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## NUTRIENT STOICHIOMETRIC RELATIONS AND BIOGEOCHEMICAL NICHE IN COEXISTING PLANT SPECIES: EFFECT OF SIMULATED CLIMATE CHANGE

**ABSTRACT:** Here we define a “biogeochemical niche” characterized by the species position in the multivariate space generated by its content not only of macronutrients like N, P or K, but also of micronutrients such as Mo, Mg and Ca, and trace toxic elements such as Pb and As. We then hypothesize that the flexibility of the species “biogeochemical niche” will influence the quality of plant tissue, which may have implications for herbivores, and will affect the species capacity to respond to disturbances and climate change and to adapt to the new climate conditions. We show with a simple multivariate procedure, a principal component analysis (PCA), first, that there is a strong differentiation in the total and relative (stoichiometry) content of the different elements in coexisting plant species, and, second, that there is species-specific plasticity in the response of this elemental composition to experimental climate change. The concentrations of foliar macro and micronutrients, as well as trace elements were measured in several tree species (*Quercus ilex* L., *Phillyrea latifolia* L. and *Arbutus unedo* L. in a Mediterranean broad leaf forest (Prades Mts) and in shrub species (*Erica multiflora* L., *Globularia alypum* L. and *Dorycnium pentaphyllum* Scop.) in a Mediterranean shrubland (Garraf Mts) in control plants and in plants grown in experimental drought and warming plots. The climate conditions were monitored during the period 1999–2005. During this period, in the Prades experiment the drought plots had on

average a soil moisture content 9% lower than the control plots, whereas in the Garraf experiment the drought treatment led to a mean reduction in soil moisture of 21% and the warming treatment to 0.9°C rise. The species with greater changes in biogeochemical niche under increased warming or drought, *Arbutus unedo*, *Erica multiflora* and *Globularia alypum*, were those that were also more affected in growth, photosynthetic capacity and other eco-physiological traits. The species differentiations indicate a strong biogeochemical niche, and that the changes in biogeochemical niche are probably an underlying factor in community structure shifts.

**KEY WORDS:** biogeochemical niche, climate change, disturbance; elemental composition, nutrients, plant species coexistence, stoichiometry

### 1. INTRODUCTION

In plant ecology we still keep asking, and have no definitive answer to, the question of how plant species coexist stably. The classical answer is based on the Lotka-Volterra competition model and states that stable coexistence between competing species requires them to occupy different niches. The niche concept was initially conceived by zoologists and emphasized the role of habitat and food

in defining an animal's niche (Chase and Leibold 2002). This does not offer an obvious explanation for coexistence among plants. All plants require water, CO<sub>2</sub>, light, nitrogen, phosphorus, potassium and a common set of minor mineral nutrients, and acquire them in similar ways. The question thus remains as to how competing species coexist apparently without the niche differences that classical ecology theory predicts to be necessary. Two answers are possible: either the classical theory is wrong or incomplete, or there are niche differences between plants that have been overlooked (Silvertown 2004).

A candidate for such a niche difference, apart from competition for vacant space and partition of light intensity and quality or water sources, is a differential use of nutrients (possibly through the mediation of species-specific microbial symbionts), similar to the way in which animals use different food in their animal niche differentiation. It is generally accepted that the realized niche (including the ways of acquiring or using nutrients) is different for the different species and that there is competitive exclusion, but in many cases it has not been possible to show the separation of niches nor to quantify how different two niches need to be in order for corresponding species to coexist (Begon *et al.* 2006).

Water is the most limiting factor in Mediterranean ecosystems. Current climate and ecophysiological models such as GOTILWA (IPCC 2007, Sabaté *et al.* 2002, Peñuelas *et al.* 2005) project increased warming and drought for the future in the Mediterranean ecosystems. The effects of this climate change on carbon, nutrients and trace element concentrations in the Mediterranean plants have been studied only recently. An experiment of drought effects has been conducted in a Mediterranean forest in Prades Mts (Prades Natural Park) since 1999 (Ogaya and Peñuelas 2007). To test drought and warming effects on Mediterranean shrublands, another experiment of drought and warming simulation is being conducted in Garraf Mts (Garraf Natural Park) (Catalonia, north-east Spain) since 1999 (Peñuelas *et al.* 2007).

Here we applied a simple multivariate procedure, a principal component analysis (PCA), first, to discern whether there is a strong differentiation in the relative content

(stoichiometry) of the different macronutrients, micronutrients, and trace elements in the coexisting dominant plant species of these Mediterranean ecosystems, and, second, how do the different species change their elemental composition in response to experimental climate change.

## 2. STUDY SITE

The study in Prades Natural Park was carried out in a natural *Quercus ilex* L. oak forest in the Prades Mts in southern Catalonia (NE Spain) (41°13'N, 0°55'E) on a south-facing slope (25%). The soil is a stony *Dystric Xerochrept* (Soil Survey Staff 1999) lying on a bedrock of metamorphic sandstone. Its depth ranges between 35 and 100 cm, with the depth of horizon A ranging between 25 and 30 cm. The average annual temperature is 12°C and the average rainfall was closed to 658 mm, with a period between September to November experiencing the maximum of rainfall. Summer drought is pronounced and usually lasts for 3 months. The vegetation consists of a dense forest with a canopy height average of 8–10 m dominated by *Quercus ilex* (20.8 m<sup>2</sup> ha<sup>-1</sup> of trunk basal area at 50 cm of height) accompanied by abundant *Phillyrea latifolia* L. (7.7 m<sup>2</sup> ha<sup>-1</sup> of trunk basal area at 50 cm of height and *Arbutus unedo* L. For details see Ogaya and Peñuelas (2007).

In Garraf Natural Park the study was carried out in a natural Mediterranean calcareous shrubland on a south-facing slope in the Garraf mountains in central-coast Catalonia (NE Spain) (41°18'N, 1°49'E). The site is located on formerly cultivated terraces – abandoned approximately a century ago – with a *Petrocalcic calcixerepts* (Soil Survey Staff 1999) soil lying on bedrock of sedimentary limestone. During the study period (1999–2005) the average annual temperature was 15.1°C (7.4°C in January and 22.5°C in July) and the average annual rainfall was closed to 580 mm. The summer drought is pronounced and usually lasts for three months. The total vegetation cover is 70% and consists of a calcareous shrubland with plants about 1 m high dominated by the shrubs *Globularia alypum* L., *Erica multiflora* L., *Dorycnium pentaphyllum* Scop., *Rosmarinus officinalis* L., *Ulex parviflorus* Pourr., and *Pistacia lentiscus* L., *Erica*

*multiflora* represents ca. 20% and *Globularia alypum* ca. 33% of the total surface area. For details see Peñuelas *et al.* (2007).

### 3. MATERIAL AND METHODS

We measured the elemental concentrations of C, N, P, K, Mg, Ca, Fe, Na, S, Mo, Al, As, Cd, Cu, Cr, Hg, Ni, Pb, Sb, Ti, Zn in the leaves of the three most abundant woody species (*Quercus ilex*, *Phillyrea latifolia*, *Arbutus unedo*) coexisting in 1 ha of Mediterranean forest (Prades mountains) and in the three most abundant woody species (*Erica multiflora*, *Globularia alypum*, *Dorycnium pentaphyllum*) coexisting in 1 ha of Mediterranean shrubland (Garraf mountains) both located in Catalonia (NE Spain). We also measured those foliar concentrations in plants of the same species in the 1 ha field sites after having subjected them for 6 years to the warming (ca. 1°C) and drought (ca. 15–20% decrease in soil moisture) projected for this region in the coming decades by climate models (IPCC 2007, Sabaté *et al.* 2002, Peñuelas *et al.* 2005).

#### 3.1. Experimental design

The experimental climate change in Prades study area consisted in eight 15 × 10 m plots established at the same altitude (930 m above sea level) on a slope. Four of the plots received the drought treatment and four plots left as controls. All the plots were established in an area with the same aspect and altitudinal level, with a minimum distance between plots of 15 m. The treatments were randomly assigned to different plots. The drought treatment consisted of partial rainfall exclusion by suspending transparent PVC strips at a height of 0.5–0.8 m above soil level and covered approximately 30% of the total soil surface. Four plastic strips 14 m long and 1 m wide were placed along the drought treatment plots and a 0.8–1 m deep ditch was dug along the entire top edge of the upper part of the treatment plots to intercept runoff water. The water intercepted by the strips and ditches was channeled to the bottom edge of the plots. The drought treatment began in March 1999 and has been lasting to the present. For details see Ogaya and Peñuelas

(2007). During the period 1999–2005 the drought plots (D) had an average soil moisture content of 15.5 ± 0.5 % (n=100), i.e. 9% lower than the control (C) plots soil moisture 19.2 ± 0.5% (n = 100, P <0.05). For details see Sardans *et al.* (2008a)

In the Garraf study area we conducted field-scale night-time warming and drought treatments with automatic roofs that covered the vegetation either when dark or when raining (Beier *et al.* 2004), and compared the responses to control plots. Each treatment (control, warming and drought) was replicated 3 times. Each plot occupied an area of 4 × 5 m, but to avoid possible disturbance edge effects, efficient area was established as 3 × 4 m. Manipulations started on March 1999 and has been lasting to the present. For details see Peñuelas *et al.* (2007). During the six years of study (1999–2005), the drought treatment led to a mean reduction in soil moisture of 21% with respect to the control treatment. A significant decrease in soil moisture occurred in drought plots in spring and autumn rainy seasons with the drought treatment running. During the period 2001–2004 the temperature increase in the warming treatment was 0.95°C at a depth of – 5 cm and 0.75°C in the air (20 cm), but the warming treatment had no effect on soil moisture during the 6 years of study. For wider and more detailed information see Sardans *et al.* (2008c) and Llorens *et al.* (2004). There were no alterations in the light conditions of the different treatments (Beier *et al.* 2004).

#### 3.2. Leaf sampling

In the Prades study area in January 2005, just 6 years after experiment initiated, eight samples of leaf from the three dominant species (*Quercus ilex*, *Phillyrea latifolia* and *Arbutus unedo*) were randomly sampled in each plot (4 samples in the sun and 4 samples in the shade). The leaves were sampled from between 1.5 to 6 m height where most foliar biomass was located. Sample collection was standardized in order to avoid bias due to differences in the age of tissues and their position with respect to sunlight. The leaves sampled were those from current year leaves grown in 1998 and 2004. All leaves were collected from different plants in each plot.

In the Garraf study area in January 2005 six years after starting the experiment we sampled 15 individual plants per plot, 5 pertaining to each one of the two dominant shrub species, *G. alypum* and *E. multiflora*, and to the N-fixing species *D. pentaphyllum*. Five branches were sampled from each plant and current-year leaves were collected.

### 3.3. Sample preparation and chemical analyses

All the samples were taken to the laboratory and stored at 4°C until analysis began. In order to analyze only the elements into the foliar tissue, the leaves were washed with distilled water as described by Porter (1986). After all samples had been washed, they were dried in an oven at 60°C to a constant weight and then ground in a CYCLOTEC 1093 (Foss Tecator, Höganäs, Sweden).

We measured the concentrations of Sb, Cd, Pb, Ni, Cu, Cr, Ti, Zn, and Al in all biomass and soil samples by ICP-MS (Mass Spectroscopy with Inductively Coupled Plasma) using a model Elan-6000 (Perkin Elmer Corp, Inc. Norwalk, CO, USA). Furthermore, to analyze As with ICP-MS a arsenic hydride generation must be performed previously and As (V) was reduced to As (III) by a mixture of HCl (30% w/v), KI (1%w/v) and ascorbic acid (0.2% w/v) as a necessary preliminary step. The solution was pumped into a gas-liquid separator and reacted with NaBH<sub>4</sub> (1.3% v/v solution in 0.1 M NaOH) to form arsenic hydride (De Gieter *et al.* 2002). We also analyzed Hg with cold vapor atomic absorption spectrometry (CVAS). We measured P, K, Ca, Mg, Fe and S by ICP-OES (Optic Emission Spectroscopy with Inductively-Coupled Plasma) using a Jobin Ivon JY 38 instrument (Longjumeau HORIBA, Jobin Ibon S.A.S. France). Before the ICP-MS and ICP-OES analyses, an acid digestion of the samples was carried out with an acid mixture of HNO<sub>3</sub> (60%) (143255, purissimum, PANREAC, Barcelona) and HClO<sub>4</sub> (60%) (141054, purissimum, PANREAC, Barcelona) (2:1) in a microwave oven (SAMSUNG, TDS, Seoul, South Korea) using Oak Ridge 50-ml teflon centrifuge tubes (Nalge Nunc International, Rochester, NY, USA). Mixed acid solution (2 ml) was added to 100 mg of

dry biomass for each sample. The digested solutions were diluted to 10 ml of final volume. During the acid digestion process, two blank solutions (2 ml of acid mixture without any sample biomass) were also analyzed. In order to assess the accuracy of digestion and the analytical biomass procedures, standard certified biomass DC73351 (leaf poplar, purchased from China National Analysis Center for Iron & Steel) was used. For C and N concentration determination, 1–2 mg of finely-sieved leaf sample plus 2 mg of V<sub>2</sub>O<sub>5</sub> (as oxidant) were used. Foliar C and N concentrations were analyzed by organic elemental analysis employing combustion coupled to gas chromatography. We used a Thermo Electron Gas Chromatograph model NA 2100 (C.E. instruments-Thermo Electron, Milano, Italy). The analytical precision was better than 5% in all leaf samples – as verified by parallel analyses of the international certified standards DC73351.

### 3.4. Data analysis

With these elemental concentration data we conducted PCA, cluster and ANOVA analyses by using the *Statistica* software package (StatSoft, Inc. Tulsa, Oklahoma). We conducted PCA analyses of nutrients and trace elements based on the correlations matrices for all species together and separately for each species with the replicated samples in control, warming and drought plots as cases. The corresponding scores of PC1 and PC2 axes of each case (control, drought or warming plot) were analyzed by an one-way ANOVA to determine the significance of PC1 and PC2 axes to separate drought or warming plants with respect to the control plants in each species and also considering all species together.

## 4. RESULTS AND DISCUSSION

### 4.1. Species-specific responses to drought and warming

In the Prades experiment, drought increased leaf P and Mg concentrations in *Quercus ilex* and Mo concentrations in *Phillyrea latifolia*, but these nutrients concentrations did not change in other dominant

species (Sardans and Peñuelas 2007a, Sardans *et al.* 2008a). Drought tended to increase some trace element concentrations, mainly leaf Cd concentrations in *Arbutus unedo* and leaf As concentrations in *Phillyrea latifolia* codominant shrubs (Sardans and Peñuelas 2007b). The PCA of nutrients for *Arbutus unedo* plants separated drought plot plants from control plots plants in the space formed by PC1 (accounting for 45% variance) and PC2 (accounting for 26% variance) (Fig. 1) because of lower leaf concentrations of C, Fe and Mo and higher concentration of Mg. *Quercus ilex* also presented a significant ( $P < 0.05$ ) separation (cluster and ANOVA analyses) between control and drought plants along the PC2 (PC accounting for 19% variance) due to the higher leaf P, Mg and S concentrations in drought than in control plants (Fig. 1). There was a general tendency to decrease Mo and to increase Mg in response to drought in both *Arbutus*

*unedo* and in *Quercus ilex*. Mo is linked to active uptake and drought reduced the plant productivity and active nutrient uptake capacity of these two species (Ogaya and Peñuelas 2007). *Phillyrea latifolia* did not show any significant difference between control and drought plants in the PCA of plant nutrient concentrations (Fig. 1), coinciding with its insensitivity to drought (Ogaya *et al.* 2003, Ogaya and Peñuelas 2007). Regarding to trace elements, *Arbutus unedo* was also the species with greater separation between drought and control plants in the space formed by PC1 (accounting for 38% variance) and PC2 (accounting for 31% variance). A significant separation ( $P < 0.01$ ) by the PC2 was observed mainly as a result of the higher Cd, and the lower Al, Hg and Ti concentrations in drought than in control plants (Fig. 2). *Quercus ilex* and *Phillyrea latifolia* did not show any clear change of trace elements concentration in drought plot

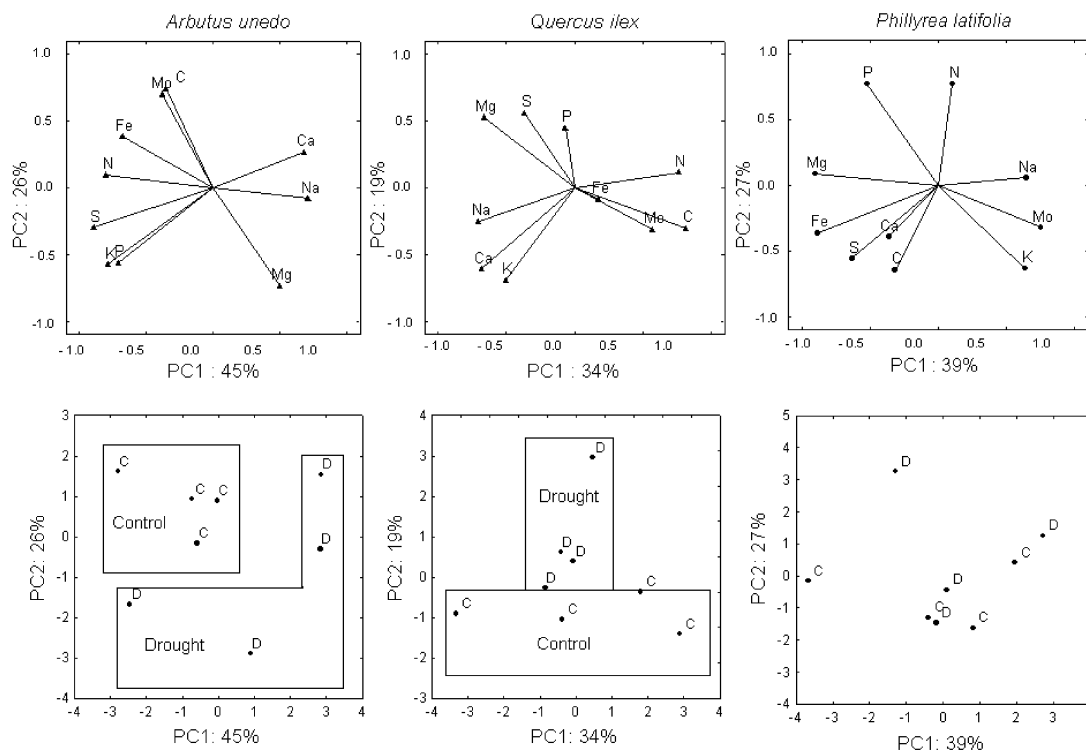


Fig. 1. Principal component analysis (PCA) of leaf concentrations of 10 nutrients in the three dominant forest species in the Mediterranean forest located in Prades Natural Park. The upper panels show the loadings of the 10 nutrients and the lower panels represent the scores of the different plots. C – control plots, and D – drought plots. Treatments are separated when significant (ANOVA,  $P < 0.05$ ) differences were found in the score values.

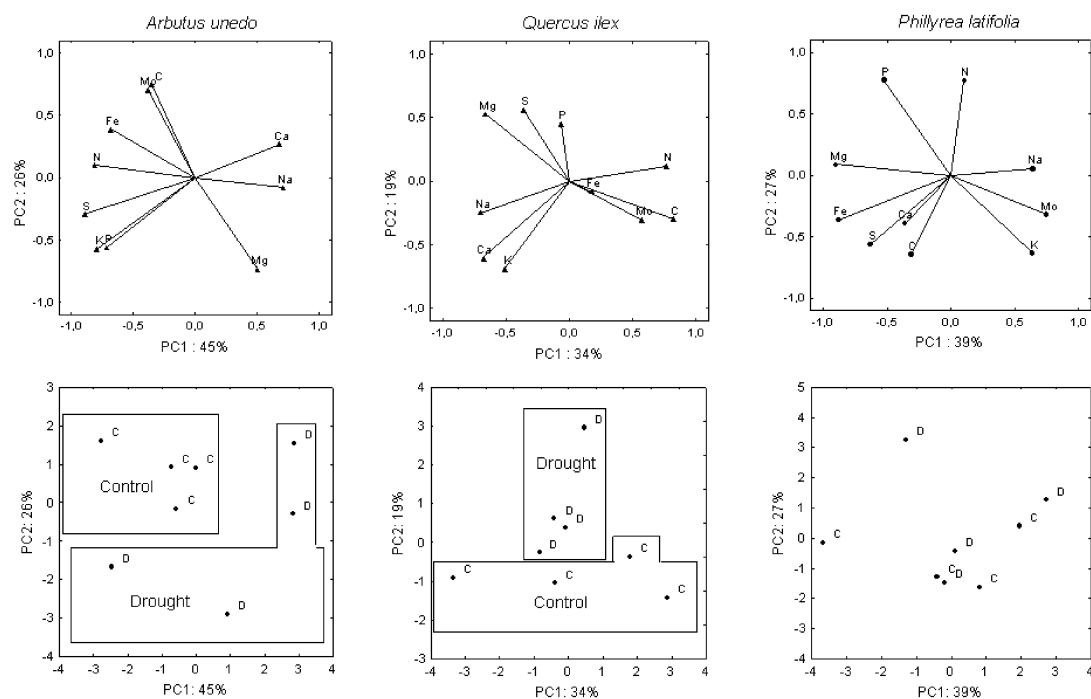


Fig. 2. Principal component analysis (PCA) of leaf concentrations of 11 trace elements in the three dominant forest species in the Mediterranean forest located in Prades Natural Park. The upper panels show the loadings of the 11 trace elements and the lower panels represent the scores of the different plots. C – control plots, and D – drought plots. Treatments are separated when significant (ANOVA,  $P < 0.05$ ) differences were found in the score values.

plants. These stronger effects of drought on biogeochemical niche in *Arbutus unedo* than in *Quercus ilex* and *Phillyrea latifolia* coincided with the stronger effects of drought on the growth, regeneration and nutrient concentration and accumulation in *Arbutus unedo* than in the other two dominant species described in previous studies (Ogaya *et al.* 2003, Ogaya and Peñuelas 2007, Sardans and Peñuelas 2007a, b, Sardans *et al.* 2008a).

In the Garraf experiment warming increased the concentrations of trace elements related to plant uptake capacity, such as Al, As, Cr, Cu, and Pb. This effect was more general in *Erica multiflora* than in *Globularia alypum*. These species-specific increases were related to greater growth responses to warming in *Erica multiflora* than in *Globularia alypum* (Sardans *et al.* 2008b). A significant separation ( $P < 0.05$ ) of drought and control plants was observed throughout PC1 (PC accounting for 29% variance) for *Erica*

*multiflora* and throughout PC2 ( $P < 0.05$ ) (PC accounting for 29% variance) for *Globularia alypum* when conducting the PCA with nutrient concentrations. As in the forest, there was a general tendency to increase Mg in response to drought. Plants from warming plots were separated by the PC2 (accounting for 22% variance,  $P = 0.07$  in *Erica multiflora* and 29%,  $P < 0.05$  in *Globularia alypum*) of the nutrients PCA (Fig. 3). This effect was due to the higher Ca and Fe concentrations and lower K concentrations in warming than in control plots (Fig. 3). In this experiment, the two dominant species, *Erica multiflora* and *Globularia alypum*, changed their nutrient stoichiometry in different way when climate conditions changed. The N fixing species, *Dorycnium pentaphyllum* did not present any clear pattern of separation among control, drought and warming plants in the PCA space of nutrients (Fig. 3). No clear pattern of separation among control, drought and warming plants was observed

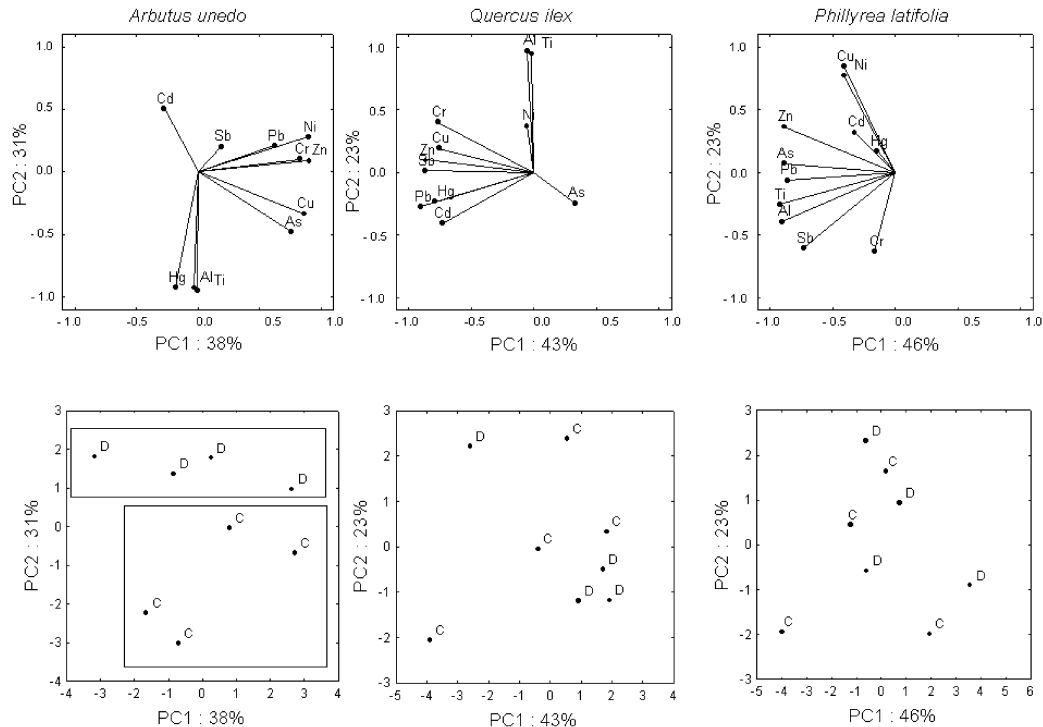


Fig. 3. Principal component analysis (PCA) of leaf concentrations of 10 nutrients and in the three dominant shrub species in the Mediterranean shrubland located in Garraf Natural Park. The upper panels show the loadings of the 10 nutrients and the lower panels represent the scores of the different plots. C – control plots, D – drought plots, and W – warming plots. Treatments are separated when significant (ANOVA,  $P < 0.05$ ; except  $P = 0.07$  for *Erica multiflora* in warming plots) differences were found in the score values.

in the PCA space of trace element concentrations in any of the three dominant shrub species (Fig. 4).

#### 4.2. Biogeochemical niche

The most outstanding pattern resulting from the overall PCA analysis was the clear and significant ( $P < 0.05$ ) separation (cluster and ANOVA analyses) of each species in the space formed by PC1 (accounting for 38% variance) and PC2 (accounting for 18% variance) in the Mediterranean forest, and by PC1 (accounting for 47% variance and mostly linked to the gradient of increasing sclerophylly from *Dorycnium pentaphyllum* to *Erica multiflora*) and PC2 (accounting for 13% variance) in the Mediterranean shrubland (Fig. 5). This species separation, also found in other studies (e.g. Garten 1978), indicates that each species uses the different nutrients and trace elements in different amounts and proportions, i.e. with different stoichiometry,

and places each species in what we here call a different “biogeochemical niche”. This “biogeochemical niche” is characterized by the species position in the multivariate space defined by the elemental concentrations not only of macronutrients like N, P or K, but also of micronutrients such as Mo, Mg or Ca, and trace toxic elements such as Pb or As. This “biogeochemical niche” occupied by the different plant species should moreover play an important role in plant succession according to resource-ratio competition hypotheses (Tilman 1985).

The results for the Mediterranean shrubland leaves (Fig. 5) point to a discrimination between *D. pentaphyllum* and the two remaining species apparently based on C content, suggesting that the reaction to drought, especially in *E. multiflora*, was imposed not by changes in nutrient content, but rather by changes in “sclerophylly” of the leaf’s tissues. To test this possibility, two separate analyses were carried out: one for macronutrients

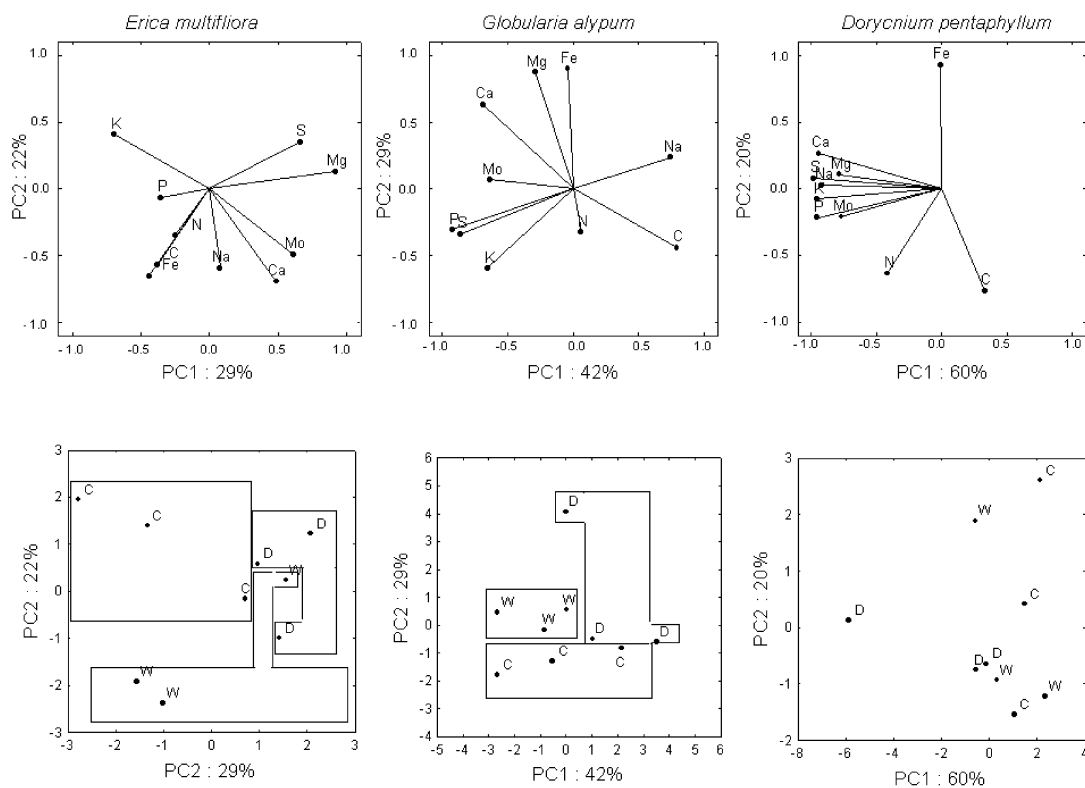


Fig. 4. Principal component analysis (PCA) of leaf concentrations of 11 trace elements in the three dominant shrub species in the Mediterranean shrubland located in Garraf Natural Park. The upper panels show the loadings of the 11 trace elements and the lower panels represent the scores of the different plots. C – control plots, D – drought plots, and W – Warming plots. Treatments are separated when significant (ANOVA,  $P < 0.05$ ) differences were found in the score values.

without C and another one with micronutrients without C. In both cases the results were similar than those with C (data not shown). It seems thus that the changes in sclerophylly indicated by the changes in C content are not the only cause of the observed separation among species in the multivariable space.

The overall PCA analyses (Fig. 5) also shows that some species, such as for example *Arbutus unedo* in the forest or *Dorycnium pentaphyllum* in the shrubland, occupy a more flexible “biogeochemical niche”. They change their position in the PCA space in response to environmental disturbances such as drought more clearly than others such as the dominant *Quercus ilex* and *Phillyrea latifolia* in the forest studied, or the dominant *Erica multiflora* and *Globularia alypum* in the shrubland studied (Fig. 5).

The climate change affects the nutrient availability and the nutrient accumulation

differently depending on the nutrient and the species (Sardans and Peñuelas 2007a; Sardans *et al.* 2008a, 2008b), which implies different changes in the biogeochemical niche of the different species. The flexibility of the species’ “biogeochemical niche” will influence the quality of plant tissues, which may have implications for herbivores and will also affect the species’ capacity to respond to disturbances and climate change and to adapt to the new climate conditions. All this may contribute to change the inter-specific competitive relations, and finally the community structure. On the other hand, and since the theory predicts positive effects of species diversity that result from functional niche complementarity (Grime 2001), the consequences of ongoing diversity loss for ecosystem functioning may also be related to incomplete use of the nutrients and trace elements in general.

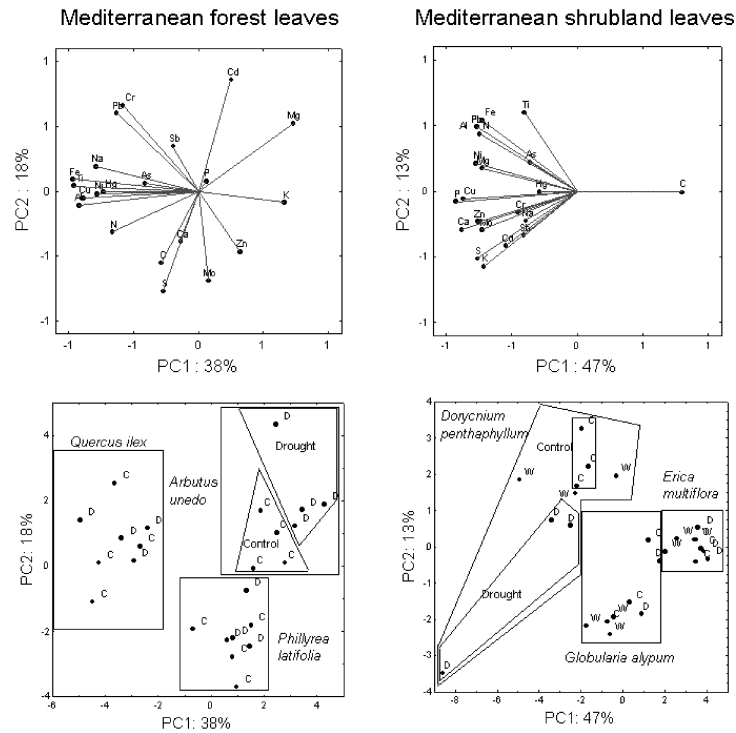


Fig. 5. Principal component analysis (PCA) of leaf concentrations of 21 mineral elements in a Mediterranean forest located in the Prades Natural Park, and in a Mediterranean shrubland in the Garraf natural park, both in Catalonia, NE Spain. There is a clear and significant ( $P < 0.05$ ) separation (cluster and ANOVA analyses) of the coexisting dominant species in the space of elemental composition formed by PC1 and PC2. In some species, *Arbutus unedo* in the forest, and *Dorycnium pentaphyllum* in the shrubland, there is also a clear and significant (ANOVA,  $P < 0.05$ ) separation between plants grown under control and under drought, i.e. they show a flexible “biogeochemical niche”.

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