, both of them shrublands (Fig. 5).

5 Conclusions

In the present deliverable we evaluate the short-term effect (1 year) of fire on soil fertility in areas burnt with different fire recurrence and same successional stage (Várzea, Portugal) and the long-term effect (>5 years) in areas with different fire recurrence and successional stage (Valencia, Spain). Trends towards soil fertility loss with increasing fire recurrence (one, two, three or four fires in 37 years) were observed at both the short- and the long-term following fire. Labile organic matter fractions were more sensitive than total amounts to fire impact suggesting their high indicatory value for this type of disturbance. Furthermore, the soil surface between shrubs, generally covered by herbaceous plants, showed a higher sensitivity to fire occurrence and recurrence. Overall, our results suggest that the current trend of increasing fire recurrence in Southern Europe may result in losses or alterations of soil organic matter, particularly when fire promotes a transition from pine woodland to shrubland. This shift promotes a soil fertility degradation trend. To what extend and time-frame is this degradation reversible is unknown and deserves further research. Our results also point to labile organic matter fractions in the intershrub spaces as potential early warning indicators for changes in soil functioning in response to fire.

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