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Drought decreases soil enzyme activity in a Mediterranean *Quercus ilex* L. forest

J. Sardans*, J. Peñuelas

Unitat d'ecofisiologia CSIC-CEAB-CREAF, Facultat de Ciencias, Edifici C, Universitat Autònoma de Barcelona, Bellaterra 08913, Barcelona, Spain

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Abstract

Longer and more severe drought periods are expected in the near future for Mediterranean ecosystems. Soil enzymes play an essential role in the nutrient mineralization and their activity is an exceptional sensor in predicting the capacity of nutrient supply to plants. We conducted an experiment of water availability manipulation in evergreen oak mountain stands with the aim to study the effects of enhanced drought on the activity of five soil enzymes. The drought treatment consisted of runoff exclusion by a ditch along the entire top edge of the upper part of treatment plots and partial rain exclusion by suspending PVC strips and funnels. The reduction of 10% of soil moisture produced by runoff exclusion decreased urease activity by 10–67%, protease activity by 15–66% and β-glucosidase activity by 10–80%, depending on annual period and soil depth. The reduction of 21% of soil moisture produced by runoff and rainfall exclusion together reduced urease activity by 42–60%, protease activity by 35–45%, β-glucosidase activity by 35–83% and acid phosphatase activity by 31–40%. No significant effects were observed on alkaline phosphatase activity. The activities of the enzymes involved in the nitrogen cycle, protease and urease, were the most affected by drought. In all cases, the activities of these enzymes strongly decreased with soil depth and they were greater in spring than in autumn. These results show the link between drought and a slower nutrient turn-over, which decreases the nutrient supply to plants.

Keywords: Phosphatases; Protease; Urease; β-glucosidase; *Quercus ilex*; Water stress; Mediterranean; Climate change; Global change

1. Introduction

Soil enzyme activities are 'sensors' of soil degradation since they integrate information about microbial status, and also, from soil physico-chemical conditions (Wick et al., 1998; Aon and Colaneri, 2001; Baum et al., 2003). They are used as sensors in studies on the influence of soil treatments on soil fertility (Chen et al., 2003). They may correlate well with nutrient availability (Asmar et al., 1994). Bacteria and fungi synthesize and secrete enzymes such as phosphatases, proteases, ureases and pectinases extracellularly. Those microbially secreted enzymes constitute an important part of the soil matrix as extra-cellular enzymes, also called abiontic enzymes (Sinsabaugh, 1994). Factors influencing soil microbial activity exert control over soil enzyme production and control on nutrient availability and soil fertility (Sinsabaugh et al., 1993).

Mediterranean ecosystems are water limited (Specht, 1979; Mooney, 1989), but frequently, they are also nutrient limited (Kruger, 1979; McMaster et al., 1982; Henkin et al., 1998). Mediterranean soils often suffer from nutrient deficiencies, especially of N and P (Specht, 1963; Zinke, 1973; Henkin et al., 1998; Hanley and Fenner, 2001; Terradas, 2001; Sardans et al., 2004). In Mediterranean ecosystems, the effects of CO_2 enhancement on plant growth, leaf composition and leaf P and N contents could affect nutrient availability (Niinemets et al., 1999). On the other hand, the increases of heavy rainfall predicted in most global circulation models (IPCC, 2001) may increase nutrient losses in the near future. A greater degree of nutrient limitation to plant growth can ensue, further slowing the regeneration process of these forests, which is already compromised due to low water and nutrient

* Corresponding author. Tel.: +34 93 581 13 12; fax: +34 93 581 41 51.
E-mail address: j.sardans@creaf.uab.es (J. Sardans).

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availability and scrub competition. P and N inputs are dependent on the mineralization capacity of soil organic matter. In areas of Central Spain soil enzyme activity decreases proportionally to the decrease of plant cover (García et al., 2002). A decrease of enzyme activity in Mediterranean ecosystems under drier conditions might be critical because of the decrease in nutrient supply and consequently, this might have a direct effect on the photosynthetic production capacity and an indirect effect on WUE (Water Use Efficiency). Moreover, the re-wetting effect (Baum et al., 2003) can reduce the bacterial population impairing soil enzyme activity. A decrease in P content of the stand biomass together with a decrease in the soil P-available forms in response to a more pronounced drought has already been observed in Mediterranean forests (Sardans and Peñuelas, 2004). The possible negative results of more frequent and stronger drought periods on nutrient availability in the Mediterranean ecosystems could compromise their current structure.

We monitored the activities of four enzyme groups: phosphatases, proteases, ureases and β-glucosidases in an evergreen holm-oak Mediterranean forest. We aimed to detect the possible changes in the activity of these soil enzymes at different layers in the soil profile in response to a drought enhancement as the one predicted by climate change models for the next decades (IPCC, 2001; Sabaté et al., 2002). We hypothesized that a slight soil moisture decrease would significantly reduce the activity of soil enzymes.

2. Material and methods

2.1. Study site

The study was carried out in a natural *Quercus ilex* oak forest of the Prades Mountains located in Southern Catalonia (North-Eastern Iberian Peninsula) (41° 13′ N, 0° 55′ E), on a south-facing slope (25%). The soil is a stony *Dystric Xerochrept* (Soil Taxonomy) on a bedrock of metamorphic sandstone. Its depth ranges from between 35 and 100 cm. Depth of Horizon A ranges between 25 and 30 cm. The average annual temperature is 12 °C and the average annual rainfall is 658 mm. Summer drought is pronounced and usually lasts for 3 months. The vegetation is a dense forest dominated by *Quercus ilex* L. as a dominant tree (20.8 $m^2\ ha^{-1}$ of trunk basal area at 50 cm of height) with abundant presence of *Phillyrea latifolia* (7.7 $m^2\ ha^{-1}$ of trunk basal area at 50 cm of height) and *Arbutus unedo* L. and other evergreen species well adapted to drought conditions (*Erica arborea* L., *Junniperus oxycedrus* L., *Cistus albidus* L.), and occasional individuals of deciduous species (*Sorbus torminalis* L. Crantz and *Acer monspessulanum* L.).

2.2. Experimental design

Eight 15 × 10 m plots were established at the same altitude (930 m above sea level) along the slope. Four the plots received the drought treatment and the other half were control plots. The drought treatment consisted of partial rain exclusion by suspending PVC strips and funnels at a height of 0.5–0.8 m above the soil surface. Strips and funnels covered approximately 30% of the total plot surface. Two plastic strips of 14 m of long and of 1 m of wide were placed along the drought treatment plots from the top until the bottom part, and 30 plastic funnels of 1 m^2 each one were randomly placed in each drought treatment plot. A 0.8–1 m deep ditch was excavated along the entire top edge of the upper part of the treatment plots to intercept runoff water supply. The water intercepted by strips, funnels, and ditches was conducted outside the plots, below their bottom edge. The drought treatment has been applied since March 1999 (Ogaya et al., 2003). Soil moisture was measured every 2 weeks throughout the experiment by time domain reflectometry (Tektronix 1502 C, Beaverton, OR, USA, Zegelin et al., 1989). Three stainless steel cylindrical rods, 25 cm long, were fully driven into the soil at randomly selected places in each plot. The time domain reflectometer was connected to the ends of the rods to determine the soil moisture.

2.3. Sampling process

We conducted soil sampling in spring (May) and autumn (October) 2003, i.e. after 5 years of drought treatment. In each control plot we sampled four soil cores from the first 30 cm of soil profile (Horizon A). In the treatment plots we distinguished two levels of drought, that of the soil between the strips (D) (runoff exclusion) and that below the strips (DD) (runoff exclusion and rainfall exclusion). In those treatment plots, eight soil cores were taken, four between strips and four below the strips, at a minimum distance of 1 m from the nearest tree or shrub. In each soil core we distinguished the 15 cm of the upper layer from the 15–30 cm of the lower layer in order to detect possible differences in enzyme activity with soil depth. We selected these two levels because in the horizon A, there could be distinguished the A_1 subhorizon (first 15 cm) rich in organic matter (7.25%, w/w), and the A_2 subhorizon (15–30 cm) with moderate amounts of organic matter (1.3%, w/w).

2.4. Enzyme activity measurements

Phosphomonoesterase activity was determined using para-nitrophenyl phosphate (*p*NPP) as an orthophosphate monoester analogue substrate (Tabatabai, 1982). We used the original method of Tabatabai (1982) modified in a few points according with Schinner et al. (1996). Briefly we took 1 g of each sample soil (<2 mm) in a 50 ml Erlenmeyer flask, added 4 ml of THAM

(Tris–hydroxymethyl-aminomethane, with the acids citric, maleic and boric) buffer (pH 6.5 for acid phosphatase assay or pH 11 for the assay of alkaline phosphatase), 1 ml of *p*-nitrophenyl phosphate solution prepared in the same buffer, and swirled the flask for a few seconds to mix the contents. After stoppering the flask, we placed it in an incubator at 37 °C. After 1 h, we removed the stopper, added 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH, swirled the flask for a few seconds, and filtered (0.45 μm HA nitrocellulose, Millipore) the soil suspension in order to prevent the interference of possible precipitates. The extinction of the yellow colour intensity of calibration standards, samples and controls was measured with a spectrophotometer Spectronic 20 Genesys (Spectronic Instruments, Inc, Rochester NY, USA) at 398 nm against the reagent blank. We calculated the *p*-nitrophenol content by referring to a calibration curve obtained with standards containing 0, 10, 20, 30, 40 and 50 ppm of *p*-nitrophenol. Then the phosphomonoesterase activity was expressed as μg *p*-nitrophenol (*p*NP) per gram dry matter and incubation time.

For Protease activity determination we used the method developed by Ladd et al. (1976). Five millilitres of the substrate solution (casein solution, 2%, w/v) was added to 1 g of field-moist soil. We added 5 ml of Tris (Tris–hydroxymethyl-aminomethane) buffer (0.05 M, pH 8.1), and then incubated for 2 h at 50 °C. After incubation, amino acids released during the incubation period were extracted, and the remaining substrate was precipitated after the addition of thichloroacetic acid. Thereafter, samples and controls were filtered immediately. For photometric analysis, we pipetted 5 ml of filtrate and 7.5 ml of alkali reagent into a test tube, mixed well, added 5 ml of Folin-Ciocalteu's phenol reagent, and mixed again. Before colour measurements, samples, controls and the standards were filtered (0.45 μm HA nitrocellulose, Millipore) in order to prevent the interference of precipitates which form the casein remainders. All were allowed to stand at room temperature for exactly 90 min for colour development. Within the following 90 min, we measured the extinction at 700 nm with a spectrophotometer Spectronic 20 Genesys (Spectronic Instruments, Inc, Rochester NY, USA) against the reagent blank. We calculated the tyrosine content by referring to a calibration curve obtained with standards containing 0, 100, 250, 1000 and 1500 μg of tyrosine. Protease activity was expressed as μg tyrosine equivalents per gram dry matter and incubation time. Tyrosine equivalents were calculated from the calibration curve.

For urease activity determination, we used the Kandeler and Gerber (1988) method. After the addition of an aqueous (controls) or a buffered urea solution (samples) to 5 g of soil samples, those were incubated for 2 h at 37 °C. Released ammonium was extracted with potassium chloride solution, and determined by a modified Berthelot reaction (Schinner et al., 1996). The solutions were shaken for 30 min and filtered (0.45 μm HA nitrocellulose, Millipore) in order to prevent the interference of possible precipitates. The determination was based on the reaction of sodium salicylate with NH_3 in the presence of sodium dichloroisocyanurate which forms a green-coloured complex under alkaline pH conditions, being the extinction measured at 690 nm with a spectrophotometer Spectronic 20 Genesys (Spectronic Instruments, Inc, Rochester NY, USA) against the reagent blank. We calculated the ammonium content by referring to a calibration curve obtained with standards containing 0, 1, 1.5, 2 and 2.5 μg NH_4^+ ml^{-1}. Sodium nitroprusside was used as a catalyst. It increased the sensitivity of the method about tenfold.

The method used to determine β-glucosidase was based on the colorimetric determination of saligenin released by β-glucosidase when 5 g of soil were incubated 3 h at 37 °C with acetate buffer (pH 6.2), and salicin (β-glucosido-saligenin) (Tabatabai, 1994). The solutions were filtered (0.45 μm HA nitrocellulose, Millipore) in order to prevent the interference of possible precipitates. Saligenin released from the substrate was determined colorimetrically after colouring with 2,6-dibromomchinon-4-chlorimide with borate buffer. At pH values above 9, saligenin forms a blue indophenol dye with 2,6-dibromchinon-4-chlorimide which is read at 578 nm in a spectrophotometer Spectronic 20 Genesys (Spectronic Instruments, Inc, Rochester NY, USA). We calculated the saligenin content by referring to a calibration curve obtained with standards containing 0, 10, 20, 50 and 100 μg of saligenin. β-Glucosidase activity was expressed as the amount of saligenin released per gram dry matter and incubation time.

2.5. Statistical analyses

We analysed soil enzyme activities by using a factorial ANOVA with soil profile levels (A_1 = 0–15 cm, and A_2 = 15–30 cm) and drought treatments, C = Control, D = drought between strips (runoff exclusion), and DD = drought below strips (runoff exclusion plus partial rainfall exclusion) as factors. We compared the different levels of each experimental factor through Bonferroni/Dunn post-hoc ANOVA test. The statistica 5.0.1 package (SAS Institute, Inc., Berkeley, CA, USA 1998) was used for all statistical tests.

3. Results

3.1. Soil moisture and temperature

In drought treatment plots the soil located between the plastic strips (D) was on average 10% drier than the soil from control plots, whereas the soil below the plastic strips (DD) was on average 21.8% drier than the control plot soils

Table 1
Activities of five soil enzymes in spring and in autumn 2003 after 3 years of water elimination

		Soil depth (cm)	Treatments		
			Control	Runoff exclusion	Runoff exclusion plus partial rainfall exclusion
Soil T (°C) (at 10 cm)	Spring		12.99 ± 0.74^{a}	13.02 ± 0.80^{a}	12.37 ± 0.79^{a}
	Autumn		10.08 ± 0.60^{a}	11.35 ± 0.95^{a}	10.89 ± 0.45^{a}
Mean soil water content (% of the first 30 cm)	Spring		28.3 ± 1.2^{a} (27.9 ± 0.4^{a})	$25.8 \pm 1.2^{a,b}$ (25.4 ± 0.4^{b})	20.3 ± 3^{b} (21.7 ± 0.8^{c})
	Autumn		24.9 ± 0.8^{a} (23.8 ± 0.5^{a})	23.4 ± 0.9^{a} (20.7 ± 0.7^{b})	18.3 ± 1.9^{b} (18.6 ± 0.8^{b})
Acid phosphatase (μg p-NP g^{-1} soil h^{-1})	Spring	0–15	13.9 ± 0.7^{a}	13.7 ± 0.7^{a}	14.3 ± 0.8^{a}
		15–30	6.45 ± 0.62^{a}	6.42 ± 0.43^{a}	7.41 ± 0.61^{a}
	Autumn	0–15	7.54 ± 0.72^{a}	$6.30 \pm 0.43^{a,b}$	5.25 ± 0.48^{b}
		15–30	4.93 ± 0.50^{a}	4.37 ± 0.34^{a}	3.09 ± 0.42^{b}
Alkaline Phosphatase (μg p-NP g^{-1} soil h^{-1})	Spring	0–15	10.3 ± 1.4^{a}	11.8 ± 1.3^{a}	11.0 ± 1.2^{a}
		15–30	4.05 ± 0.73^{a}	3.66 ± 0.80^{a}	4.61 ± 0.61^{a}
	Autumn	0–15	4.09 ± 0.59^{a}	4.64 ± 0.58^{a}	4.28 ± 0.73^{a}
		15–30	3.15 ± 0.76^{a}	2.27 ± 0.50^{a}	2.37 ± 0.59^{a}
Urease (μg N g^{-1} soil h^{-1})	Spring	0–15	0.449 ± 0.022^{a}	0.417 ± 0.045^{a}	0.254 ± 0.035^{b}
		15–30	0.305 ± 0.011^{a}	0.129 ± 0.023^{b}	0.165 ± 0.018^{b}
	Autumn	0–15	0.218 ± 0.033^{a}	0.120 ± 0.012^{b}	0.095 ± 0.012^{b}
		15–30	0.112 ± 0.013^{a}	0.081 ± 0.009^{b}	0.052 ± 0.008^{c}
Protease μg tyrosine (g^{-1} soil h^{-1})	Spring	0–15	27.0 ± 3.1^{a}	23.4 ± 3.6^{ab}	18.5 ± 3.0^{c}
		15–30	6.9 ± 1.3^{a}	3.6 ± 1.2^{b}	3.1 ± 1.1^{c}
	Autumn	0–15	19.0 ± 3.0^{a}	14.8 ± 2.4^{a}	15.1 ± 3.7^{a}
		15–30	8.6 ± 1.99^{a}	2.9 ± 1.1^{b}	$4.2 \pm 2.1^{a,b}$
β-Glucosidase μg saligenin (g^{-1} soil h^{-1})	Spring	0–15	13.0 ± 1.57^{a}	11.7 ± 2.36^{a}	6.85 ± 1.04^{b}
		15–30	13.8 ± 1.35^{a}	2.57 ± 0.73^{b}	2.24 ± 1.79^{b}
	Autumn	0–15	4.66 ± 0.79^{a}	3.52 ± 0.42^{a}	4.05 ± 0.73^{a}
		15–30	2.84 ± 0.57^{a}	2.27 ± 0.38^{a}	1.86 ± 0.48^{a}

Soil acid and alkaline phosphatase activity (mg *p*-NP g^{-1} soil h^{-1}), soil urease activity (mg N g^{-1} soil h^{-1}), soil protease activity (mg tyrosine g^{-1} soil h^{-1}), and soil β-glucosidase activity (mg saligenin g^{-1} soil h^{-1}) in spring and in autumn in the upper A_1 0–15 cm layer and in the lower A_2 15–30 cm layer. (Different letters represent significant differences at $p < 0.05$ among drought treatments). The mean soil water contents that are shown in brackets are average values of current plus three previous years (1999–2003) of the corresponding seasons, and those shown without brackets are the corresponding values on the day of soil samplings.

(Table 1). The average soil temperatures in spring were 12.9 ± 0.4 °C and in autumn 10.7 ± 0.2 °C.

3.2. Acid and alkaline phosphatase activities

Acid and alkaline phosphatase activities were ca. twice as high in spring than in autumn (Table 1). In the spring sampling no effects of drought on acid phosphatase and on alkaline phosphatases were observed in the upper 0–15 cm. layer nor in the lower 15–30 cm. layer (Table 1). In autumn, the acid phosphatase activity was lower in the DD soil than in the control soils both in the 0–15 cm. layer (31%) and in the 15–30 cm layer (40%) (Table 1). The acid phosphatase activity in D soil samples was also higher than in the DD soil samples (39%) in the 15–30 cm layer (Table 1). In the autumn sampling, no significant effects of drought treatments were observed on soil alkaline phosphatase activity (Table 1). In spring and in autumn, significantly higher acid (Table 1) and alkaline (Table 1) phosphatase activities were observed in the 0–15 cm soil layer than in the 15–30 cm soil layer in all treatments (Table 1). No interactions between soil layers and treatments were observed in the ANOVA analysis.

3.3. Urease and protease activities

The urease and protease activities were also ca. twice as high in spring than in autumn (Table 1). In spring, the soil below strips (DD) presented lower urease activity than the control soil both in the 0–15 cm soil layer (42%), and in the 15–30 cm soil layer (45%) (Table 1). In spring, urease activity in the first 15 cm of soil layer was lower (36%) in the below strips soil samples (DD) than in the between strips soil samples (D) (Table 1). A statistically significant interaction ($p < 0.01$) between drought treatment and soil layer factors was detected in the factorial ANOVA test. This interaction was due to the higher negative effect of drought on soil urease activity at the 15–30 cm layer than at the 0–15 cm layer (Table 1). In autumn, control soils presented higher urease activity than the D and DD soil samples, both in the 0–15 cm layer and in the 15–30 cm layer (Table 1). In the 15–30 cm layer, the D soil samples had higher urease activity than the DD soil samples (Table 1). Urease activity

was always higher in the upper 0–15 cm layer than in the lower 15–30 cm layer and both in spring and in autumn soil samples in all treatments (Table 1).

In spring, the DD soils presented lower protease activity (35%) than the control soils in the 0–15 cm layer but this did not occur in the 15–30 cm layer (Table 1). In autumn, the D soils presented significantly lower protease activity (66%, $p<0.05$) than the Control soils in the 15–30 cm layer (Table 1). Lower protease activity was observed in the 15–30 cm layer than in the 0–15 cm layer in the D soils ($p<0.001$) and in a marginally significant way in the DD soils ($p=0.08$) and the control soils ($p=0.07$) (Table 1). No interactions between soil layers and treatments were observed in the ANOVA analysis.

3.4. β-Glucosidase activity

The β-glucosidase activities were ca. three times higher in spring than in autumn (Table 1). In spring, β-glucosidase activity significantly decreased in response to the drought treatments. In the 0–15 cm layer, β-glucosidase activity of DD soils was lower (47%) than in the control soils and also lower (36%) than in the D soils (Table 1). In the 15–30 cm layer, β-glucosidase activity of control soils was higher than in D soils (80%) and in DD soils (83%) (Table 1). In spring, a significant interaction ($p=0.004$) between soil layer and treatment effects was observed in the factorial ANOVA test: the lower soil β-glucosidase activity in the 15–30 cm layer than in the 0–15 cm layer was only observed in the drought treatments (D and DD) but not in the Control soils (Table 1). In autumn, no significant effects of drought treatments on soil β-glucosidase activity were observed (Table 1). The β-glucosidase activity of the 0–15 cm layer was higher than in the 15–30 cm layer in the D soils ($p<0.05$), in the DD soils ($p<0.05$), and in the control soils ($p=0.07$) (Table 1). No interactions were observed between soil layers and treatments in the ANOVA analysis.

4. Discussion

Soil enzyme activity showed a considerable sensitivity to slight decreases in water availability. An average of 10% of reduction in soil moisture produced by runoff exclusion, D soils, was sufficient to reduce markedly the activity of three of the five enzymes analyzed in the studied evergreen Mediterranean forest at least in some periods of the year, up to 67, 66 and 80% in urease, protease and β-glucosidase activities, respectively. An average of 21% of reduction in soil moisture produced by runoff and rainfall exclusion, DD soils, had stronger effects, reducing the activity of the three previously mentioned enzymes and moreover the activity of acid phosphatase. Our results thus show a decrease in soil enzyme activity with drought increase and are in agreement with those reported by Li and Sarah (2003) who reported decreased enzyme activities with increasing activity along a climatic transect in Israel. The reductions of the enzyme activity show the critical role of water for microbial enzyme activity in this Mediterranean soil. The decrease in the activity of soil enzymes involved in the recycling of phosphorus, nitrogen and carbon will affect, in the long term, soil nutrient availability, reducing the nutrient supply to plants. The greatest effect of our experimental drought on enzyme activity was observed in the enzymes involved in nitrogen cycling. This can be explained by the limiting role of N in the holm-oak forest of the Prades mountains reported in previous studies (Mayor et al., 1994). The limiting role of N would imply a higher sensitivity to drought.

The drought effect was higher for acid phosphatase than for alkaline phosphatase. In general, the acid phosphatase activity was higher than the alkaline phosphatase activity, this result being in agreement with the soil pH (between 6.5 and 7), i.e. closer to the optimal for acid phosphatase activity (6.5) than to the optimal for alkaline phosphatase activity (11). Our results are in agreement with many others reported in the literature, showing that phosphatase activity, both alkaline and acid, is well correlated with soil water availability (Kramer and Green, 2000). However, the activity of other enzymes such as urease has not always been reported as correlated with soil water availability (Sall and Chotte, 2002).

Although the drought treatment soils had more litter than the control soils (Ogaya and Peñuelas, 2004), the drought soil enzymes tended to reduce their activities, which is in contrast with other studies where the increase of organic matter enhanced soil enzyme activities (Zaman et al., 1999; Dodor and Tabatabai, 2003). In particular, phosphatase production and activity have been reported to be very sensitive to soil organic matter concentration (Goldstein et al., 1988). Phosphatases are involved in the transformation of organic and inorganic phosphorus compounds in soil (Amador et al., 1997), and their activities are an important factor in maintaining and controlling the rate of P cycling through soils. The observed tendency to a decrease of Phosphatase activities as a consequence of the slight drought is a negative trait in this Mediterranean context. A possible decrease of P available forms may affect negatively the water use efficiency aggravating by an indirect way the drought effects. On the other hand, the decrease of β-glucosidase activity by the slight drought may have negative effects on the activity capacity of the others enzymes since it is one of the enzymes that break down labile cellulose and other carbohydrate polymers. Furthermore their action is fundamental in order to liberate nutrients, to reduce the molecular size or organic structures and thus facilitates future microbe enzyme activities.

Enzyme activities can vary depending on the sampling date in zones with a seasonal climate (Watanabe and Hayano, 1995; Baum et al., 2003). In Mediterranean ecosystems, the highest activities occur in spring together with the most active growth of plants and microbial activity

(García et al., 1997, 2002). Autumn is the second most active season in activity in those Mediterranean areas. Similar patterns have been observed in other not Mediterranean forest. Thus, Dilly and Munch (1996) in a Germany Alder forest have reported a positive relation between water content in litter and β-glucosidase and protease activities, and an increase of protease activity in spring when soil moisture and temperature conditions are optimal. In contrast, when the winter is warm and the climate dry, the enzyme activity is ususally higher in autumn–winter period, intermediate in spring and lowest in summer (Fioretto et al., 2000, 2001), showing once more that climatic conditions determine the phenology of soil enzyme activities. Our results show a significant activity of the five enzymes analysed in both spring and in autumn, but in agreement with those previous studies, the highest activity of all enzymes occurred in spring when, in addition to optimal temperatures and water availability there was also a higher quantity of litter in the holm oak forest (Ogaya and Peñuelas, 2004).

Phosphatases activities obtained in this study were higher in the first 15 cm layer of soil profile than the values reported by other authors, but this was not the case for the 15–30 cm layer (Turner et al., 2002; Baum et al., 2003). The activity of the five enzymes analysed also decreased with soil depth, confirming a result that has been widely observed (Chen, 2003). The asymmetrical inputs of litter through soil profile due to the aboveground litter incorporation on the soil surface together with a better soil aeration account for the higher soil enzyme activity in the 0–15 cm than in the lower 15–30 cm. Decreases in soil enzyme activities through soil profile have been observed in forest and agriculture soils when there are organic inputs are in the soil surface (Aon et al., 2001; Taylor et al., 2002; Zaman et al., 2002; Chen, 2003).

Reduction in soil enzyme activities observed in this study is in agreement with the decrease in P-available forms in the drought treatment soils that had been observed in this forest in a previous study (Sardans and Peñuelas, 2004). The experiment results, although dealing with potential transformation rates rather than in situ liberation rates, point to an indirect effect of drought on community productivity capacity in Mediterranean forests through a decreased soil enzyme activity and thus a reduced potential transformation rate of organic matter. This effect can be a key factor in the possible degradation process that drought can have on Mediterranean plant communities. In the Mediterranean areas of the Iberian Peninsula, plant decline is well correlated with soil enzyme activity decline (García et al., 2002).

In conclusion, aside from reduced water availability, drought may have a negative effect on soil enzyme activities and therefore on nutrient availability in Mediterranean ecosystems. Therefore, the decrease in water availability currently occurring (Piñol et al., 1998; Peñuelas et al., 2002) and expected to be further magnified in the immediate future in the Mediterranean forests (Sabaté et al., 2002; Peñuelas et al., 2004) can have other negative indirect effects on plant growth apart from the direct ones of the reduction in water availability and uptake.

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