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Soil microbiological properties and enzymatic activities of long-term post-fire recovery in dry and semiarid Aleppo pine (*Pinus halepensis* M.) forest stands

J. Hedo¹, M. E. Lucas-Borja², C. Wic², M. Andrés Abellán², and J. de Las Heras¹

¹Department of Plant Production and Agricultural Technology, School of Advanced Agricultural Engineering, Castilla La Mancha University, Campus Universitario s/n, CP 02071, Albacete, Spain

²Department of Agroforestry Technology and Science and Genetics, School of Advanced Agricultural Engineering, Castilla La Mancha University, Campus Universitario s/n, CP 02071, Albacete, Spain

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Correspondence to: J. Hedo (javier.hedo@gmail.com)

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Abstract

Wildfires affecting forest ecosystems and post-fire silvicultural treatments may cause considerable changes in soil properties. The capacity of different microbial groups to re-colonize soil after disturbances is crucial for proper soil functioning. The aim of this work

- ⁵ was to investigate some microbial soil properties and enzyme activities in semiarid and dry Aleppo pine (*Pinus halepensis* M.) forest stands. Different plots affected by a wildfire event 17 years ago without or with post-fire silvicultural treatments five years after the fire event were selected. A mature Aleppo pine stand unaffected by wildfire and not thinned was used as a control. Physicochemical soil properties (soil texture, pH,
- ¹⁰ carbonates, organic matter, electrical conductivity, total N and P), soil enzymes (urease, phosphatase, β -glucosidase and dehydrogenase activities), soil respiration and soil microbial biomass carbon were analysed in the selected forests areas and plots. The main finding was that long time after this fire event produces no differences in the microbiological soil properties and enzyme activities of soil after comparing burned and
- thinned, burned and not thinned, and mature plots. Thus, the long-term consequences and post-fire silvicultural management in the form of thinning have a significant effect on the site recovery after fire. Moreover, significant site variation was generally seen in soil enzyme activities and microbiological parameters. We conclude that total vegetation restoration normalises microbial parameters, and that wildfire and post-fire silvicultural treatments are not significant factors of soil properties after 17 years
- ²⁰ silvicultural treatments are not significant factors of soil properties after 17 years.

1 Introduction

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Fire is one of the most important disturbances in the Mediterranean region as it shapes and structures many plant communities, forest ecosystems and landscapes (Boydak et al., 2006). After a fire event, forest functions, nutrient cycling, and the physical, chemical and biological properties of soils are significantly affected (Wic-Baena et al., 2013). Also, increasing runoff and surface erosion rates have been exposed (Alegre-Prats





et al., 2013). On this context, post-fire forest management is useful to accelerate the recovery of soil forest functions, and to improve health, growth and reproductive processes (Moya et al., 2008). For fire-adapted pines, such as *Pinus pinaster* Ait. (Maritime pine) and *Pinus halepensis* Mill. (Aleppo pine), three main forest management
⁵ guidelines have been proposed as proper post-fire silvicultural treatments. The guidelines are in accordance with the success of natural regeneration: (1) no treatments if natural regeneration is achieved after the fire event, (2) assisted natural regeneration or (3) active restoration (De las Heras et al., 2012). Moreover, several studies have shown that "thinning in young" reduces both intra-specific competition and fire recur¹⁰ rence events (Espelta et al., 2008).

Soil plays an essential role in the forest ecosystem's fertility and stability (Smith et al., 1993) and specifically soil microorganisms, which accomplish reactions to release soil nutrients for vegetation development (Hannam et al., 2006). Forest fires and post-fire silvicultural treatments may significantly change forest and soil properties (Grady and Hart, 2006; Wic-Baena et al., 2013). After forest fires, changes in

- ties (Grady and Hart, 2006; Wic-Baena et al., 2013). After forest fires, changes in vegetation dynamics and soil properties are expected to occur due to the plant-soil feedback (Van der Putten et al., 2013; Brandt et al., 2013). Soil erosion is a key process as redistribute the soil particles, the seeds and the nutrients (Cerdà and Lasanta, 2005; Lasanta and Cerdà, 2005). Fire may alter physical–chemical soil properties
- (i.e., soil organic matter content and structure, hydrophobicity, pH and nutrient cycles), and microbiological or biochemical soil properties (i.e., microbial biomass, microbial activity, soil enzymes activities) (Mataix-Solera et al., 2009). These changes mostly occur below 5 cm of the surface, where the soil temperature rarely overtakes 100 °C (Úbeda and Outeiro, 2009; Aznar et al., 2013). Post-fire silvicultural treatments may also modify the soil microbiological and biochemical variables, such as belowground
- 25 also modify the soli microbiological and biochemical variables, such as belowground biological activity and soil nutrients availability (Grady and Hart, 2006) or enzyme activities (Wic-Baena et al., 2013). Tree felling or shrub clearing modifies microclimatic conditions at the ground level, as well as the amount and quality of potential organic inputs to soil (Grady and Hart, 2006). The magnitude of the changes occurring after



wildfire events or post-fire silvicultural treatments depends on forest characteristics, such as the recovery capacity of vegetation (Irvine et al., 2007), climatic factors (Almagro et al., 2009) and post-fire soil rehabilitation management (Fernández et al., 2012; Alegre-Prats et al., 2013).

- ⁵ Given the fundamental importance of soil microbial communities in soil ecosystem sustainability, information on how microbial functionality is affected by fire or post-fire silvicultural treatments under semiarid climatic conditions is required. Estimation of microbiota and soil status are necessary to determine optimal management strategies (Mabuhay et al., 2003; Mataix-Solera et al., 2009). In this context, the use of one
- ¹⁰ parameter is not consistent because soil quality depends on a wide range of chemical, physical, biochemical and microbiological variables (Nannipieri et al., 1990). Thus, many authors have proposed using a combination of several variables as indicators of soil status (Dick et al., 1996). Specific indicators of microbial activity, such as variables relating to nutrient cycles (nitrogen, carbon and phosphorus) and enzymatic activi-
- ties (urease, β-glucosidase and phosphatase), have been proposed to evaluate soil status (Trasar-Cepeda et al., 1998). Moreover, general indicators of microbial activity have been extensively used in forest and agricultural soil status characterization (Armas et al., 2007; García-Orenes et al., 2010; Fterich et al., 2014; Câmara-Ferreira et al., 2014).
- Long-term studies into soil quality or those that evaluate soil recovery capacity are scarce. However, long term studies are necessary to reach reasonable conclusions on the impacts that fire events and post-fire silvicultural treatments have on soil properties, particularly in Mediterranean ecosystems (Wic-Baena et al., 2013). Some long-term studies appreciated that soil organic matter and microbial communities can recover to
- the pre-fire levels (Guénon et al., 2013). The aim of this study is to investigate soil microbiological and soil enzymatic activities in different semiarid and dry Aleppo pine forest ecosystems affected by: (i) a wildfire event 17 years earlier, (ii) a wildfire event 17 years ago and treated with early thinning 12 years earlier, (iii) an Aleppo pine mature stand not affected by wildfire with no silvicultural treatments. We hypothesised that: (1)





microbiological soil properties and enzymatic activities are influenced by the climatic conditions recorded at each semiarid and dry location, (2) there are no significant differences among treatment (burned and thinned plots), control (burned and no thinned plots) and mature (unburned and unthinned plots) because of the soil/vegetation recovery capacity. It is noteworthy that we define recovery as a scenario which returns to the same soil functioning activity levels between the burnt or thinned and mature plots.

2 Material and methods

2.1 Study area

The study was conducted at two sites burnt in the summer of 1994; Yeste and Calas parra (in the provinces of Albacete and Murcia, respectively) in SE Spain. The total burnt area covered about 44 000 ha in both provinces. The forest tree composition in the study area was dominated by mature even-aged Aleppo pine stands, with shrubs and herbaceous vegetation in the understory (Table 1). Natural post-fire regeneration took place at both sites (45 000 saplings ha⁻¹ in Calasparra and 7000 saplings ha⁻¹ in Yeste) (Table 1). The climate of both experimental areas is classified as Mediterranean (Allué, 1990), with Yeste and Calasparra classed as a dry site and a semi-arid ombro-

- climate site, respectively (Rivas-Martínez, 1987). Average annual rainfall and temperature for the last 30 years were respectively 503 mm and 13.5 °C in Yeste as compared to 282 mm and 16.3 °C in Calasparra. According to the Spanish Soil Map, Yeste and Calasparra soils are classified as Inceptisols and Aridisols, respectively. Soil texture at
- ²⁰ Calasparra soils are classified as Inceptisols and Aridisols, respectively. Soil both sites is classified as loam/clay-loam (Table 1).

2.2 Experimental design

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Two experimental sites of 3 ha were selected in both Yeste $(2^{\circ}20' \text{ W } 38^{\circ}21' \text{ S})$ and Calasparra $(1^{\circ}38' \text{ W } 38^{\circ}16' \text{ S})$. Three plots were set up inside each site, one of which (1 ha) was naturally burnt in summer 1994 and was then occupied by high Aleppo





pine post-fire natural. The second plot of 1 ha was naturally burnt in summer 1994 and then thinned in 1999. The post-fire silvicultural treatment and thinning operations left 1600 saplings ha⁻¹ at both the Calasparra and Yeste sites. The third plot was a mature stand of 1 ha used as a control. The mature Aleppo pine stand was located adjacent to the fire perimeter at both the Calasparra and Yeste sites and has not been affected by either forest-fire or silvicultural treatments in the last 20 years. All the plots were selected in areas with a low slope (< 5 %).

In December 2011, six soil samples (1000 g) were randomly taken from each plot: (i) the plot affected by a wildfire event and post-fire silvicultural treatments 17 and

- 10 12 years earlier (burned and thinned, hereafter named "BT"), (ii) the plot affected by a wildfire event 17 years earlier with no post-fire silvicultural treatments (burned and not thinner, hereafter named "BNOT"), (iii) the plot occupied by a mature Aleppo pine stand (hereafter named "MAT"). Each soil sample was composed of six subsamples collected in a 5m × 5m subplot area, which were thoroughly mixed to obtain a composite sample (10 to 10 to 10
- (Andrés et al., 2011). The results shown are the average of the samples taken at each subplot. Soil samples were taken from the uppermost mineral layer (0–10 cm) after removing litter. Samples were passed through a 2 mm sieve and were kept at 4 °C during one month to avoid any influence on the parameters analysed in the laboratory (Andrés et al., 2011).

20 2.3 Physical and chemical variables

Five hundred grams of the collected soil samples were used to analyse some physical and chemical soil properties. pH and electrical conductivity (EC) were measured in a 1/5 (w/v) aqueous solution using a pH-meter (Navi Horiba model). Total organic carbon (TOC) was determined by wet oxidation with K₂CrO₇ and titration of dichromate excess with Mohr's salt (Yeomans and Bremner, 1989), while organic matter (OM) was inferred by multiplying the TOC content by 1.728. Total carbonates ($CO_3^{2^-}$) were measured in a Bernard calcimeter according to the method of Guitián & Carballas (1976). Bioavailable phosphorus (P) was determined using the method described by Olsen and





Sommers (1982). Total nitrogen (total N) was measured following Kjeldhal's method modified by Bremner (1965). The texture analysis was performed using the method of Guitián and Carballas (1976). Soil moisture and temperatures were recorded during the sampling season (winter 2011) using a soil moisture sensor (ECHO EC-10 model), a soil temperature sensor (TMC6-HD model) and a data-logger (Hobo U12-006 model).

Soil temperature and humidity sensors were installed at a depth of 10 cm in each plot.

2.4 Biochemical and microbiological variables

Soil dehydrogenase activity (DHA) was determined by using 1 g of soil, and the reduction of *p*-iodonitrotetrazolium chloride (INT) to *p*-iodonitrotetrazolium formazan was measured by a modified version of the method reported by García et al. (1993). Soil dehydrogenase activity was expressed as µmol INTF g⁻¹ soil h⁻¹. Urease activity (UA) was determined as the NH₄⁺ released in the hydrolysis reaction (Kandeler et al., 1999). Alkaline phosphatase (PA) and β -glucosidase (BA) activities were measured following the methods reported by Tabatabai and Bremner (1969) and Tabatabai (1982), respectively. Basal soil respiration (RESP) was analysed by placing 50 g of soil moistened to 40–50 % of its water-holding capacity (water potential: 0.055 MPa) in hermetically

sealed flasks and by incubating for 20 days at 28 °C. Released CO₂ was periodically measured (daily for the first 4 days and then weekly) using an infrared gas analyzer (Toray PG-100, Toray Engineering Co. Ltd., Japan). The data were summed to give
 a cumulative amount of released CO₂ after a 20 day incubation. Basal soil respiration was expressed as mg CO₂-C kg⁻¹ soil day⁻¹. Microbial biomass carbon (CB) was determined by Vance et al. (1987) following the method adapted by García et al. (2003).

2.5 Statistical analysis

Data were analysed by a two-way ANOVA at which site level (Yeste and Calasparra) and the silvicultural management level ("BT", "MAT" and "BNOT") were selected as the factors. All the subplots were assumed to be spatially independent. The post hoc



test applied was Fisher's least significant difference. A P < 0.05 level of significance was adopted throughout, unless otherwise stated. Moreover, a multivariate statistical method using a principal component analysis (PCA) was carried out to study the structure of the dependence and correlation between the physicochemical and microbiological soil properties at the different sites and for the various treatments. To satisfy the assumptions of the statistical test (equality of variance and normal distribution), variables were square root-transformed whenever necessary. The statistical analyses were done with the Statgraphics Centurion software.

3 Results

3.1 Physical and chemical variables

Soil temperatures and soil moisture differed significantly (P < 0.05) between both experimental sites (Yeste and Calasparra), but not between different treatments ("BT", "MAT" and "BNOT") (Table 1). Soil texture (Table 1) and electrical conductivity (Table 2) were also similar for both study sites and for the different treatments. The percentage of carbonates, organic matter, phosphorus and total nitrogen differed between sites, with higher values recorded for Yeste. Significant differences were also observed (P < 0.05) in the pH values and C/N ratio between sites, with Yeste obtaining lower values. Under the experimental conditions, the physical and chemical variables showed a different behaviour depending on the site (Yeste and Calasparra; Table 2).

20 3.2 Biochemical and microbiological variables

The experimental treatments considered in this study and the interaction between sites and experimental treatments did not significantly (P < 0.05) influence the microbiological properties and enzyme activities (Table 3). The experimental site was the only influential factor (P < 0.05) found for microbial biomass carbon, soil respiration and enzymatic activities (Table 3). Uncase activity showed higher values in Calasparra than

²⁵ zymatic activities (Table 3). Urease activity showed higher values in Calasparra than





in Yeste, whereas β -glucosidase and dehydrogenase activities displayed higher values in Yeste than in Calasparra (Fig. 1). No significant differences for phosphatase activity were found (Fig. 1). In relation to the experimental treatments, enzymatic activities presented similar values in the "BT", "MAT" and "BNOT" plots (Fig. 1).

5 3.3 Correlation analysis

Positive and significant correlation coefficients were found between organic matter and some microbiological and biochemical variables (dehydrogenase, β -glucosidase and soil respiration). Negative and significant correlation coefficients were observed between organic matter and the physical–chemical variables, such as pH and C/N ratio, and also among the microbiological variables, such as urease activity (Table 4). pH also showed a positive correlation and a significant coefficient with urease activity. pH negatively and significantly correlated with soil respiration, dehydrogenase and β -glucosidase activity. Urease activity presented different correlation coefficients, and positively and significant correlated with phosphatase activity, pH and C/N ratio, while a negative and significant correlation was observed with dehydrogenase and β -glucosidase activities and total carbonates, phosphorus and total nitrogen. Conversely, a positive and significant correlation was seen between dehydrogenase and β -glucosidase activity. pH and C/N ratio correlated significantly and negatively with

dehydrogenase and β -glucosidase activities (Table 4).

20 3.4 PCA analysis

The multivariate PCA analysis showed differences between the two study sites by separating into homogeneous groups (Fig. 3). Conversely, the PCA did not separate among different treatments. The PCA analysis clustered the plots located in Yeste on the negative axis of PC 2 (Fig. 3), which explained about 13.81 % of variability. PC 2 explained around 42.22 % of variability. The plots located in Calasparra were clustered on

²⁵ plained around 42.22 % of variability. The plots located in Calasparra were clustered on the positive axis of PC 2. Urease activity, C/N ratio and pH had a positive weight on PC





1, whereas dehydrogenase, β -glucosidase and organic matter had a negative weight (Table 5). Moreover, respiration, phosphatase and electrical conductivity had a positive weight on PC 2, while phosphorus and biomass carbon had a negative weight. The other loading factors of the different variables appear in Table 5.

5 4 Discussion

Vegetation and soil type are key factors that can modify soil characteristics and are responsible for maintaining a stable microbial community (Bastida et al., 2008). Since Aleppo pine forest dominates both experimental sites, variations in soil properties can be related mainly to site-specific differences, such as soil temperature and moisture and soil type (soil organic matter, C/N ratio, pH and P, soil texture). Micro-climatic factors influence microbial enzymes, and also change the quality and quantity of the substrate upon which they act (Kumar et al., 1992). Different authors have demonstrated that higher soil temperatures and scarce soil moisture generate lower soil respiration rates, microbial biomass carbon values and dehydrogenase, phosphatase and

- β -glucosidase enzymatic activities (Criquet et al., 2004; Sardans and Peñuelas, 2005; Baldrian et al., 2010; Lucas-Borja et al., 2012). Our results coincide with these trends since Calasparra (higher temperatures at lower soil moisture values) obtained lower values of microbiological parameters, β -glucosidase and dehydrogenase activities, but higher values for urease and phosphatase enzymes. The latter may be explained by
- quantity of total N and P present at each site. Given the lower total N and P values found in Calasparra, greater urease and phosphatase activity may be required to produce inorganic N and P ready for plant development. Gutknecht et al. (2010) recently showed that decreased N and P results in greater urease and phosphatase activity and higher enzyme production through soil microorganisms. Furthermore, Bastida et al. (2008)
- indicated that seasonality affects enzymatic activities or microbial biomass, and in this work only we sampled in early winter, so it would be suitable to conduct sampling in different seasons.





In relation to fire and post-fire silvicultural treatments, soil moisture and temperature showed no significant differences in the "BT", "MAT" and "BNOT" plots, thus may explain in a large part similar microbiological parameters values and enzymatic activities. Moreover, Aleppo pine is a pyrophyte species that exhibits good post-fire natural regeneration, being observed good post-fire seedling recruitment during the first growth

- season after the wildfire event (Leone et al., 2000). Thus, initial vegetation recovery is promptly ensured after a wildfire event (De las Heras et al., 2012). In this context, temporary plant cover loss and subsequent plant recruitment after a fire event may enhance the microbiological soil properties recovery. According to our results, the micro-
- biological soil properties and enzymatic activities capacity recovery should be achieve 15 years after the wildfire event and the post-fire silvicultural treatment. This long-term study demonstrated that soil parameters might recover to the pre-fire levels 15 years after the fire event and thinning operations. Wic-Baena et al. (2013) have recently shown that soil enzymatic activities did not diminish 6 years after thinning.
- ¹⁵ The organic matter greatly differed, obtaining higher values for Yeste than for Calasparra. Higher values for the general soil microbial activity indicators (i.e., soil respiration and dehydrogenase activity) and for β -glucosidase and phosphatase activity have been reported by Lucas-Borja et al. (2010, 2011) in forest soil at a higher organic matter concentration. Some organic matter fractions contain readily metabolis-
- able compounds, which can act as energy sources for microorganisms. In relation to fire and post-fire silvicultural treatments, the organic matter content was similar when comparing "BT", "MAT" and "BNOT" plots, which may be explained by the Aleppo pine post-fire initial recruitment. The organic matter derived from new trees may be the responsible of the similarities comparing "BT", "MAT" and "BNOT" plots. We found sig-
- ²⁵ nificant positive correlations between microbiological measurements (soil respiration) and enzymatic activities (dehydrogenase and β -glucosidase activities) and organic matter content. Our results also indicate lower C/N values at Yeste, but no significant differences among treatments. We found significant negative correlations between microbiological measurements and enzymatic activities (except urease enzyme) with the





C/N ratio. As Merilä et al. (2002) have shown, substrate quality, as determined by C/N, generally influences microbial biomass and respiration. Lower C/N rates have been associated with higher respiration rates and microbiological properties (Schmitz et al., 1998). Regarding pH, some authors have denoted its influence on soil microbial biomass properties (Bååth and Anderson, 2003). According to Sinsabaugh (2008), soil pH has direct biochemical effects on the activity of the extracellular enzymes immobilised in the soil matrix. The same author has also argued that soil pH reflects climatic controls in soil and plant community composition, which may affect the large-scale distribution of extracellular enzymatic activities through changes in nutrient availability, soil organic composition and microbial community composition. Our results agree with this

¹⁰ organic composition and microbial community composition. Our results agree with this trend and indicate that pH correlates negatively with soil enzymes activities (except urease activity), soil respiration and organic matter.

Finally, the PCA results reveal that the sites were significantly discriminated. The higher soil temperatures and lower soil moisture values recorded at Calasparra provide

¹⁵ unfavourable conditions for balanced soil functional diversity, as reflected by poorer enzyme activities, soil respiration and biomass carbon if compared with Yeste. On the contrary, treatments were not significantly discriminated, which reflects that vegetation recovery after a wild-fire event and the time elapsed since the post-silvicultural treatments applied were enough to achieve the initial soil property values found in mature and unaffected plots.

5 Conclusions

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Biochemical, microbiological and physicochemical variables are affected by site, but not by post-fire silvicultural treatment, under dry and semiarid conditions. Seventeen years after the wildfire event and the post-fire silvicultural treatment, microbiological soil properties may recover the initial status and values shown for mature and undisturbed Aleppo pine forest stands. The micro-climatic conditions, higher soil temperature and lower soil moisture values obtained at Calasparra indicate unfavourable conditions for





microbiological properties and enzyme activities if compared with Yeste. Our results provide data on the long-term recovery pattern of microbiological and enzymatic activities, and clearly distinguish between sites with different microclimatic conditions (temperature and moisture), but not among burnt/unburnt or post-fire thinned/unthinned
 Aleppo pine forests stands for more than 17 years after the wildfire and silvicultural treatment. Forest management guidelines should consider the effect of thinning treatments and forest site in order to preserve soil quality under the adaptative forest management context.

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Discussion

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6, 3025-3050, 2014

Soil microbiological

properties and

enzymatic activities

of long-term post-fire

recovery

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Table 1. Soil, climatic and stand characteristics of each experimental site^{*}.

400

45 000

1600

500

7000

1600

Aleppo pine

density (trees ha⁻¹)

Т

(°C)

 12.0 ± 1.1

 9.2 ± 1.8

 9.5 ± 1.4

 8.0 ± 1.2

 7.1 ± 0.9

 7.5 ± 1.3

Н

(%)

 5.9 ± 2.0

 7.5 ± 1.1

 5.2 ± 0.9

Forest

Yeste

Calasparra

site

Plot

MAT

BT

MAT

BT

BNOT 860

BNOT 430

Altitude

(m)

330

330

1010

1010

Vegetation

90 % Ph

80 % Ph

70 % Ph

90 % Ph

80 % Ph

70% Ph

herbaceous

herbaceous

herbaceous

herbaceous

10% Shrub and

20% Shrub and

30 % Shrub and

10% Shrub and

20% Shrub and

30 % Shrub and

herbaceous

herbaceous

cover

* Ph: Aleppo pine; T: soil temperature (mean ± standard error) during the sampling period; H: soil moisture (mean ± standard error) during the season of sampling (Winter, 2011).



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Table 2. Soil physicochemical parameters for each site and experimental condition.

Site	Exp. condition	рН	Electrical conductivity (µS cm ⁻¹)	Organic matter (%)	Total carbonates (%)	Р	Total N (mg kg ⁻¹)	C/N (%)
Calasparra	BT	8.66 (0.07) aA	21.15 (0.78) aA	6.73 (0.66) aB	2.72 (0.07) aB	11.32 (1.35) aB	0.18 (0.00) aB	53.5 (4.26) bA
	BNOT	8.75 (0.06) aA	20.28 (1.03) aA	5.87 (0.38) bB	2.13 (0.02) bB	12.74 (3.84) aB	0.11 (0.00) bB	83 (5.95) aA
	MAT	8.39 (0.02) bA	23.48 (2.69) aA	5.35 (0.68) bB	1.92 (0.20) bB	16.95 (1.12) aB	0.20 (0.03) aB	44 (3.73) bA
Yeste	BT	8.30 (0.17) aB	20.85 (0.02) aA	8.24 (0.60) aA	2.94 (0.01) aA	27.99 (0.57) aA	0.98 (0.22) aA	16.5 (4.22) aB
	BNOT	7.83 (0.17) aB	21.15 (0.73) aA	9.17 (0.19) aA	2.94 (0.01) aA	14.24 (2.38) cA	1.09 (0.26) aA	15 (3.71) aB
	MAT	8.37 (0.22) aB	21.89 (2.28) aA	6.42 (0.22) bA	2.88 (0.02) bA	20.63 (2.67) bA	0.76 (0.27) aA	33 (12.07) aB

For each parameter values represent mean (standard error). Data followed by the same small letter are not significantly different according to the LSD test (P < 0.05) for each experimental condition. For each experimental site, data followed by the same capital letter are not significantly different according to the LSD test (P < 0.05).

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Table 3. Result of the two-factor ANOVA (site and experimental condition) for the microbiological properties and enzymatic activities analysis.

	Dehydrogenase activity		Urease activity		Phospha	atase activity	β –Gluco	sidase activity	Soil re	spiration	C-Bi	omass
	F ratio	P value	F ratio	P value	F ratio	P value	F ratio	P value	F ratio	P value	F ratio	P value
Factors												
S	170.21	0.0001	45.15	0.0001	0.37	0.5486	65.14	0.0001	14.88	0.0006	4.61	0.0399
Т	0.34	0.7137	0.01	0.9932	0.29	0.754	1.70	0.1993	1.35	0.2741	0.20	0.8202
$S \times T$	2.16	0.1334	0.02	0.9819	0.05	0.9519	0.72	0.4948	0.01	0.9885	0.07	0.9363

S: Site; T: Experimental treatment; $S \times T$: interaction between S and T.

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able	4. Cor	relation	DHA	k betwe	BC	e differe	nt varia	ables d	letermi	pH	EC	Total N	CO32-	Discussion	enzymatic of long-ter reco J. Hed	m post-fir very o et al.
PA DHA CB	UA 0.38* -0.66*** -0.58*** -0.26ns	PA -0.14ns -0.06ns -0.11ns	DHA 0.67***	BA 0.28ns	BC	e differe RESP	nt varia	ables d	P P	pH	EC	Total N	CO ₃ ²⁻	Discussion Paper	enzymatic of long-tern reco J. Hed	m post-fir very o et al.
PA DHA BA CB RESP	UA 0.38 [*] -0.66 ^{***} -0.58 ^{***} -0.26ns -0.12ns	PA -0.14ns -0.06ns -0.11ns 0.18ns	DHA 0.67*** 0.36* 0.61***	6 betwee BA 0.28ns 0.42*	BC 0.10ns	e differe RESP	nt varia	ables d	letermi P	ned. _{рн}	EC	Total N	CO ₃ ²⁻	Discussion Paper	enzymatic of long-tern reco J. Hed	m post-fir very o et al. Page
PA DHA BA CB RESP H OM	4. Corr UA 0.38* -0.66** -0.58** -0.26ns -0.12ns -0.62** -0.38*	PA -0.14ns -0.06ns -0.11ns 0.18ns 0.18ns 0.18ns	DHA 0.67*** 0.36* 0.61*** 0.50**	6 betwee BA 0.28ns 0.42* 0.76***	BC 0.10ns 0.28ns 0.02ns	e differe RESP	nt varia	ables d	letermi P	ned. _{рн}	EC	Total N	CO ₃ ²⁻	Discussion Paper	enzymatic of long-tern reco J. Hed	m post-fir very o et al. Page
PA DHA BA CB RESP H OM P	UA 0.38* -0.66*** -0.26ns -0.26ns -0.22ns -0.62*** -0.38* -0.41*	PA -0.14ns -0.06ns -0.11ns 0.18ns 0.01ns 0.18ns -0.13ns	DHA 0.67*** 0.36* 0.61*** 0.50** 0.26ns	0.28ns 0.42* 0.76*** 0.52**	BC 0.10ns 0.28ns 0.02ns 0.19ns	e differe RESP 0.41* 0.54*** 0.10ns	nt varia H	OM 0.11ns	letermi P	pH	EC	Total N	CO ₃ ²⁻	Discussion Paper 1	enzymatic of long-tern reco J. Hed Title	m post-fir very o et al. Page
PA DHA BA CB RESP H OM P P	UA 0.38* -0.66*** -0.58*** -0.26ns -0.12ns -0.62*** -0.38* -0.41* 0.39*	PA -0.14ns -0.06ns -0.11ns 0.18ns 0.18ns -0.13ns -0.13ns -0.15ns	DHA 0.67*** 0.36* 0.61*** 0.77*** 0.26ns -0.39*	0.28ns 0.42* 0.76*** 0.52** -0.57***	0.10ns 0.28ns 0.02ns 0.19ns -0.19ns	0.41* 0.54*** 0.10ns -0.40*	0.47** 0.43** -0.50*	0.11ns -0.63***	P -0.11ns	ned. _{рн}	EC	Total N	CO ₃ ²⁻	Discussion Paper Di	enzymatic of long-tern reco J. Hed Title Abstract	m post-fir very o et al. Page Introduction
PA DHA BA CB RESP H OM P P DH EC	UA 0.38° -0.66° -0.58° -0.26ns -0.26ns -0.26ns -0.62° -0.38° -0.41° 0.39° 0.18ns 0.41°	PA -0.14ns -0.06ns -0.11ns 0.18ns 0.18ns 0.18ns -0.13ns -0.13ns -0.15ns 0.10ns	DHA 0.67*** 0.36* 0.61*** 0.77*** 0.26ns -0.39* 0.10ns 0.20**	0.28ns 0.42* 0.76*** 0.56*** -0.57*** -0.13ns	0.10ns 0.28ns 0.02ns 0.19ns -0.19ns -0.03ns	0.41* 0.54*** 0.10ns -0.40* 0.43**	0.47** 0.43** -0.50* -0.09ns	0.11ns -0.63''' 0.07ns	-0.11ns -0.16ns	pH 0.16ns	EC	Total N	CO ₃ ²⁻	Discussion Paper Disc	enzymatic of long-tern reco J. Hed Title Abstract	m post-fir very o et al. Page Introduction
PA DHA BA CB RESP H OM P P PH EC Total N Total N	UA 0.38° -0.66° -0.58° -0.26ns -0.26ns -0.12ns -0.26rs -0.38° -0.41° 0.39° 0.18ns -0.51° 0.58°	PA -0.14ns -0.06ns -0.11ns 0.18ns 0.01ns 0.13ns -0.13ns -0.15ns 0.10ns -0.07ns	DHA 0.67 ^{***} 0.36* 0.61*** 0.77*** 0.26ns -0.39* 0.10ns 0.73*** 0.61***	0.28ns 0.42° 0.76°° 0.52° -0.57°° -0.13ns 0.54°°	0.10ns 0.28ns 0.02ns 0.19ns -0.19ns -0.03ns 0.37c	e differe RESP 0.41 [*] 0.54 ^{**} 0.10ns -0.40 [°] 0.43 ^{**} 0.64 ^{**}	0.47** 0.43** -0.50* -0.09ns 0.62*** 0.46**	0.11ns -0.63*** 0.07ns 0.27ns	-0.11ns -0.16ns 0.06ns	0.16ns	EC 0.33*	Total N	CO ₃ ²⁻	Discussion Paper Discus	enzymatic of long-ter reco J. Hed Title Abstract Conclusions	m post-fir very o et al. Page Introduction References

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Table 5. Weights of principal components analysis.

	PC 1	PC 2
Dehydrogenase	-0.351	-0.072
β -glucosidase	-0.341	-0.092
Moisture	-0.334	-0.103
Organic matter	-0.283	0.359
Total nitrogen	-0.273	-0.079
Soil respiration	-0.247	0.338
Phosphorus	-0.161	-0.298
Carbon biomass	-0.143	-0.271
Electrical conductivity	-0.021	0.317
Phosphatase	0.017	0.444
рН	0.264	-0.168
Total carbonates	-0.271	-0.093
C/N	0.284	0.058
Urease	0.288	0.312

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Figure 1. Dehydrogenase activity (μ g (INTF) g⁻¹ soil h⁻¹), β -glucosidase activity (μ moles PNP g⁻¹ dry soil h⁻¹), phosphatase activity (μ moles PNP g⁻¹ dry soil h⁻¹) and urease activity (μ mol N-NH4+ g⁻¹ dry soil h⁻¹) in relation to the experimental site. Error bars are the LSD intervals at P < 0.05.







Figure 2. Soil respiration (mg CO_2 kg⁻¹ soil) and microbial biomass carbon (mg kg⁻¹) in relation to the experimental site. Error bars are the LSD intervals at P < 0.05.



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Figure 3. Principal components analysis of the experimental sites Yeste and Calasparra.



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