Original article

Field-simulated droughts affect elemental leaf stoichiometry in Mediterranean forests and shrublands

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Abstract

This study evaluated the change induced by the year season and by experimentally induced drought on foliar element stoichiometry of the predominant woody species (Quercus ilex and Erica multiflora) in two Mediterranean ecosystems, a forest and a shrubland. This study is based in two long-term (11 yr) field experiments that simulated drought throughout the annual cycle.

The effects of experimental droughts were significant but weaker than the changes produced by ontogeny and seasonality. Leaf N and P concentrations were higher in spring (the main growing season) in E. multiflora and, in Q. ilex in autumn (a period of additional growth). Leaf N:P ratios were lower in spring. In Q. ilex, the highest leaf K concentrations and leaf K:P ratios, and the lowest leaf C:K and N:K ratios, occurred in summer, the season when water stress was greatest. In E. multiflora, leaf K concentrations and K:P ratios were highest, and leaf C:K and N:K ratios were lowest in the plants from the drought-treated plots.

The plant capacity to change K concentrations in response to seasonality and to drought is at least as great as the capacity to change N and P concentrations. The results underscore the importance of K and its stoichiometry relative to C, N and P in dry environments. These results indicate first, that N:P ratio shifts are not uniquely related to growth rate in Mediterranean plants but also to drought, and second, that there is a need to take into account K in ecological stoichiometry studies of terrestrial plants.

1. Introduction

The C:N:P ratios in organisms have been proven to be associated with important ecological processes, such as litter decomposition (D’Annunzio et al., 2008; Gusewelle and Gessner, 2009), plant-herbivore-predator interactions (Ngai and Jefferies, 2004; Kagata and Ohgushi, 2006), N2 fixation (Sañudo-Wilhelmy et al., 2001), responses to environmental stress (Woods et al., 2003; Sardans and Peñuelas, 2007a; Sardans et al., 2008a), and ecosystem composition and diversity (Roem and Berendse, 2000; Gusewelle et al., 2005). Experiments have shown that soils litter and humus, water and an organism’s C:N ratios are correlated with important ecological processes and ecosystem traits, which have raised stoichiometry to occupy a fundamental central role in ecological research (Elser et al., 1996, 2000; Sterner and Elser, 2002; Raubenheimer and Simpson, 2004; Sardans et al., 2012a,b).

In Mediterranean ecosystems, water is the primary limiting factor, and climate and ecophysiological models have predicted increases in temperature and drought in these ecosystems (IPCC, 2007; Sabaté et al., 2002; Peñuelas et al., 2005). In plants, drought can increase C:N and C:P ratios (Sardans et al., 2008a,b), which is associated with sclerophyllly (Buissotti et al., 2000; Sardans et al., 2006a). However, few studies have examined the long-term effects of drought on ecosystem N and P cycles and N and P status of Mediterranean plants and the results have been inconclusive (Sardans et al., 2008a,b; Matías et al., 2010; Sardans et al., 2012a,b), although negative relationships between water availability and foliar N:P ratio have been evidenced in Mediterranean forest along precipitation gradients (Sardans et al., 2011, Sardans and Peñuelas, 2013). The few available studies suggest that in the medium-term drought reduces enzyme activity and nutrient availability in soils (Sardans and Peñuelas, 2005a; Sardans et al., 2008c,d) and reduces the N and P concentrations in stand biomass (Sardans and Peñuelas, 2004a; Sardans et al., 2008a,b). Low nutrient availability can reduce...
water use efficiency (WUE) (Payne et al., 1995; Harvey and Van den Driessche, 1999; Ruiz-Lozano et al., 2001; Mohammad and Zuraigi, 2003; Hobbie and Colpaert, 2004) and, ultimately, plant fitness because it reduces the capacity to adapt to droughts. One of the most widely examined paradigms in ecological stoichiometry is the Growth Rate Hypothesis (GRH), which posits that, to meet the elevated demands for protein synthesis required for rapid growth, organisms must increase the relative allocation of P to P-rich ribosomal RNA. Thus, greater allocation to P-rich ribosomal RNA is possible under low N:P ratios in the environment, which promotes high growth rates (Main et al., 1997; Sterner and Elser, 2002). Through its links with organism’s growth rate, C:N:P stoichiometry of organisms has also a relationship with ecosystem’s structure and functions because it favors species that have different growth rate (Elser et al., 2000; Sardans et al., 2012b). The GRH appears to be widely applicable in freshwater ecosystems (Elser and George, 1993; Elser and Urabe, 1999; Elser et al., 2000; Hessen et al., 2007) but, although some studies have identified relationships between low organism N:P stoichiometry and high growth rates in terrestrial plants (Niklas et al., 2005; Elser et al., 2003; Zhang and Han, 2010), the general applicability of the GRH in terrestrial ecosystems is uncertain (Matzek and Vitousek, 2009; Sardans et al., 2012a). In terrestrial ecosystems N:P stoichiometry could be determined by water availability, which is frequently limiting. Drought could impact differently N and P cycle. Under drought, the enhanced carbon allocation to C-rich compounds such as phenolics and tannins (Peñuelas and Estiarte, 1998) should reduce growth rate capacity and increase leaf N:P ratio. Recently, Rivas-Ubach et al. (2012) have observed a decrease in plant primary metabolism associated to an increase in leaf N:P ratio and to a secondary antistress metabolism increase during drought season. Moreover, the changes of N:P ratio throughout year in Mediterranean ecosystems, with a strong seasonal contrast of water availability and with seasons with growth and other seasons without growth can provide some clues of the links between N:P ratio and drought in terrestrial plants. In addition to those of C, N, and P, the biomass concentrations of other elements such as K and trace elements can vary in response to drought in ways that have a positive effect on a plant’s resistance to the projected climate change. The tissue concentrations of K are important in plant biology (Paoli et al., 2005), particularly, in controlling leaf water loss (Babita et al., 2010) through its positive effects on stomatal function (Khosravifar et al., 2008) and the control of osmosis (Babita et al., 2010; Laus et al., 2011). Given its role in the water retention, K is especially important in dry environments. K can have a significant positive effect on plant drought resistance both in natural ecosystems (Egilla et al., 2005) and in crops (Stone and Moreira, 1996). In Mediterranean plants, the use and remobilization of K is associated with the requirements of osmosis control (Milla et al., 2005). In Mediterranean pine Pinus pinaster in natural ecosystems in the Iberian Peninsula, a reduction in the availability of K led to an increase in stomatal conductance and, consequently, a reduction in Water Use Efficiency (WUE) (Fernández et al., 2006). During the early stages of tree establishment in a young silvopastoral system in Greece, Gakis et al. (2004) observed a significant positive correlation between tree growth and leaf K concentrations. Rivas-Ubach et al. (2012) reported increases in leaf K concentrations of Erica multiflora in summer (the driest period) related to metabolic balance shifts toward increasing concentrations of metabolites linked to osmosis control. Metadata analysis of forest Catalan inventory has provided evidence that Mediterranean forests have higher leaf K concentrations in summer coinciding with drought period (Sardans et al., 2012a,b,c). The changes in plant concentrations of K and in its ratios with N and P in response to drought merits further research to reach a better knowledge of terrestrial plant stoichiometry relationships with environmental changes. In recent decades, increases in the number and intensity of anthropogenic pollution, e.g., rubbish tips, smelters, fertilizers, pesticides, waste incineration, mining, electro-industry, vehicle exhaust, and sewage, have increased the environmental levels of some trace elements (Peñuelas and Filella, 2002; Autier and White, 2004; Sardans and Peñuelas, 2005b). Frequently, human related factors are the cause of Cd, Cu, Pb, and Zn pollution in natural ecosystems (Wilcke et al., 1998). Those anthropic activities have greatly increased in recent decades, particularly, in urban and adjacent areas, which have led to increases in the concentrations of trace elements in some regions of the Mediterranean Basin (Peñuelas and Filella, 2002; Sardans and Peñuelas, 2005b, 2006; Achotegui-Castells et al., 2013). Drought affects the release of soluble trace elements into the soil solution via the lysis of bacterial cells and the destruction of soil aggregates during drying-rewetting events (Turner and Haygarth, 2001). In addition, drought can affect plant metabolism and the internal distribution of elements, which alters the mobilization and translocation of elements within plant organs (Gavito et al., 2005; Jonsdottir et al., 2005). Furthermore, most trace elements tend to form stable complexes with biomolecules, which persist in plant tissues and trophic webs. Drought can change the biomass concentrations of some trace elements (Sardans and Peñuelas, 2007b; Sardans et al., 2008e), but experiments are needed to identify the medium- and long-term effects of drought on trace elements contents in plants to take a general overview of drought impacts on plant elemental composition. The most representative natural plant communities in European Mediterranean areas are forest and shrublands both dominated by sclerophyllous species. Generally, shrublands are present in drier areas or in areas where forest should be dominant but that suffered a disturbance. The differences in water requirements could imply different responses of these two communities to a moderate drought. In two field experiments; one in a Mediterranean forest and the other in a Mediterranean shrubland, that simulated drought conditions in Catalonia, NE Spain, we observed at medium-term (5 yr) that drought tended to alter the leaf concentrations of C, N, P, Mg, Fe, Na, and Mo in both experiments (Sardans and Peñuelas, 2007a; Sardans et al., 2008a,fg). In those studies, leaves were sampled once per year, only; however, the Mediterranean climate varies seasonally, with warm-dry summers and moderately wet springs and autumns. In this study, we assess the medium-long term (11 years) effects of simulated drought conditions on the concentrations and stoichiometry of the nutrients, micronutrients, and trace elements in two dominant woody species, one from Mediterranean forest, Quercus ilex, and the other from Mediterranean shrublands, E. multiflora, throughout the year seasons. We have aimed to investigate the possible similarities in the stoichiometry response to long term drought between two species growing in soils with different nutrient availabilities and also to compare the changes in stoichiometry produced by drought treatment with those produced by natural summer drought. Since few studies have examined the effects of drought on K concentrations and particularly the stoichiometry between K and other important nutrients such as N and P, in this study we have also aimed to include K in the research of C:N:P:K stoichiometry shifts of Mediterranean plants in response to seasonality and drought.

2. Materials and methods

2.1. Study sites

The study sites were two contrasting Mediterranean ecosystems in Catalonia, NE Spain: one side is on a south-facing slope (25%) in a natural Quercus ilex oak forest in Prades Mountains Natural Park.
(41°21′ N, 1°2′ E) and a shrubland in Garraf Mountains Natural Park. In Prades Mountains NP, the soil is a stony Dystric Cambisol (Soil Survey Staff, 1998) that lies on a bedrock of metamorphic sandstone. The soil is 35–100 cm deep and horizon A lies between 25 and 30 cm. The average annual temperature is 12 °C and the average rainfall is 658 mm. The summer drought is pronounced and usually lasts 3 months. The vegetation is a dense forest (8–10 m high) dominated by Quercus ilex L. (20.8 m² ha⁻¹ of trunk basal area at a height of 50 cm), and accompanied by an abundance of Phillyrea latifolia (7.7 m² ha⁻¹ of trunk basal area at 50 cm of height) and Arbustus unedo L. For details, see Ogaya and Peñuelas (2004, 2007). In Prades plots the total aboveground biomass was 155 Mg ha⁻¹ and Q. ilex represented 89 Mg ha⁻¹. The maximum Q. ilex canopy height was 10 m.

The other side was placed in Garraf Natural Park, the experiment was performed in a natural calcareous Mediterranean shrubland on a south-facing slope in the Garraf Mountains in the central-coastal part of Catalonia (NE Spain) (41°18′ N, 1°49′ E). The site is on formerly cultivated terraces (abandoned about a century ago) that have a Petrocalcic calcixerupt (Soil Survey Staff, 1998) soil underlain by a bedrock of sedimentary limestone. During the study period (1999–2007), the average annual temperature was 15.1 °C and the average annual rainfall was 580 mm Typically, the pronounced summer drought lasts for three months. The total vegetation cover was 75% and consisted of a calcareous shrubland (plants ~ 1 m high) dominated by the shrubs E. multiflora, Globularia alypum, Dorycnium pentaphyllum Scop., Rosmarinus officinalis L., Ulex parviflorus Pourr., and Pistacia lentiscus L., E. multiflora covered ~20% of the area (see Peñuelas et al., 2007).

2.2. Experimental design

The experiment in Prades consisted of eight 15 × 10 m plots that were established from March 1999 to now on a slope at 930 m a.s.l., and the minimum distance between plots was 15 m. To avoid the edge effect the outer 1 m of each plot was excluded of sampling. The drought treatment was randomly assigned to four of the plots and four plots were left untreated. The drought treatment involved the partial exclusion of rainfall by suspending transparent PVC plastic curtains when it rained. The average soil water content was reduced by 17% in the drought plots (see Beier et al., 2004; Peñuelas et al., 2004, 2007). Since 1999, the drought treatment has been applied in spring and autumn by automatically covering the plots with plastic curtains when it rained. The average soil water content was reduced by 17% in the drought plots (see Beier et al., 2004; Llorens et al., 2004; Peñuelas et al., 2004, 2007). Soil moisture was measured following the Time Domain Reflectometry method (TDR). This method consists in the measurement of electric conductivity through the soil, which depends largely on soil water content and the influence of other factors are very low and negligible (Zegelin et al., 1989). Measurements have been conducted since 1999 twice each month.

2.3. Plant sampling

In each plot in the two study sites, either five Q. ilex or five E. multiflora plants were chosen randomly as subject of the study. Once per season; May-2009 (spring), August-2009 (summer), November-2009 (autumn), and February-2010 (winter), several branches were collected from each of the marked plants. Thus, leaves of 40 trees and 30 shrubs were included in the analyses in each season. We sampled the youngest cohorts. The youngest leaves were the spring leaves and the oldest were winter leaves. The sampled leaves were sun-leaves and oriented to south. In Q. ilex leaves were sampled at 2–3 m of height (sufficient to collect sun leaves since the slope allows that the canopy exposed to south receive sunlight during several hours) and in E. multiflora leaves were sampled in the top crown position. Leaves were dry frozen and ground with a ball-mill grinder.

2.4. Chemical analyses of leaves

The leaf concentrations of the macro- and microelements (P, K, Mg, Ca, Fe, Na, Mo) and the trace elements (As, Cd, Cr, Cu, Ni, Mn, Pb, V) in leaves were quantified after the samples were exposed to an acid digestion in a MARSXpress microwave (CEM, Mattheus, USA) at high pressure and temperature (see Peñuelas et al., 2010). Briefly, 250 mg of leaf powder, 5 mL of nitric acid, and 2 mL of H₂O₂ were placed into Teflon tubes. The products of the digestions were placed in 50-ml flasks and diluted using Milli-Q water to a volume of 50 mL. The concentrations of As, Cd, Cu, Cr, Ni, Mo, Pb, and Zn in the dilutions were determined using ICP-MS (Mass Spectrometry with Inductively Coupled Plasma), and the concentrations of P, Ca, K, Mg, Mn, Fe were determined using ICP-OES (Optic Emission Spectrometry with Inductively Coupled Plasma). To assess the accuracy of the digestions and the analytical biomass procedures, we used a certified standard biomass (NIST 1573a, tomato leaf, NIST, Gaithersburg, MD) as a reference.

To quantify the concentrations of C and N, 1.4 mg of leaf powder was placed in a tin microcapsule and subjected to elemental analysis using combustion coupled to gas chromatography in a CHNS-O Elemental Analyser (EuroVector, Milan, Italy).

2.5. Data analyses

To study the seasonality effects we analyzed the differences of control plots among different seasons by a general linear model using seasons as independent fixed factor and plots as independent random factor by using IBM SPSS 19.0 (IBM Corporation, Armonk, NY, USA). To analyze the treatment effects we conducted firstly repeated measures ANOVA with climatic treatments as independent variables and the results of each variable in different seasons in each plot as repeated dependent variable. Thereafter we conducted one-way ANOVAs with the climatic treatments as independent variables and the concentration and concentration ratio values in each season as dependent variables. When there were not significant interaction effects between seasons and treatment, we assessed the effects of simulated drought within each season by comparing the leaf concentrations and stoichiometry of plants in the control plots and the drought-treated plots within each season. Bonferroni Tests were used for post-hoc analyses. To detect patterns of sample ordination with respect to element concentrations and ratios on season and simulated drought conditions, the data were subjected to Principal Component Analyses (PCA). We conducted PCA analyses using all leaf elemental concentrations and nutrient ratios and, thereafter, selected those that had the highest loadings on the main axes. To detect differences among PC axes among leaves of different seasons or between leaves of control and drought plots we conducted one-way ANOVAs of the scores. The repeated-measures and one-way ANOVAs, post-hoc and PCAs tests were performed using Statistica v8.0 (Statsoft, Tulsa, USA).
3. Results

3.1. Soil moisture during the experiment

In Prades experiment in the Mediterranean Holm oak forest, drought plots had lower soil water content during the whole year but the differences were significant in spring, autumn and winter (13%, 11% and 8% decreases, respectively), the raining seasons (Fig. 1). These values are in the range of drought effects observed on average since the beginning of the experiment, and the precipitations were also similar than those of the average of previous years (Ogaya et al., 2011). In Garraf experiment in the Mediterranean shrubland, drought plots had lower soil water content during the whole year but the differences were significant in spring and autumn (20% and 26% decreases, respectively), the raining seasons (Fig. 1). These data are also similar to the average of previous years (Sardans et al., 2008a).

3.2. Leaf elemental concentrations

3.2.1. *Quercus ilex*

Leaf N and P concentrations were highest in autumn, but K concentration was highest in summer (Fig. 2). Consequently, leaf C:N and C:P ratios were lowest in autumn, leaf C:K and N:K ratios were lowest in summer, and leaf K:P ratio was highest in summer (Table 1). In the control plots, the leaf N:P ratio was lowest in spring, autumn and winter (Fig. 3).

Simulated drought conditions increased leaf C and P concentrations and decreased leaf C:P and N:P ratios throughout the year, as observed in repeated-measure ANOVA in different seasons (Table 2). Leaf P concentrations were higher in autumn than in summer, and winter (Fig. 2). Drought reduced Na concentrations in autumn and Pb concentrations in spring, autumn and winter (Table 1). Drought increased leaf C:N ratio in autumn, and decreased leaf C:P ratio in summer and winter, and leaf N:P ratio in

![Fig. 1. Soil water content in the year seasons in control (white) and in drought (black) plots (Mean ± S.E. of the period 1999–2010) and both in Prades and in Garraf study sites. (*) indicate significant (P < 0.05) differences between treatments.](#)

![Fig. 2. Leaf elemental concentrations of C, N, P, K, and Mg throughout the year in *Quercus ilex* trees in Prades Mountains, Spain. Different letters indicate significant (P < 0.05) differences among seasons. An asterisk indicates significant (P < 0.05) differences between control and drought-treated plots within a season. (n = 4 plots).](#)
In drought-treated plots, the leaf N:P ratio was very low and did not vary significantly between control and simulated drought treatments within each season. Different letters indicate significant differences \((P < 0.05)\) between seasons \((n = 4\) plots). summer, autumn, and winter (Fig. 3, Table 1). In the drought-treated plots, the leaf N:P ratio was very low and did not vary significantly throughout the year (Fig. 3).

3.2.2. *E. multiflora*

Foliar N, P, and K concentrations were highest, and C and Fe concentrations were lowest in spring (Fig. 4, Table 3). Among the trace elements, the leaf concentrations of Pb, Cd, As, and V were highest in summer, but Cu concentrations were highest in spring (Table 3). Leaf C:N, C:P, N:P, and K:P ratios were highest in summer, whereas C:N, C:P, N:P, and K:P ratios were lowest in spring followed by autumn (Fig. 3, Table 3). Simulated drought conditions reduced leaf Fe concentrations and increased marginally \((P = 0.068)\) leaf K concentrations throughout the year, as observed

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<table>
<thead>
<tr>
<th>Element</th>
<th>Treatment</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe ((\text{mg g}^{-1}))</td>
<td>Control</td>
<td>0.14 (0.01)</td>
<td>0.18 (0.02)</td>
<td>0.19 (0.02)</td>
<td>0.15 (0.02)</td>
</tr>
<tr>
<td>Drought</td>
<td>0.17 (0.02)</td>
<td>0.18 (0.02)</td>
<td>0.17 (0.02)</td>
<td>0.15 (0.02)</td>
<td></td>
</tr>
<tr>
<td>Mn ((\text{mg g}^{-1}))</td>
<td>Control</td>
<td>0.53 (0.17)</td>
<td>1.32 (0.14)</td>
<td>1.38 (0.16)</td>
<td>1.40 (0.18)</td>
</tr>
<tr>
<td>Drought</td>
<td>1.28 (0.20)</td>
<td>1.16 (0.16)</td>
<td>1.18 (0.16)</td>
<td>1.22 (0.16)</td>
<td></td>
</tr>
<tr>
<td>Na ((\text{mg g}^{-1}))</td>
<td>Control</td>
<td>0.02 (0.02)</td>
<td>0.07 (0.02)</td>
<td>0.07 (0.03)</td>
<td>0.06 (0.03)</td>
</tr>
<tr>
<td>Drought</td>
<td>0.05 (0.03)</td>
<td>0.10 (0.02)</td>
<td>0.10 (0.02)</td>
<td>0.15 (0.15)</td>
<td></td>
</tr>
<tr>
<td>Cu ((\mu \text{g g}^{-1}))</td>
<td>Control</td>
<td>4.00 (0.11)</td>
<td>3.76 (0.13)</td>
<td>4.16 (0.12)</td>
<td>3.97 (0.10)</td>
</tr>
<tr>
<td>Drought</td>
<td>3.92 (0.10)</td>
<td>4.60 (0.55)</td>
<td>4.05 (0.12)</td>
<td>3.89 (0.09)</td>
<td></td>
</tr>
<tr>
<td>Zn ((\mu \text{g g}^{-1}))</td>
<td>Control</td>
<td>24.4 (1.4)</td>
<td>24.9 (1.2)</td>
<td>25.0 (1.7)</td>
<td>23.6 (1.5)</td>
</tr>
<tr>
<td>Drought</td>
<td>21.5 (1.7)</td>
<td>22.8 (1.5)</td>
<td>23.3 (1.9)</td>
<td>22.2 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Pb ((\mu \text{g g}^{-1}))</td>
<td>Control</td>
<td>0.55 (0.06)</td>
<td>0.49 (0.03)</td>
<td>0.48 (0.04)</td>
<td>0.46 (0.04)</td>
</tr>
<tr>
<td>Drought</td>
<td>0.40 (0.03)</td>
<td>0.50 (0.13)</td>
<td>0.39 (0.02)</td>
<td>0.36 (0.03)</td>
<td></td>
</tr>
<tr>
<td>Cd ((\mu \text{g g}^{-1}))</td>
<td>Control</td>
<td>0.19 (0.03)</td>
<td>0.19 (0.02)</td>
<td>0.19 (0.02)</td>
<td>0.15 (0.01)</td>
</tr>
<tr>
<td>Drought</td>
<td>0.14 (0.01)</td>
<td>0.17 (0.14)</td>
<td>0.14 (0.01)</td>
<td>0.14 (0.01)</td>
<td></td>
</tr>
<tr>
<td>Mo ((\mu \text{g g}^{-1}))</td>
<td>Control</td>
<td>0.04 (0.01)</td>
<td>0.04 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.01)</td>
</tr>
<tr>
<td>Drought</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.04 (0.01)</td>
<td>0.04 (0.01)</td>
<td></td>
</tr>
<tr>
<td>Cr ((\mu \text{g g}^{-1}))</td>
<td>Control</td>
<td>0.91 (0.03)</td>
<td>0.96 (0.05)</td>
<td>0.99 (0.06)</td>
<td>0.99 (0.06)</td>
</tr>
<tr>
<td>Drought</td>
<td>1.05 (0.11)</td>
<td>0.97 (0.06)</td>
<td>0.99 (0.05)</td>
<td>1.07 (0.10)</td>
<td></td>
</tr>
<tr>
<td>As ((\mu \text{g g}^{-1}))</td>
<td>Control</td>
<td>0.05 (0.01)</td>
<td>0.05 (0.01)</td>
<td>0.05 (0.01)</td>
<td>0.05 (0.01)</td>
</tr>
<tr>
<td>Drought</td>
<td>0.05 (0.01)</td>
<td>0.04 (0.01)</td>
<td>0.05 (0.01)</td>
<td>0.05 (0.01)</td>
<td></td>
</tr>
<tr>
<td>V ((\mu \text{g g}^{-1}))</td>
<td>Control</td>
<td>0.38 (0.05)</td>
<td>0.40 (0.05)</td>
<td>0.37 (0.05)</td>
<td>0.35 (0.05)</td>
</tr>
<tr>
<td>Drought</td>
<td>0.39 (0.05)</td>
<td>0.37 (0.04)</td>
<td>0.35 (0.04)</td>
<td>0.32 (0.04)</td>
<td></td>
</tr>
<tr>
<td>Ni ((\mu \text{g g}^{-1}))</td>
<td>Control</td>
<td>2.05 (0.29)</td>
<td>2.32 (0.19)</td>
<td>2.45 (0.34)</td>
<td>2.30 (0.25)</td>
</tr>
<tr>
<td>Drought</td>
<td>0.219 (0.35)</td>
<td>2.71 (0.35)</td>
<td>2.41 (0.40)</td>
<td>2.47 (0.37)</td>
<td></td>
</tr>
<tr>
<td>C:N</td>
<td>Control</td>
<td>41.1a (0.9)</td>
<td>40.5a (0.9)</td>
<td>36.4b (0.7)</td>
<td>40.4a (0.8)</td>
</tr>
<tr>
<td>Drought</td>
<td>40.8 (1.1)</td>
<td>40.7 (1.1)</td>
<td>39.0 (1.1)</td>
<td>40.1 (1.1)</td>
<td></td>
</tr>
<tr>
<td>C:P</td>
<td>Control</td>
<td>554bc (12)</td>
<td>599ab (18)</td>
<td>516c (18)</td>
<td>604a (13)</td>
</tr>
<tr>
<td>Drought</td>
<td>512 (25)</td>
<td>532 (25)</td>
<td>492 (21)</td>
<td>510 (17)</td>
<td></td>
</tr>
<tr>
<td>N:P</td>
<td>Control</td>
<td>13.6b (4.4)</td>
<td>17.6a (5.2)</td>
<td>14.2b (5.1)</td>
<td>15.0b (4.5)</td>
</tr>
<tr>
<td>Drought</td>
<td>12.5 (5.5)</td>
<td>13.1 (5.3)</td>
<td>12.7 (5.5)</td>
<td>12.8 (5.4)</td>
<td></td>
</tr>
<tr>
<td>C:K</td>
<td>Control</td>
<td>108a (6)</td>
<td>76.7b (4.4)</td>
<td>87.0b (5.3)</td>
<td>119a (7)</td>
</tr>
<tr>
<td>Drought</td>
<td>119 (6)</td>
<td>81.4 (4.2)</td>
<td>98.1 (7.8)</td>
<td>129 (8)</td>
<td></td>
</tr>
<tr>
<td>K:P</td>
<td>Control</td>
<td>5.4b (0.3)</td>
<td>8.3a (0.5)</td>
<td>6.4b (0.5)</td>
<td>5.9b (0.4)</td>
</tr>
<tr>
<td>Drought</td>
<td>4.6 (0.3)</td>
<td>7.0 (0.6)</td>
<td>5.6 (0.5)</td>
<td>4.3 (0.3)</td>
<td></td>
</tr>
<tr>
<td>N:K</td>
<td>Control</td>
<td>2.6ab (0.1)</td>
<td>1.9c (0.1)</td>
<td>2.4b (0.1)</td>
<td>3.0a (0.2)</td>
</tr>
<tr>
<td>Drought</td>
<td>3.0 (0.2)</td>
<td>2.1 (0.1)</td>
<td>2.6 (0.2)</td>
<td>3.3 (0.2)</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 3.** Leaf N:P ratios throughout the year in *Quercus ilex* and *Erica multiflora*. Different letters indicate significant \((P < 0.05)\) differences among seasons. An asterisk indicates significant \((P < 0.05)\) differences between control and drought-treated plots within a season.
3.3. Overall effects of season and drought on leaf nutrient concentrations

3.3.1. Q. ilex

Using leaf N, P, Mg, and K concentrations and leaf C:N, C:P, C:K, K:P, N:P, and N:K ratios as variables, the PCA distinguished between the leaves collected in summer and those collected in spring \((P = 0.0001)\), and winter \((P = 0.0001)\) on the PC1 axis which explained 42.7% of the overall variance. Leaf K concentrations and the ratios between K and C, N, and P were the most important factors (highest loadings) in PC1, and the leaves collected in summer had the highest K concentrations (Fig. 5).

In the PCA of the samples of each season, individually, the Q. ilex leaves from the control and drought-treated plots were distinguished in all seasons along the PC1 axis \((P = 0.03, P = 0.04<0.00001, \text{and } P = 0.03 \text{ in spring, summer, autumn and winter, respectively})\), which was most strongly influenced by leaf P concentrations and N:P ratios in spring, summer and winter, with highest P concentrations in the leaves of the plants from the drought-treated plots (Fig. 6). The PC1 distinguished between the control and drought-treated plots in autumn, primarily, because of the higher leaf C:N ratio and the lower leaf N concentrations and leaf N:P ratios in the leaves of the plants subjected to drought conditions (Fig. 6). The PC3 distinguished between the leaves collected from the control and the drought-treated plots, both in autumn \((P = 0.02)\) and in winter \((P = 0.04)\), largely because of the higher leaf C and lower leaf Pb concentrations in the leaves from the plants in the drought-treated plots (Fig. 6).

3.3.2. E. multiflora

With leaf C, N, P, K, Cu, Cd, As, V, Pb, and Fe concentrations and leaf C:N, C:P, C:K, K:P, N:P, and N:K content ratios as the variables, the PC1 axis (which explained 46.9% of the overall variance) separated E. multiflora leaves collected in spring from autumn, winter, and summer \((P = 0.0001, \text{in all three cases})\). In addition, the PC1 axis distinguished between summer and both autumn \((P = 0.01)\) and winter \((P = 0.02)\) (Fig. 5). The PC1 was most strongly influenced by leaf N and P concentrations and leaf C:P and N:P ratios. The highest N and P concentrations and lowest N:P ratios occurred in the leaves collected in spring, and the lowest N and P concentrations and the highest N:P ratios occurred in summer (Fig. 5). Moreover, along the PC2 axis (which explained 17.9% of the overall variance) leaves collected in summer were separated from...
those in autumn (P = 0.008), winter (P < 0.0001), and spring (P = 0.009). The highest K:P concentration ratio and the lowest C:K and N:P ratios occurred in the leaves collected in summer (Fig. 5).

In the PCA of the samples of each season, individually, the E. multiflora leaves from the control and drought-treated plots were distinguished in all seasons along the PC1 (P < 0.0001; P = 0.0002, and P = 0.003, in spring, summer, autumn, and winter, respectively). In summer, the PCA was most strongly influenced by leaf K concentrations with the leaves from the plants in the drought-treated plots tended to have the highest K concentrations (Fig. 7). In autumn and winter, the PCA was most strongly influenced by leaf N and P concentrations and the C:N and C:P ratios, and the leaves from the plants in the drought-treated plots tended to have the highest C:N and C:P ratios and the lowest N and P concentrations (Fig. 7). In spring, the leaves from the drought-treated plots were distinguished from the leaves of the plants in the control plots (P = 0.03), primarily, because of the high concentrations of Mg, As, and V (Fig. 7).

4. Discussion

4.1. Seasonality and drought

We have observed significant changes in leaf nutrient concentrations at the study sites in an oak forest in Prades Mountains, Catalonia, N.E. Spain, due both to seasonality and experimentally induced drought simulating the projected climate for the future decades (Sabal et al. 2002; IPCC 2007). For example, in Q. ilex, the largest difference in leaf K concentrations among the seasons was 40%, and the largest difference between treated and untreated plots within a season was 10% (Fig. 2). In E. multiflora, the range of leaf K concentrations among seasons was 66% and the range of differences among control and drought plots was 25% (Fig. 4). The seasonally and experimentally induced ranges of variation were similar for most other elements.

In Q. ilex, but not in E. multiflora, leaf K concentrations were highest in summer. In Q. ilex, the highest K concentrations and K:P ratios, and the lowest leaf C:K and N:P ratios occurred in summer, the drought period. Conversely, in E. multiflora, the highest leaf K concentrations occurred in spring, which coincided with the highest concentrations of N and P and the growing season. In the PCA axis, the E. multiflora leaves collected in spring were distinguished from those collected in the other seasons by their higher leaf K concentrations from the control plots and those from the drought-treated plots (Fig. 7). Moreover, in E. multiflora, along the PC2 axis the leaves collected in summer have the highest proportion of K respect to C, N and P (Fig. 5). Those results could be related...
Fig. 5. Principal component analyses with leaf element concentrations and ratios as variables and leaves of control plots sampled in different seasons as cases. The significant differences ($P < 0.05$) among scores of the different seasons are depicted along the PC axes.

Fig. 6. Principal component analyses with leaf element concentrations and ratios as variables and leaves of different climate plots sampled in different seasons as cases in Quercus ilex. The significant differences ($P < 0.05$) of the PC scores of the different treatments are depicted along the PC axes.
to a water conservation strategy by the role of K in stomatal functioning. Thus, the overall results underscore further the importance of leaf K concentrations and its ratios with the other main nutrients in the responses of Mediterranean plants to summer drought (see also, Sardans and Peñuelas, 2007a; Sardans et al., 2008h).

Simulated drought increased the concentrations of P in Q. ilex leaves in all seasons and reduced leaf N concentrations in autumn, whereas in the shrubland of the Garraf Mountains, E. multiflora experienced optimum conditions for growth in spring, when the N, P, and K concentrations were highest and the leaf N:P ratio was the lowest. The elevated P concentrations in Q. ilex leaves that grew under simulated drought conditions probably were the result of a concentration effect, which coincided with a reduction in growth (Ogaya et al., 2003; Ogaya and Peñuelas, 2007).

In both woody species, the lowest leaf N:P ratios occurred in spring and autumn (Fig. 3), the two growing seasons, which is consistent with the predictions of the GRH, which posits that higher N and P concentrations and lower N:P ratios maximize the growth rate capacity (Main et al., 1997; Sterner and Elser, 2002). The reduction in the leaf N:P ratio in Q. ilex in the drought-treated plots probably was not associated with growth rate but, rather, with the differential effects of drought on N and P uptake or with a concentration effect in P (as in Q. ilex forest, where it was not limiting), which was absent in N (limiting at both sites), resulting from a decrease in growth rates and or to a mechanism for improving WUE (Ruiz-Lozano et al., 2001; Mohammad and Zuraiqi, 2003). In dry environments, the N:P ratio of an organism is not solely associated with growth rate, because it can be influenced passively by water availability or actively through the maximization in the investment in mechanisms to cope with drought stress (Sardans et al., 2012a,b). Thus in resource-limited ecosystems, where high growth rates are difficult to achieve, other factors such as an anti-stress mechanism can have a strong influence on plant N:P when plants need higher investment in anti-stress functions.

4.2. Effects of soil nutrient availability

The soils in the Prades Mountains are not P-limited (Sabaté et al., 1992) and the concentrations of available-P are in the normal range (Olsen-P ca. 10 ppm) (Sardans et al., 2008h). Higher leaf P concentrations in the drought plots were already evident within the first five years of the simulated drought experiment (Sardans and Peñuelas, 2007a). High concentrations of P can improve WUE during periods of drought (Ruiz-Lozano et al., 2001; Mohammad and Zuraiqi, 2003; Jones et al., 2005) and even under absence of water stress (Payne et al., 1992; Graciano et al., 2005; Brown et al., 2011). The increase of P availability and plant P status can have a positive effect on WUE through several processes, as for example by improving photosynthetic efficiency (Reich et al., 1989; Jacob and Lawlor, 1992; Olszewksa et al., 2008) and by increasing plant transpiration control capacity (Payne et al., 1992; Singh et al., 2000; Olszewksa et al., 2008).

Unlike Q. ilex, leaf P concentrations in E. multiflora did not change under drought-simulated conditions. In the Garraf Mountains, E. multiflora grows on calcareous soils that have low P availability (Olsen-P 1–2 ppm) (Sardans et al., 2008h), and leaf concentrations are naturally lower than those in the species that grow in the Prades Mountains; e.g., in spring, 0.48 ± 0.03 mg g⁻¹ in E. multiflora and 0.91 ± 0.02 mg g⁻¹ in Q. ilex (Figs. 2 and 4) which probably reflects a natural adaptation of E. multiflora to low water and low P availability. The low leaf P concentrations in E. multiflora under drought conditions probably are associated with a decrease in root–phosphatase activity, which has been described in other species in the Garraf plant community, e.g., Globularia alypum (Sardans et al., 2007), and reductions in soil phosphatase activity in some seasons during drought conditions (Sardans et al., 2006b). The results also show that by growing on a soil with greater N and P availability than the soil where E. multiflora grows, Q. ilex changes less its foliar N:P contents ratio through the year than E. multiflora.

Fig. 7. Principal component analyses with leaf element concentrations and ratios as variables and leaves of different climate plots sampled in different seasons as cases in Erica multiflora. The significant differences (P < 0.05) of the PC scores of the different treatments are depicted along the PC axes.
than the observed (Sardans and Peñuelas, 2007b). Conversely, in tions of As, Cd, Cu, Ni, and Zn increased from 1999 (Sardans et al., spring (Table 3). Although it is unclear why the concentrations of 2010. In Q. ilex, Pb concentrations tended to be lowest in the leaves of plants in the drought-treated plots, and the effect was statisti-
centrations for high growth rates in the growing seasons. Probably,
was to adapt leaf composition to seasonal changes in the environment,
cofactor for several enzymes. The summer increases in leaf As,
concentrations in spring, the most productive season, might have
is a cofactor for several enzymes. The summer increases in leaf As,
concentrations for high growth rates in the growing seasons. Probably,
the need to resist drought and to take advantage of the annual period
of water availability has led Mediterranean species to acquire such
natural plasticity. The results indicate that the capacity to adapt
the stoichiometry to drought was influenced by the availability of nu-
trients in the soil and/or by species-specific responses. The results
also showed significant changes in the leaf K concentrations and
stoichiometry relative to other main nutrients. Moreover, the leaf K
concentrations changes tend to be different and more intense than
those changes in leaf N and P concentrations, mainly in Q. ilex
throughout year and in E. multiflora in response to drought treat-
ment. These results emphasize the importance of including K in
ecological stoichiometric studies in terrestrial ecosystems.

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