

# Drought's impact on Ca, Fe, Mg, Mo and S concentration and accumulation patterns in the plants and soil of a Mediterranean evergreen *Quercus ilex* forest

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**Abstract** We conducted a 6-year field manipulation drought experiment in an evergreen *Quercus ilex* forest where we simulated the drought predicted by GCM and ecophysiological models for the coming decades (an average of 15% soil moisture reduction). We thereby tested the hypothesis that enhanced drought will change Ca, Fe, Mg, Mo and S availability, concentrations and accumulation patterns in Mediterranean ecosystems. The strongest effects of drought occurred in the soil. Drought increased the total soil concentrations of S, the soil extract concentrations of Fe, Mg and S, the Mg saturation in the soil exchangeable complex and tended to increase the percentage base saturation of the soil exchangeable complex. These increased soil concentrations were related to a decrease of plant uptake capacity and not to an increase of soil enzyme activity, which in fact decreased under drier conditions. Drought increased leaf Mg concentrations in the three dominant species although only significantly in *Quercus ilex* and *Arbutus unedo* (20 and 14%, respectively). In contrast, drought tended to decrease Ca in *Phillyrea latifolia* (18%) and Ca and Fe

concentrations in the wood of all three species. Drought increased Ca and Fe concentrations in the roots of *Quercus ilex* (26 and 127%). There was a slight general trend to decrease total biomass accumulation of nutrients that depend on water flux such as Mg, Fe and S. This effect was related to a decrease of soil moisture that reduced soil flow, and to a decrease in photosynthetic capacity, sap flow, transpiration and growth, and therefore plant uptake capacity under drought observed in *Quercus ilex* and *Arbutus unedo*. On the contrary, drought increased Mo accumulation in aboveground biomass in *Phillyrea latifolia* and reduced Mo accumulation in *Arbutus unedo* by reducing growth and wood Mo concentrations (51%). *Phillyrea latifolia* showed a great capacity to adapt to drier conditions, with no decrease in growth, an increase of Mo uptake capacity and a decrease in leaf Ca concentration, which was related to a decrease in transpiration under drought. The results indicate asymmetrical changes in species capacity to accumulate these elements, which are likely to produce changes in inter-specific competitive relations among dominant plant species and in their nutritional quality as food sources. The results also indicate that drought tended to decrease nutrient content in aboveground biomass, mainly through the decrease in growth and transpiration of the most sensitive species and caused an increase in the availability of these nutrients in soil. Thus, drought decreased the ecosystem's capacity to retain Mg, Fe and S, facilitating their loss in torrential rainfalls.

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## Introduction

Water is the most limiting factor in Mediterranean ecosystems. In the Mediterranean Basin there has been increasing aridity in recent decades (Piñol et al. 1998; Peñuelas et al. 2002). Global circulation models (IPCC 2001) and ecophysiological models predict drier conditions for the coming decades. For instance the model GOTILWA which takes into account the predicted changes in air temperatures, annual precipitations, CO<sub>2</sub> concentrations and plant physiology, predicts a scenario of 15% decrease in soil moisture over the next 2–3 decades in the Mediterranean areas of Catalonia (NE Spain) which will reach 25% in 2040 (Sabaté et al. 2002; Peñuelas et al. 2005). Together with water, nutrients can also be limiting in Mediterranean ecosystems (Kruger 1979; Specht 1979; Carreira et al. 1992; Henkin et al. 1998; Sardans et al. 2004, 2005a, b). Few experiments have studied the effects of drought on nutrient contents and availability in these ecosystems. The interaction of drought with other important or critical factors for ecosystem functioning such as nutrient availability needs to be studied to gain insight into the impacts of the predicted drought enhancement on Mediterranean ecosystems. Among plant nutrients, N and P are the most widely studied since plants need them in larger quantities. Recent studies have shown drought's impact on P and N cycles and availability in Mediterranean ecosystems (Sardans and Peñuelas 2004, 2005, 2007). These studies observed that N, P and K contents tended to decrease in aboveground biomass and tended to increase in soil in response to drought (Sardans and Peñuelas 2004, 2007). Drought also decreased soil enzyme activity and soil P availability (Sardans and Peñuelas 2004, 2005). There are no studies as far as we know on the effects of drought on other nutrients necessary for plant life and growth such as Ca, Fe, Mg, Mo and S in Mediterranean ecosystems.

Although not very frequent, plant Ca deficiency has been observed in some studies (Ouimet and Camire 1995; Gulpen and Feger 1998; Pintro and Taylor 2005) and can limit growth in forest ecosystems (Rodenkirchen 1998). In Mediterranean ecosystems, Ca can play a significant role because it is involved in

the regulation of turgor pressure (Takagi and Nagai 1992), and thus is important in water loss regulation. In addition, root Ca content is a significant factor for the capacity to absorb NH<sub>4</sub><sup>+</sup> in several plant species (Fenn and Feagley 1999). All these properties mean Ca plays an important role in water use efficiency (WUE).

Fe is essential for plant growth and its deficiency occurs especially, but not only, in calcareous soils (Shenker and Chen 2005) that are widely found in the Mediterranean Basin. Drastic changes in the soil Fe<sup>2+</sup>/Fe<sup>3+</sup> ratio are common in Mediterranean areas. The Fe soil content and availability are dependent on soil water content. The Fe<sup>2+</sup>/Fe<sup>3+</sup> ratio is higher in wet soils thus permitting greater availability of Fe for plants, whereas in drought conditions the greater presence of O<sub>2</sub> in soil favors a decrease in the Fe<sup>2+</sup>/Fe<sup>3+</sup> ratio and therefore a decrease in available Fe for plant absorption since Fe<sup>2+</sup> is more soluble than Fe<sup>3+</sup>, as observed in a Mediterranean *Quercus suber* forest in South France (Orgeas et al. 2002).

Low Mg contents have been observed in several Mediterranean forests (Montès et al. 2002), but there is also a lack of experimental data on the effects of drought on the Mg cycle in Mediterranean ecosystems. The capacity to maintain high Mg biomass contents under drought conditions is important to sustain an adequate production capacity and metabolic status that then allows plants to take advantage of the rewetting periods.

With some exceptions, Mo availability is not limiting in Mediterranean soils (Rashid and Ryan 2004). Mo is fundamental for plant growth (Vankova-Radeva et al. 1997; Kaiser et al. 2005) because it is used by several enzymes (nitrate reductase, xanthine dehydrogenase, aldehyde oxidase and sulfite oxidase), however, when Mo availability exceeds certain levels it can become toxic to plants (Gupta 1997) and even to most mammal herbivores (Albasel and Pratt 1989).

S is essential to the growth of higher plants (Browder et al. 2005). S availability is also partly related to soil moisture such as observed in rainfall gradients in tropical areas of Africa (Itanna 2005). In Mediterranean ecosystems the information about S is scarce. Sardans et al. (2006a) reported a negative correlation between leaf sclerophylly and leaf S concentration in a Mediterranean forest. As sclerophylly is important for dealing with dry conditions in

Mediterranean environments (Sardans et al. 2006a), reductions in S concentrations and contents in above-ground biomass are likely in response to drier conditions.

Drought can change the capture of these five nutrients by several processes such as by reducing soil water content, by affecting soil properties and enzyme activity, by reducing plant growth, or by changing the photosynthetic and metabolic status of the plant and therefore its capacity to capture nutrients. Most studies have observed that by reducing soil moisture and transpiration rates, drought reduced plant growth and nutrient uptake capacity (Dambrine et al. 1993; Grabarova and Martinkova 2001; Wang et al. 2005; Protocic et al. 2005; Sing and Singh 2006; Hu et al. 2007). Moreover, soil moisture reduction decreases soil diffusion capacity, thus hindering plants' capture of these nutrients (Baranowski et al. 2005), mainly for the nutrients that strongly depend on transpiration fluxes for their plant absorption (Protocic et al. 2005; Comerford et al. 2006). As a consequence of soil water reduction under drought, soil enzyme activity may also decrease (Sardans and Peñuelas 2005), decreasing the liberation and therefore the availability of nutrients in the soil. This availability can also be affected by leaching changes in a drier soil scenario. In addition, drought can change other soil properties such as cation exchangeable complex (CEC) capacity that can also affect the cycling of nutrients. For example, a decrease of soil organic matter or clay formation can reduce the CEC and thus the ecosystem's capacity to prevent nutrient losses through leaching during the torrential rainfalls frequent in Mediterranean areas. If plants reduce their transpiration rates in response to drought, the amount of those nutrients such as Ca and Mg that depend mainly on mass flow, should decrease. Thus, drought can cause nutrient unbalances in plant by affecting asymmetrically the uptake of different nutrients (Dambrine et al. 1992; Yavitt et al. 2004; Inclan et al. 2005; Hu and Schmidhalter 2005). On the other hand, drought can generate asymmetrical changes among different plant tissues by changing internal remobilization patterns (Wang et al. 2005). In addition, nutrient plant concentrations may increase under drought as a result of a concentration effect due to a growth decrease and as a result of an accumulation in some plant tissues as a drought avoidance mechanism (Antolín and Sánchez Díaz 1992).

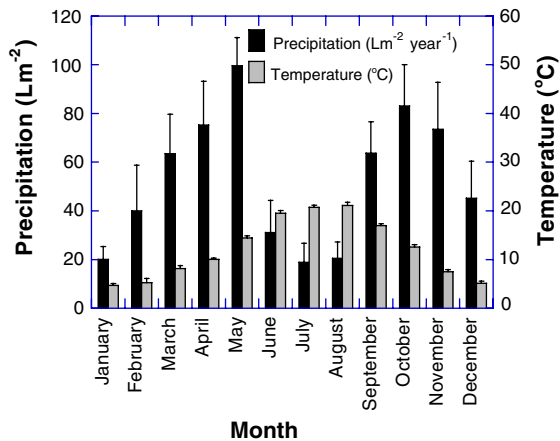
All these effects vary depending on the nutrient. Some of them like Mo are more dependent on an active capture and seem less directly dependent on the soil water content. In addition the response to drought of different plant species varies in function of their capacity to adapt their metabolic and physiological status to drought conditions. Some plants can allocate more resources to capture nutrients such as Mo that are necessary for photosynthetic N fixation and can contribute to enhance plant photosynthetic efficiency. If drought results on a decrease in nutrient content in plants or in soil availability, then the direct negative effects of water deficit on ecosystem productivity will be enhanced.

We hypothesized that changes in soil moisture, soil properties, soil microbial activity, plant photosynthetic capacity, transpiration and plant growth produced by drought would lead to changes in the concentration and the accumulation of Ca, Fe, Mg, Mo and S in the different species and ecosystem compartments (biomass fractions of the different species and soil). To test this hypothesis, we conducted a 6-year field experiment in an evergreen Mediterranean holm oak forest where we simulated the drought predicted by GCM and ecophysiological models for the oncoming decades (IPCC 2001; Sabaté et al. 2002; Peñuelas et al. 2005). We investigated the impacts of this simulated drought on: (i) Ca, Fe, Mg, Mo and S concentrations, accumulation in the biomass and losses in litter for the three dominant species of the ecosystem (98% of the total ecosystem biomass), (ii) total and available forms of Ca, Fe, Mg, Mo and S in the soil, and (iii) soil exchangeable basic cations, soil cation exchange capacity and % base saturation of the CEC.

## Material and methods

### Field site

The study was carried out in a natural *Quercus ilex* oak forest of the Prades mountains located in Southern Catalonia (NE Spain) (41°13' N, 0°55' E), on a south-facing slope (25%) at 930 m altitude. The soil is a stony *Dystric Xerochrept* (Soil Taxonomy) lying on bedrock of metamorphic sandstone. Its depth ranges from 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 average



**Fig. 1** Monthly mean precipitations ( $\pm$ SE,  $1 \text{ m}^{-2}$ ) and temperatures ( $\pm$ SE,  $^{\circ}\text{C}$ ) during the period 1999–2005 in the experimental area

rainfall is 658 mm (Fig. 1). Summer drought is pronounced and usually lasts for 3 months. The main soil traits are shown in Table 1. The vegetation consists of a dense forest dominated by *Quercus ilex* L. (*Fagaceae*) ( $20.8 \text{ m}^2 \text{ ha}^{-1}$  of trunk basal area at 50 cm of height) accompanied by abundant presence of *Phillyrea latifolia* (*Oleaceae*) ( $7.7 \text{ m}^2 \text{ ha}^{-1}$  of trunk basal area at 50 cm of height) and *Arbutus unedo* L. (*Ericaceae*) and a number of other evergreen species well-adapted to drought conditions such as *Erica arborea* L. (*Ericaceae*), *Juniperus oxycedrus* L. (*Cupressaceae*), *Cistus albidus* L. (*Cistaceae*), and occasional individuals of deciduous species such as *Sorbus torminalis* L. Crantz (*Rosaceae*) and *Acer monspessulanum* L. (*Aceraceae*). In winter 1999, above ground biomass of *Quercus ilex* represented

77.1% of the total above ground biomass while *Phillyrea latifolia* represented 12.6% and *Arbutus unedo* 7.8%: the sum of above ground biomass of these three species represented 97.6% of the whole ecosystem tree aboveground biomass. In winter 2005, the figures for the same species were 75.6, 13.3 and 8.7%, respectively, representing in total 97.6% of the total aboveground biomass.

### Experimental design

Eight  $15 \times 10 \text{ m}$  plots were established at the same altitude (930 m above sea level) on the slope. Four of the plots received the drought treatment and four were left as control plots. The drought treatment consisted of partial rain exclusion by suspending PVC strips at a height of 0.5–0.8 m above soil surface that covered approximately 30% of the total soil surface and 0.8–1 m deep ditches were dug along the entire top edge of the upper part of the treatment plots to intercept runoff water (Sardans and Peñuelas 2007). The drought treatment began in March 1999 (Ogaya et al. 2003). Soil moisture was measured every 2 weeks throughout the experiment, as described in Sardans and Peñuelas (2007).

### Sampling process

At the beginning of the experiment (January 1999) and after 6 years of drought treatment application (January 2005), we conducted a general biomass and soil sampling, in order to estimate the total contents in stand biomass and in soil. Eight samples of leaves and stems from the three dominant species (*Quercus ilex*, *Phillyrea latifolia* and *Arbutus unedo*) were randomly sampled in each plot (four samples in the sun and four samples in the shade). The leaves were sampled between 1.5 and 6 m of height where most foliar biomass was located. The sample collection was standardized in order to avoid bias results due to different age of tissues and position with respect to sunlight. The leaves sampled were from cohorts flushed in 1998 and 2004 that represented the majority of the leaves of the plants of these three species when sampling. Stems were collected separately and stems of 0.3–2 cm and more than 2 cm in diameter were differentiated. We collected four samples of each stem

**Table 1** Soil properties at 0–15 cm soil depth

|   |                     |
|---|---------------------|
| pH (in water extracts)                                      | $6.5 \pm 0.5$       |
| pH (in 0.1 M KCl extracts)                                  | $6.2 \pm 0.5$       |
| CEC (cation exchange capacity) (meq/100 g soil)             | $14.5 \pm 0.9$      |
| Base exchangeable (meq/100 g soil)                          | $10.0 \pm 0.8$      |
| Percent base saturation (%)                                 | $70.0 \pm 7.1$      |
| Total soil N ( $\text{mg g}^{-1}$ )                         | $2.46 \pm 1.32$     |
| $\text{NH}_4^+$ in 1 M KCl extracts ( $\text{mg kg}^{-1}$ ) | $2.7 \pm 1.4$       |
| Total soil P ( $\text{mg g}^{-1}$ )                         | $0.60 \pm 0.03$     |
| Olsen- $\text{P}_i$ ( $\text{mg g}^{-1}$ )                  | $0.0055 \pm 0.0006$ |
| Total soil K ( $\text{mg g}^{-1}$ )                         | $15.5 \pm 0.3$      |

Values are mean  $\pm$  SE ( $n = 4$ )

diameter class per plot. We only sampled the trees and shrubs of the diameter class between 2 and 12 cm of BD (at 5 cm), that represent the greater portion of the community biomass (Ogaya and Peñuelas 2003; Ogaya et al. 2007).

Litterfall was collected in 20 circular baskets of 27 cm diameter with 1.5 mm mesh diameter, randomly distributed on the ground of each of the eight plots. The fallen litter was collected every 15 days during 1999 and every 2 months during 2004. We took separately five leaf litter samples of each species (*Quercus ilex*, *Phillyrea latifolia*, *Arbutus unedo*) in each plot. Leaf litter that represented 87% of the total litter mass was analyzed like biomass.

We randomly sampled five cores from the first 30 cm of soil profile (Horizon A) in control plots and ten in each drought plot. In the drought plots, we distinguished two levels of drought: that of the soil between the strips (D) (runoff exclusion) and that under the strips (DD) (runoff exclusion plus rainfall exclusion). We analyzed these two soil fractions separately because we had previously observed that soil moisture decreased more under plastic strips than between plastic strips, these differences being significant in winter (27%). Five soil cores were taken between strips and five under the strips, at a minimum distance of 1 m from the nearest tree or shrub; in each control plot only five soil cores were randomly sampled. We collected and analyzed separately the 0–15 cm-deep soil and the 15–30 cm-deep soil in each soil core, since horizon A had an A<sub>1</sub> subhorizon (first 15 cm) rich in organic matter (7.25% W/W) and an A<sub>2</sub> subhorizon (15–30 cm) with only moderate amounts of organic matter (1.3% W/W).

Additionally five soil holes per plot below *Quercus ilex* were scratched and roots ( $\phi > 5$  mm) of this species were sampled in order to have a reference for the effects of drought on root nutrient concentrations. Ten bedrock samples were also collected and their nutrient concentrations were analyzed.

#### Biomass and litter determination

Just before the treatment was begun, in January 1999, all living stems of the three dominant species with a diameter of over 2 cm were tagged at 50 cm height above the ground. Circumferences were measured at this 50 cm height with a metric tape. In January 2005,

the circumferences of the stems were measured again to calculate the annual stem diameter increment.

The aboveground biomass of *Q. ilex*, *P. latifolia* and *A. unedo* was estimated using allometric relationships between aboveground tree biomass and the diameter at 50 cm (D50) described in Sardans and Peñuelas (2007).

Total litterfall was estimated by the proportion of the surface area of the plots covered by the collecting baskets.

#### Sample preparation

All the samples were carried to the laboratory and stored at 4°C prior to analysis (no longer than 3 weeks). For all samples, 3 weeks was the maximum time from sampling until acid digestion. In order to analyze only the nutrients in foliar tissues, leaves were washed with distilled water as in Porter (1986). Biomass samples were washed and then dried along with soil samples in an oven at 60°C until constant weight. Then, they were ground up in a CYCLOTEC 1093 (Foss Tecator, Höganäs, Sweden)—in the case of the biomasses—or in a FRITSCH Pulverisette (Rudolstadt, Germany)—in the case of the soils and bedrocks.

#### Soil exchangeable basic cations and soil cation exchange capacity

Exchangeable basic cations (Na, K, Ca and Mg), cation exchange capacity (CEC) and the percent base saturation were analyzed using 1 M NH<sub>4</sub>OAc extracting solution as in Chapman (1965). Briefly, 10 g of soil were washed four times with 50 ml additions of NH<sub>4</sub>OAc, allowing each addition to filter through but not allowing the soil to crack or dry, washing the excess of NH<sub>4</sub>OAc in soil with ethanol, and replacing the NH<sub>4</sub> in the soil change complex with 1 M KCl. Exchangeable cations were analyzed with ICP-OES (optic emission spectrometry with inductively coupled plasma). CEC was calculated analyzing NH<sub>4</sub> by the Berthelot reaction (Schinner et al. 1996) using an UNICAM 8675 visible Spectrometer (UNICAM, Cambridge, UK). To calculate the percent base saturation, we divided the sum of the Ca, K, Mg and Na in meq/100 g soil by CEC.



## Soil and bedrock extracts

Total concentrations and the extractable fractions were analyzed in each soil and bedrock sample. The soil extracts were obtained by shaking 2 g of soil (or pulverized bedrock) with 12 ml of solvent (0.01 M  $\text{NaNO}_3$ ) following Yin et al. (2002) and van Elteren and Budic (2004). The soil and the 0.01 M  $\text{NaNO}_3$  solvent were mixed in 50-ml plastic centrifuge tubes and a soil/water ratio of 1/6 was used as in Blaser et al. (2000). Two soil suspensions were prepared for each sample. The soil mixtures were equilibrated by shaking in a reciprocal shaker at 100 strokes  $\text{min}^{-1}$  for 5 h, a technique based on batch extraction studies by Gupta and Mackay (1986). After equilibrium, soil solids were separated from the solution by centrifugation and then by filtration through a 0.45  $\mu\text{m}$  pore-size membrane filter. The concentrations of Ca, Fe, Mg, Mo and S in the filtrates were determined as described below for biomass and soil digestion.

## Chemical analyses

We measured the concentration of Mo in all biomass, litter, soil, soil extract, and bedrock extract samples using ICP-MS (mass spectroscopy with inductively coupled plasma) using a model Elan-6000 (Perkin Elmer Corp, Inc., Norwalk, CO, USA). We used ICP-OES (optic emission spectroscopy with inductively coupled plasma) to analyze the concentrations of Ca, Fe, Mg and S in biomass, litter, soil, soil extract and bedrock extract samples and of K and Na in soil extracts using a model JOBIN IBON JY 38 (Longjumeau, HORIBA Jobin Ibon S.A.S., France).

Before the biomass ICP-MS and ICP-OES analyses, we carried out an acid digestion of the samples with an acid mixture of  $\text{HNO}_3$  (60%) and  $\text{HClO}_4$  (60%) (2:1) in a microwave oven (SAMSUNG, TDS, Seoul, South Korea). Two milliliter of the mixed acid solution were added to 100 mg of dry biomass for each sample. The digested solutions were adjusted 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 analytical procedures of biomasses, we used standard certified biomass (DC73351). For the soil samples,

digestion was carried out with 0.25 g of ground sample in 9 ml of  $\text{HNO}_3$  (65%) and 4 ml HF (40%) in a microwave oven at 120°C for 8 h (Bargagli et al. 1995). The digested solutions were adjusted to 50 ml final volume. The digested solutions were filtered with a Millex 0.45  $\mu\text{m}$  filter and stored at 4°C until their determination. Analytical precision for soil and bedrock analyses, as verified by parallel analyses of an international (GSR-6) standard, was better than 5% for all elements analyzed.

## Soil pH and element solubility

We measured soil pH in a 1:2.5 soil solution (in both water and 0.01 M KCl) using a glass electrode (ORION 960 Autochemistry SYSTEM, Thermo Electron Corporation, MA, USA) in all the soil samples. We experimentally determined the solubility of the different elements by calculating the following ratio:

$$\frac{[\text{Element}] \text{ in } 0.01 \text{ M } \text{NaNO}_3}{\text{soil extracts}/[\text{Element}] \text{ in soil}}$$

## Statistical analyses

The effects of the drought treatment on each variable studied were investigated by means of ANOVA analyses. A post-hoc test (Bonferroni/Dunn) was also conducted to compare results of the analyses of control soil variables with those from the two soil drought levels of drought plots. These analyses were conducted with the Statview 5.01 programme (Abacus Concepts, SAS Institute Inc., Berkeley, CA, USA).

## Results

### Soil moisture and pH

During the period 1999–2005 the soil in drought treatment plots with runoff exclusion (D) had an average soil moisture of  $17.5 \pm 0.5\%$  ( $n = 100$ ) that was 9% lower than in the soil from control plots, which had an average soil moisture of  $19.2 \pm 0.5\%$  ( $n = 100$ ) ( $P = 0.026$ ). The soil submitted to both

runoff and rainfall exclusion (DD) had an average soil moisture of  $15.4 \pm 1.0\%$  ( $n = 100$ ) that was 20% lower than in the soil from control plots ( $P = 0.0018$ ), and 15% lower than in the soil submitted only to runoff exclusion ( $P = 0.083$ ). Detailed soil water content changes among C, D and DD soils during the period 1999–2005 are shown in Fig. 2. Drought had no effects on soil pH neither in water extracts nor in KCl extracts (data not shown).

#### Aboveground biomass accumulation (1999–2005)

Drought reduced *Quercus ilex* leaf biomass accumulation by  $15,378 \pm 11,125 \text{ kg ha}^{-1}$  whereas in control plots leaf biomass accumulation increased by  $41 \pm 179 \text{ kg ha}^{-1}$ , although this drought effect was only marginally significant ( $P = 0.07$ ). Drought had no effects on *Quercus ilex* wood biomass accumulation nor on *Phillyrea latifolia* leaf and wood biomass accumulation. In drought plots *Arbutus unedo* leaf, wood and total biomass accumulation increased by  $29 \pm 7$ ,  $311 \pm 156$  and  $580 \pm 162 \text{ kg ha}^{-1}$ , respectively, whereas the corresponding increases were significantly higher in control plots ( $153 \pm 42$ ,  $2,452 \pm 1,026$  and  $2,605 \pm 1,077 \text{ kg ha}^{-1}$ , respectively). The growth level observed in *Quercus ilex* plants during this period was similar than those observed by Mayor and Rodà (1994) in a near

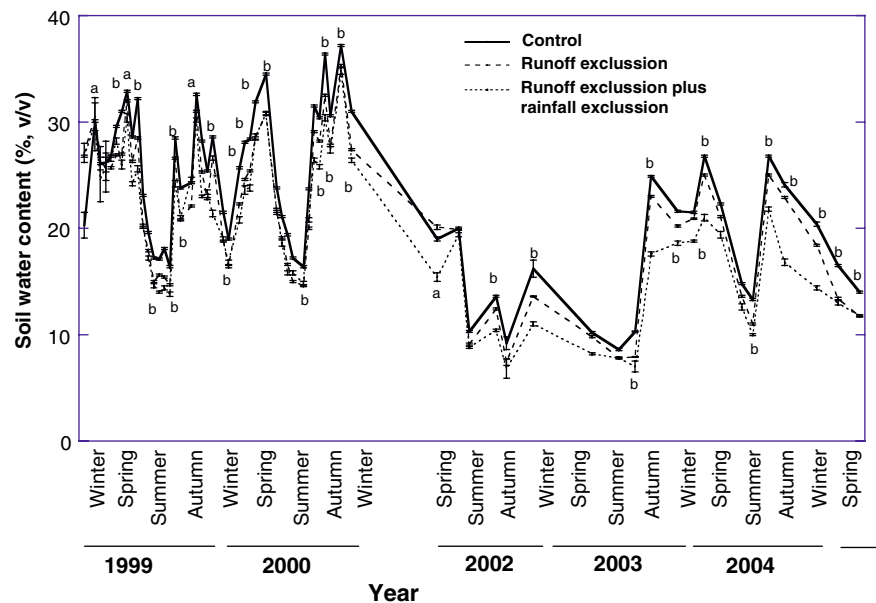
*Quercus ilex* forest located at 300 m of the experimental plots.

#### Biomass concentrations

The biomass concentrations of the five elements studied during 1999 were similar in all plots, those that would be used as control and those that would be used as drought plots (Table 2). The concentrations in the stems of diameter 0.3–3 cm did not differ from those with a diameter of more than 3 cm and thus only one stem concentration was calculated for all stem diameters, which was considered as wood concentration.

Drought decreased leaf Ca concentrations in *Phillyrea latifolia* and in *Arbutus unedo* (18 and 38%, respectively) and increased root Ca concentrations (26%) in *Quercus ilex* (Table 2). Drought decreased leaf Fe concentrations (30 and 65%, respectively) and increased root Fe concentrations (127%) in *Quercus ilex* (Table 2). Drought decreased wood Mg concentrations (27%) in *Arbutus unedo* and increased leaf Mg concentrations in *Quercus ilex* and in *Arbutus unedo* (20 and 14%, respectively). Regarding leaf litter, only an increase in Mg concentrations was observed in *Quercus ilex* in drought plots (Table 2). Drought decreased wood Mo concentrations (51%) in *Arbutus unedo* (Table 2)

**Fig. 2** Mean soil moisture ( $\pm$ SE, % soil water content v/v) in control soils (C), soils without runoff (D) and soils without runoff plus partial rainfall (DD) throughout the period (1999–2005). No data available from winter 2001 to winter 2002 due to a TDR instrumentation fault. a, indicates statistically significant differences at  $P < 0.05$  ( $t$ -student test between DD and C); b, indicates statistically significant differences at  $P < 0.05$  ( $t$ -student test between C and D)



**Table 2** Concentrations of Ca, Fe, Mg, Mo and S in  $\text{mg g}^{-1}$  ( $\text{*mg kg}^{-1}$ ) in the biomass fractions of *Arbutus unedo*, *Phillyrea latifolia*, and *Quercus ilex*, in March 1999 and January 2005

| Element                  | Treatment | Species              |                |               | <i>Phillyrea latifolia</i> |               |               | <i>Quercus ilex</i> |               |               |                   |  |  |
|--------------------------|-----------|----------------------|----------------|---------------|----------------------------|---------------|---------------|---------------------|---------------|---------------|-------------------|--|--|
|                          |           | <i>Arbutus unedo</i> |                |               | Leaves                     | Wood          | Litter        | Leaves              | Wood          | Litter        | Roots             |  |  |
|                          |           | Leaves               | Wood           | Litter        |                            |               |               |                     |               |               |                   |  |  |
| 1999 (before treatment)  |           |                      |                |               |                            |               |               |                     |               |               |                   |  |  |
| Ca                       | Control   | 7.85 ± 0.56          | 9.48 ± 1.30    | 18.8 ± 0.6    | 9.4 ± 0.7                  | 6.49 ± 0.51   | 17.0 ± 1.0    | 7.97 ± 0.95         | 16.8 ± 1.6    | 10.8 ± 0.6    |                   |  |  |
|                          | Drought   | 8.31 ± 0.72          | 9.46 ± 0.80    | 16.7 ± 1.4    | 10.3 ± 1.0                 | 6.33 ± 0.39   | 18.4 ± 1.9    | 8.26 ± 0.57         | 16.3 ± 1.1    | 11.9 ± 1.1    |                   |  |  |
| Fe                       | Control   | 0.090 ± 0.011        | 0.120 ± 0.040  | 0.129 ± 0.025 | 0.174 ± 0.037              | 0.138 ± 0.009 | 0.229 ± 0.035 | 0.219 ± 0.074       | 0.257 ± 0.018 | 0.303 ± 0.024 |                   |  |  |
|                          | Drought   | 0.065 ± 0.011        | 0.118 ± 0.043  | 0.103 ± 0.012 | 0.143 ± 0.023              | 0.135 ± 0.010 | 0.344 ± 0.084 | 0.134 ± 0.007       | 0.221 ± 0.041 | 0.392 ± 0.049 |                   |  |  |
| Mg                       | Control   | 1.50 ± 0.09          | 0.999 ± 0.139  | 1.93 ± 0.14   | 1.188 ± 0.053              | 0.999 ± 0.119 | 1.40 ± 0.09   | 1.07 ± 0.06         | 1.26 ± 0.09   | 0.91 ± 0.16   |                   |  |  |
|                          | Drought   | 1.38 ± 0.13          | 1.010 ± 0.149  | 1.65 ± 0.12   | 1.178 ± 0.085              | 0.952 ± 0.140 | 1.43 ± 0.15   | 1.12 ± 0.08         | 1.19 ± 0.04   | 1.17 ± 0.08   |                   |  |  |
| Mo*                      | Control   | 0.159 ± 0.035        | 0.077 ± 0.029  | 0.337 ± 0.123 | 0.337 ± 0.118              | 0.054 ± 0.004 | 0.216 ± 0.091 | 0.126 ± 0.046       | 0.033 ± 0.001 | 0.248 ± 0.147 |                   |  |  |
|                          | Drought   | 0.107 ± 0.019        | 0.069 ± 0.037  | 0.289 ± 0.103 | 0.222 ± 0.101              | 0.053 ± 0.003 | 0.160 ± 0.094 | 0.137 ± 0.044       | 0.030 ± 0.002 | 0.152 ± 0.079 |                   |  |  |
| S                        | Control   | 1.88 ± 0.16          | 1.11 ± 0.22    | 1.48 ± 0.11   | 1.27 ± 0.12                | 0.909 ± 0.115 | 1.59 ± 0.10   | 1.06 ± 0.08         | 0.724 ± 0.045 | 1.11 ± 0.08   |                   |  |  |
|                          | Drought   | 1.46 ± 0.10          | 1.13 ± 0.18    | 1.77 ± 0.10   | 1.34 ± 0.05                | 0.877 ± 0.109 | 1.56 ± 0.08   | 1.10 ± 0.03         | 0.718 ± 0.039 | 1.09 ± 0.09   |                   |  |  |
| 2005 (6 years treatment) |           |                      |                |               |                            |               |               |                     |               |               |                   |  |  |
| Ca                       | Control   | 10.2 ± 0.4           | 9.51 ± 0.85a   | 18.3 ± 1.1    | 13.0 ± 0.7a                | 4.54 ± 0.21   | 20.0 ± 1.1    | 11.9 ± 0.7          | 19.5 ± 1.8    | 16.1 ± 0.9    | 12.5 ± 0.6b       |  |  |
|                          | Drought   | 10.6 ± 0.4           | 5.94 ± 0.49b   | 17.5 ± 1.7    | 10.7 ± 0.5b                | 5.37 ± 0.48   | 20.6 ± 1.1    | 10.6 ± 0.5          | 15.8 ± 1.3    | 14.9 ± 0.9    | 15.8 ± 0.8a       |  |  |
| Fe                       | Control   | 0.107 ± 0.007a       | 0.343 ± 0.105a | 0.414 ± 0.072 | 0.138 ± 0.011              | 0.191 ± 0.022 | 0.807 ± 0.156 | 0.284 ± 0.016       | 0.242 ± 0.015 | 1.073 ± 0.161 | 1.098 ± 0.105b    |  |  |
|                          | Drought   | 0.075 ± 0.004b       | 0.120 ± 0.013b | 0.712 ± 0.402 | 0.114 ± 0.008              | 0.183 ± 0.016 | 0.835 ± 0.242 | 0.286 ± 0.023       | 0.222 ± 0.017 | 0.835 ± 0.133 | 2.593 ± 0.600a    |  |  |
| Mg                       | Control   | 2.49 ± 0.06b         | 0.813 ± 0.109a | 2.40 ± 0.16   | 2.08 ± 0.09                | 0.743 ± 0.059 | 2.29 ± 0.10   | 1.66 ± 0.11b        | 1.35 ± 0.02   | 1.77 ± 0.10b  | 1.68 ± 0.16       |  |  |
|                          | Drought   | 2.83 ± 0.07a         | 0.595 ± 0.042b | 2.75 ± 0.13   | 1.97 ± 0.09                | 0.850 ± 0.152 | 2.23 ± 0.15   | 2.00 ± 0.08a        | 1.52 ± 0.16   | 2.03 ± 0.10a  | 1.72 ± 0.20       |  |  |
| Mo*                      | Control   | 0.121 ± 0.029        | 0.101 ± 0.011a | 0.095 ± 0.012 | 0.191 ± 0.033              | 0.051 ± 0.011 | 0.146 ± 0.019 | 0.120 ± 0.009       | 0.053 ± 0.006 | 0.096 ± 0.013 | 0.00042 ± 0.00004 |  |  |
|                          | Drought   | 0.078 ± 0.007        | 0.049 ± 0.006b | 0.115 ± 0.019 | 0.150 ± 0.020              | 0.076 ± 0.009 | 0.209 ± 0.051 | 0.126 ± 0.012       | 0.062 ± 0.009 | 0.108 ± 0.010 | 0.00045 ± 0.00006 |  |  |
| S                        | Control   | 1.53 ± 0.05          | 0.934 ± 0.214  | 0.914 ± 0.076 | 1.96 ± 0.08                | 0.853 ± 0.038 | 1.50 ± 0.07   | 1.41 ± 0.06b        | 0.635 ± 0.036 | 1.31 ± 0.07   | 0.619 ± 0.029b    |  |  |
|                          | Drought   | 1.42 ± 0.07          | 0.623 ± 0.052  | 1.068 ± 0.184 | 1.84 ± 0.06                | 0.941 ± 0.070 | 1.70 ± 0.09   | 1.56 ± 0.04a        | 0.853 ± 0.078 | 1.39 ± 0.08   | 0.784 ± 0.037a    |  |  |

Different letters indicate significant differences between control and drought concentrations at  $P < 0.05$ . They are highlighted in bold type

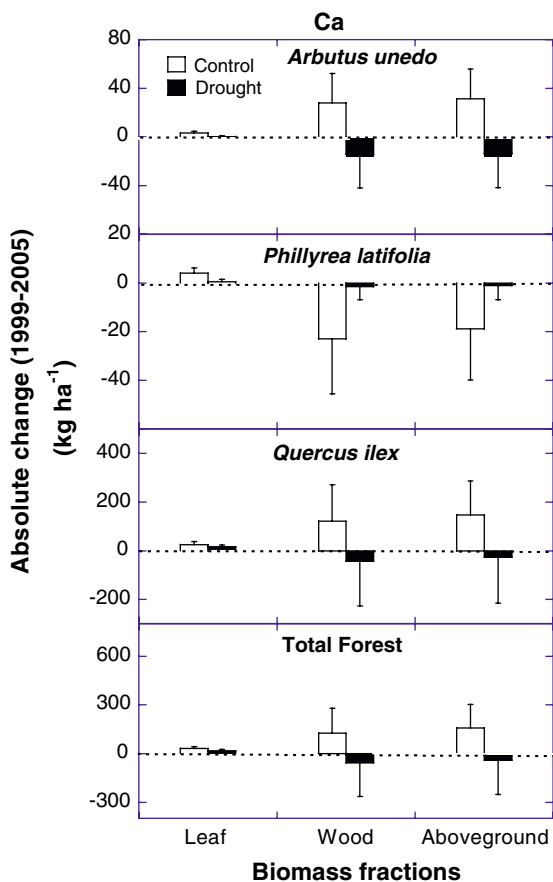


and increased leaf and root S concentrations (11 and 27%, respectively) in *Quercus ilex*.

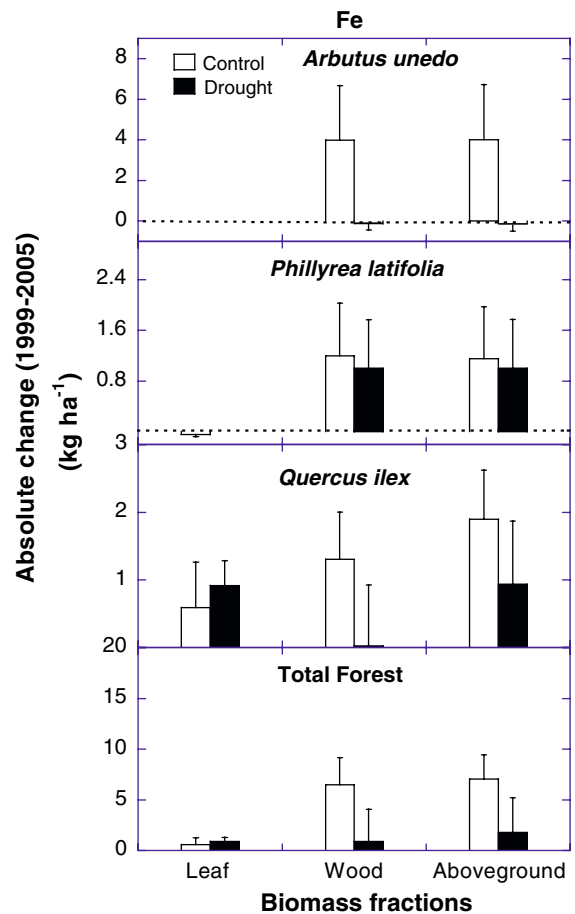
### Biomass nutrient accumulation

Drought had no significant effects on the Ca, Fe, Mg, Mo and S bioaccumulation of *Quercus ilex* neither on total aboveground biomass of the ecosystem (*Quercus ilex* plus two dominant tall shrubs) (Figs. 3–7).

Drought significantly decreased wood and total aboveground Mo content in *Arbutus unedo* (Fig. 6). In general, drought tended to reduce the mineralomass accumulation of Ca, Fe, Mg and S in total aboveground



**Fig. 3** Absolute ( $\text{g ha}^{-1}$ ) change of Ca content in leaf, stems and aboveground biomass of *Arbutus unedo*, *Phillyrea latifolia*, *Quercus ilex* and the overall forest (the three dominant species together which represent 98% of total aboveground ecosystem biomass) in control and in drought plots in the period 1999–2005. Different letters indicate statistically different values at  $P < 0.05$  between control and drought plots ( $t$ -student test between DD and C)

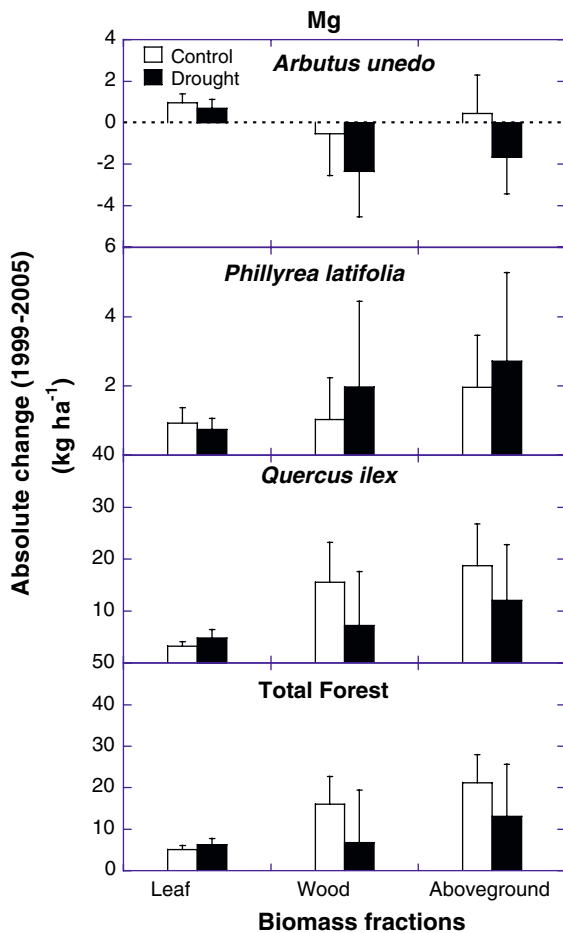


**Fig. 4** Absolute ( $\text{g ha}^{-1}$ ) change of Fe content in leaf, stems and aboveground biomass of *Arbutus unedo*, *Phillyrea latifolia*, *Quercus ilex* and the overall forest (the three dominant species together which represent 98% of total aboveground ecosystem biomass) in control and in drought plots in the period 1999–2005. Different letters indicate statistically different values at  $P < 0.05$  between control and drought plots ( $t$ -student test)

biomass of *Arbutus unedo* but these effects were in most cases not significant because of the high variability of the results (Figs. 3–7). Drought increased Mo mineralomass accumulation in the aboveground biomass of *Phillyrea latifolia*, but had no effects on any other mineralomass of this species (Figs. 3–7).

### Litter content

Drought did not change the Ca, Fe, Mg, Mo and S losses in leaf litter in any species in the year that treatments started, 1999 (Table 3). Drought reduced the losses in

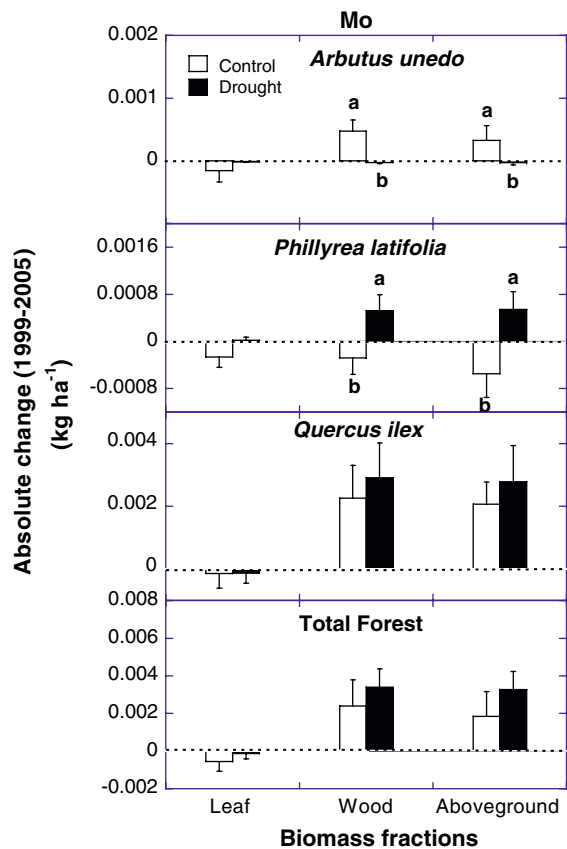


**Fig. 5** Absolute ( $\text{g ha}^{-1}$ ) change of Mg content in leaf, stems and aboveground biomass of *Arbutus unedo*, *Phillyrea latifolia*, *Quercus ilex* and the overall forest (the three dominant species together which represent 98% of total aboveground ecosystem biomass) in control and in drought plots in the period 1999–2005. Different letters indicate statistically different values at  $P < 0.05$  between control and drought plots ( $t$ -student test)

leaf litter of the five elements in 2005 in *Arbutus unedo*, this effect significant being for Ca, Mg and S (73, 61 and 66%, respectively) (Table 3). This effect was mainly due to the reduction in leaf litter production induced by drought in this species. Drought had no significant effects on the element losses in leaf litter in *Phillyrea latifolia* and *Quercus ilex* during 2005 (Table 3).

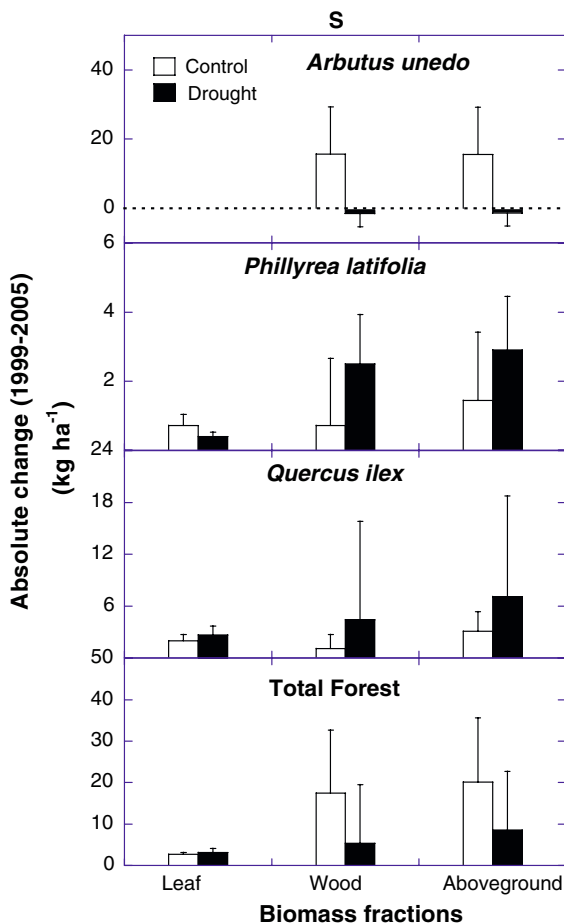
#### Soil concentrations

Drought increased the  $\text{NaNO}_3$  extractable-Fe at 0–15 cm of soil depth and at 15–30 cm of soil depth



**Fig. 6** Absolute ( $\text{g ha}^{-1}$ ) change of Mo content in leaf, stems and aboveground biomass of *Arbutus unedo*, *Phillyrea latifolia*, *Quercus ilex* and the overall forest (the three dominant species together which represent 98% of total aboveground ecosystem biomass) in control and in drought plots in the period 1999–2005. Different letters indicate statistically different values at  $P < 0.05$  between control and drought plots ( $t$ -student test)

(Fig. 8). The ratio between these  $\text{NaNO}_3$  extractable-Fe and the corresponding total soil Fe also increased (Fig. 8). Drought raised the  $\text{NaNO}_3$  soil extractable-Mg both at 0–15 and at 15–30 cm of soil depth and the ratio between these  $\text{NaNO}_3$  extractable-Mg and the corresponding total soil Mg both at 0–15 cm and at 15–30 cm of soil depth also increased (Fig. 9). Drought decreased the total soil Mg concentration at 0–15 cm of soil depth (Fig. 9). Drought increased both total soil S and soil  $\text{NaNO}_3$  extractable-S at 0–15 cm and total soil S at 15–30 cm (Fig. 10). Drought decreased the ratio between these  $\text{NaNO}_3$  extractable-S and the corresponding total soil S (Fig. 10). Drought had no significant effects on soil Mo and Ca concentration ( $0.0006$  and  $0.3 \text{ mg g}^{-1}$ ,



**Fig. 7** Absolute ( $\text{g ha}^{-1}$ ) change of S content in leaf, stems and aboveground biomass of *Arbutus unedo*, *Phillyrea latifolia*, *Quercus ilex* and the overall forest (the three dominant species together which represent 98% of total aboveground ecosystem biomass) in control and in drought plots in the period 1999–2005. Different letters indicate statistically different values at  $P < 0.05$  between control and drought plots ( $t$ -student test)

respectively) nor on the  $\text{NaNO}_3$  extractable concentrations ( $0.0003$  and  $0.2 \text{ mg ml}^{-1}$ , respectively).

Soil exchangeable basic cations and soil cation exchange capacity (CEC)

Runoff exclusion had no significant effects on exchangeable Ca, K and Na, and neither on total exchangeable cations nor CEC (Table 4). Soil with runoff exclusion plus partial rainfall exclusion had higher Mg exchangeable content (23%) (Table 4). There was a tendency to increase the percent base

saturation of the soil exchangeable complex in drought soils, but this tendency was not significant (Table 4).

## Discussion

Ca, Fe, Mg, Mo and S in soil and plants: a general overview

After 6 years of drought, and except in the case of Mo, the effects of drought were higher at soil level than at biomass level. The drought treatments increased total soil S concentration and decreased total soil Mg concentration. Drought increased the concentrations of Fe, Mg and S in soil  $\text{NaNO}_3$  water extracts. Moreover, in the case of Fe and Mg, drought increased the ratio soil soluble content/total soil content. Thus drought increased soil Fe, Mg and S availability when soil is rewetting. Drought increased the Mg concentrations in soil exchangeable complex in spite of the total soil Mg reduction, showing an increase of the soil Mg mobilization capacity of this element in drought plots. The rewetting effects on soil microbes can contribute to explain a greater liberation of Fe, Mg and S to soil water (Turner and Haygarth 2001).

The observed foliar concentrations and the fact that drought did not reduce the litter concentrations indicate that the five elements studied are currently not limiting. The biomass concentrations which were generally slightly lower or similar to most values reported in the literature for shrubs and trees of the Mediterranean Basin (Table 5), responded to drought depending on the species.

The greatest asymmetrical effect of drought on species biomass concentrations occurred at the wood level. Drought did not change concentrations of all five analyzed elements in the wood of *Quercus ilex* and *Phillyrea latifolia*, but their concentration decreased in the wood biomass of *Arbutus unedo*, this effect being significant for Ca, Fe, Mg and Mo. At the species level, *Arbutus unedo* was the species that presented the greatest changes in biomass accumulation of practically all the elements due to drought coinciding with a large decrease in growth. The significant decrease in wood growth observed as response to drought in *Arbutus unedo* was correlated with a reduction in the wood concentration of the five elements analyzed

**Table 3** Ca, Fe, Mg, Mo and S content in the litter (mean kg ha<sup>-1</sup>, \*g ha<sup>-1</sup> ± SE) in 1999 and in 2005 in the three dominant species

| Species   | Treatment | Element           |               |                     |               |                       |
|---|-----------|-------------------|---------------|---------------------|---------------|-----------------------|
|   |           | Ca                | Fe            | Mg                  | Mo*           | S                     |
| 1999  |           |                   |               |                     |               |                       |
| <i>Arbutus unedo</i>  | C         | 5.1 ± 1.8         | 0.033 ± 0.014 | 0.498 ± 0.170       | 0.084 ± 0.052 | 0.372 ± 0.019         |
|   | D         | 5.1 ± 1.7         | 0.022 ± 0.008 | 0.518 ± 0.173       | 0.087 ± 0.045 | 0.537 ± 0.177         |
| <i>Phillyrea latifolia</i>  | C         | 3.8 ± 2.2         | 0.039 ± 0.014 | 0.316 ± 0.191       | 0.082 ± 0.063 | 0.358 ± 0.214         |
|   | D         | 6.7 ± 2.8         | 0.095 ± 0.021 | 0.510 ± 0.209       | 0.075 ± 0.063 | 0.533 ± 0.204         |
| <i>Quercus ilex</i>   | C         | 7.8 ± 2.9         | 0.223 ± 0.081 | 0.563 ± 0.145       | 0.233 ± 0.182 | 0.827 ± 0.316         |
|   | D         | 9.0 ± 3.5         | 0.248 ± 0.078 | 0.785 ± 0.248       | 0.077 ± 0.032 | 0.781 ± 0.286         |
| <i>Arbutus unedo</i> + <i>Phillyrea latifolia</i> + <i>Quercus ilex</i> | C         | 16.7 ± 2.4        | 0.294 ± 0.080 | 1.38 ± 0.15         | 0.399 ± 0.153 | 1.56 ± 0.25           |
|   | D         | 20.7 ± 1.5        | 0.371 ± 0.074 | 1.81 ± 0.18         | 0.240 ± 0.025 | 1.85 ± 0.21           |
| 2005  |           |                   |               |                     |               |                       |
| <i>Arbutus unedo</i>  | C         | <b>8.9 ± 2.4a</b> | 0.175 ± 0.061 | <b>1.14 ± 0.20a</b> | 0.043 ± 0.017 | <b>0.441 ± 0.064a</b> |
|   | D         | <b>2.4 ± 0.4b</b> | 0.071 ± 0.025 | <b>0.40 ± 0.20b</b> | 0.018 ± 0.010 | <b>0.148 ± 0.071b</b> |
| <i>Phillyrea latifolia</i>  | C         | 11.2 ± 5.9        | 0.370 ± 0.150 | 1.29 ± 0.67         | 0.069 ± 0.032 | 0.92 ± 0.52           |
|   | D         | 18.3 ± 10.0       | 0.460 ± 0.140 | 1.73 ± 0.83         | 0.219 ± 0.163 | 1.50 ± 0.80           |
| <i>Quercus ilex</i>   | C         | 11.1 ± 4.9        | 0.695 ± 0.237 | 1.26 ± 0.56         | 0.057 ± 0.014 | 0.92 ± 0.42           |
|   | D         | 13.9 ± 4.9        | 0.867 ± 0.314 | 1.83 ± 0.62         | 0.099 ± 0.033 | 1.32 ± 0.46           |
| <i>Arbutus unedo</i> + <i>Phillyrea latifolia</i> + <i>Quercus ilex</i> | C         | 31.3 ± 6.1        | 1.23 ± 0.27   | 3.69 ± 0.63         | 0.165 ± 0.039 | 2.28 ± 0.46           |
|   | D         | 34.7 ± 6.3        | 1.39 ± 0.24   | 3.96 ± 0.41         | 0.336 ± 0.132 | 2.96 ± 0.48           |

Different letters indicate significant differences between control and drought concentrations at  $P < 0.05$  ( $t$ -student test). They are highlighted in bold type

showing that growth reduction capacity is related to the capacity to absorb these elements in response to drought conditions. Moreover, drought decreased litterfall production in *Arbutus unedo* diminishing the amounts of these five elements lost in litterfall, an effect that was not observed in the other two species.

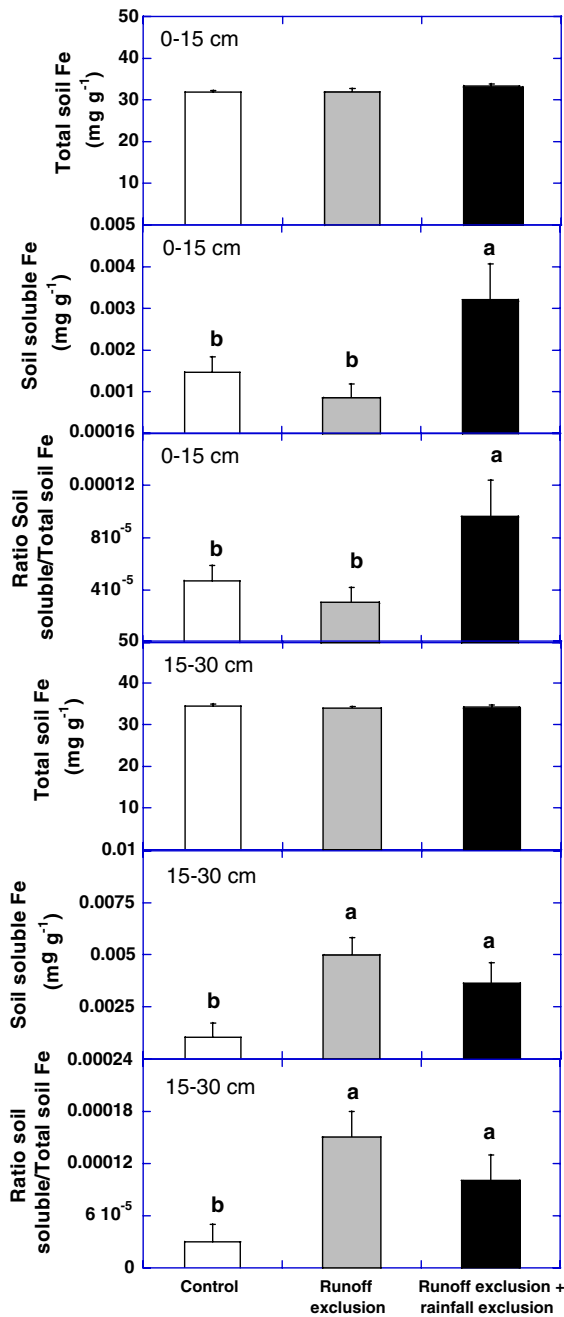
In control plots aboveground biomass accumulation, taking all species together, has been  $7,490 \pm 2,560$  kg ha<sup>-1</sup> in the period 1999–2005. This accumulation agrees with that observed for the first 2 years after the beginning of the experiment ( $1,900$  kg ha<sup>-1</sup>) (Ogaya et al. 2003). During the first 5 years of drought treatment, drought reduced the diameter increment of *Quercus ilex* by 41% and that of *Arbutus unedo* by 63% (Ogaya and Peñuelas 2007). The decrease of plant growth is the main effect that explains the tendency for a reduction in total Fe, Mg and S accumulation in the aerial tissues of those two species observed in drought plots. But drought had weak effects on bioaccumulation of the five elements analyzed at the ecosystem level because of its minor impact on the growth of the dominant tree species, *Quercus ilex*, which represents the greatest part of the ecosystem biomass. The

strongest effects of drought on aboveground content of the different elements were seen in Mo. Drought increased Mo accumulation in wood and total aboveground biomass in *Phillyrea latifolia* and decreased it in wood and total aboveground biomass in *Arbutus unedo*. In general, drought tended to reduce the total contents of the elements Ca, Fe, Mg and S which are transported by transpiration, but increased total Mo contents in aboveground biomass. Taking into account the global drought effects at ecosystem level, drought increased the soil availability of Fe, Mg and S whereas it tended to decrease the total contents of these three nutrients in aboveground biomass. However, the concentration of leaves and litter does not demonstrate a definite effect of drought on retranslocation patterns.

#### Specific nutrient responses

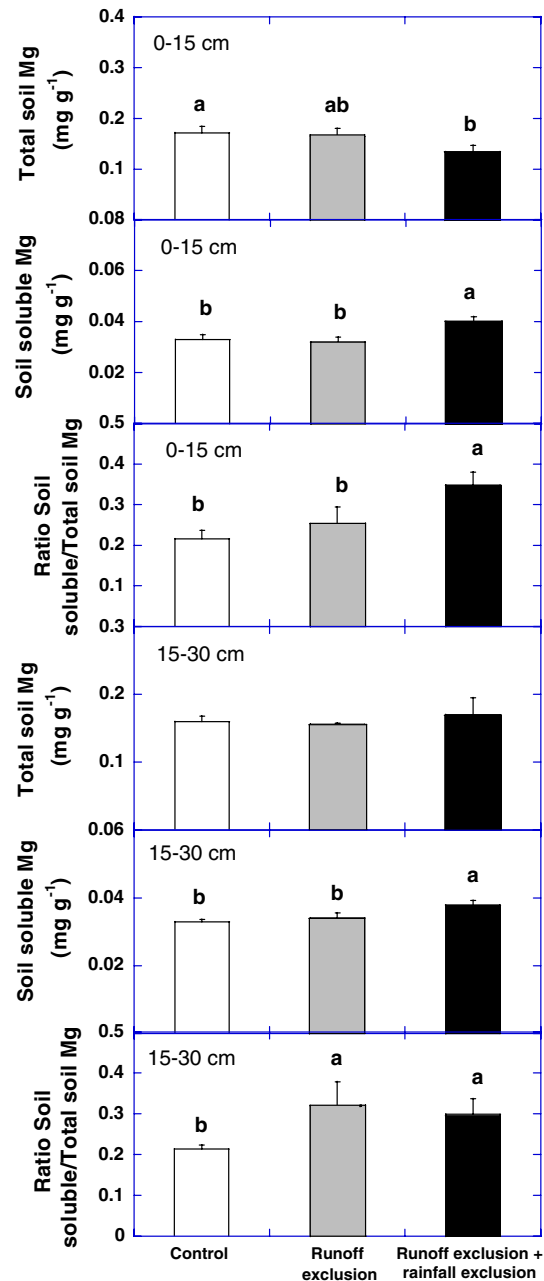
##### Calcium

Drought tended to decrease Ca concentrations in aboveground biomass. This was significant for the



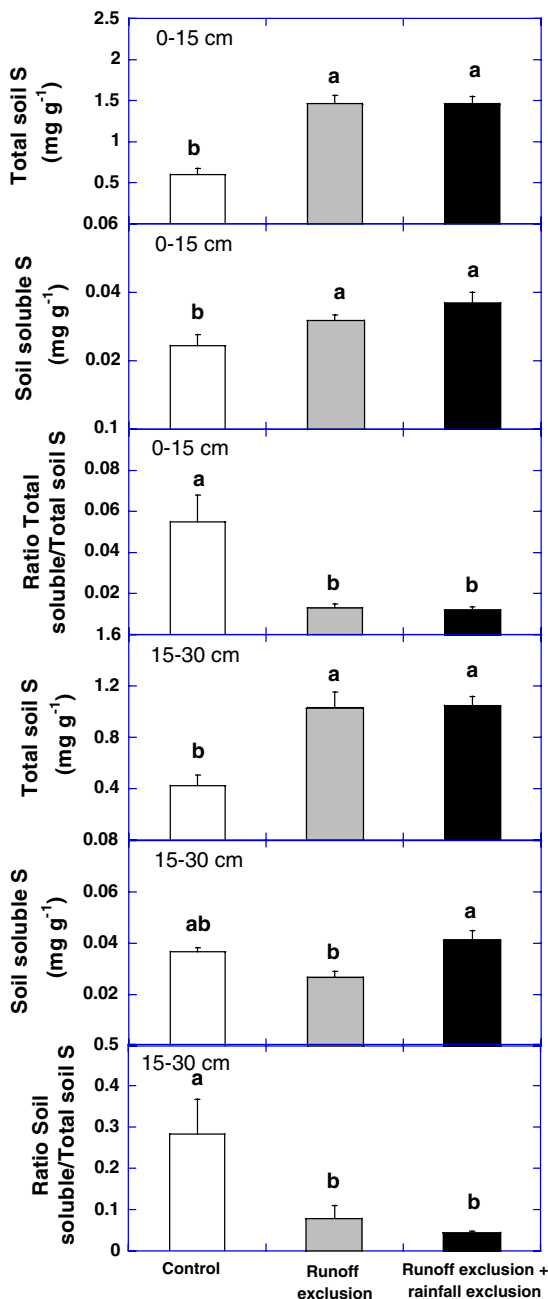
**Fig. 8** Concentration of Fe in soil (mg g<sup>-1</sup>) and in soil extracts (mg ml<sup>-1</sup>) (0.01 M NaNO<sub>3</sub>), and the ratio between concentration in soil extract/concentration in soil (mean  $\pm$  SE) of control plots and of drought plots at 0–15 cm and at 15–30 cm. Different letters indicate statistically significant differences at  $P < 0.05$ ; Bonferroni post-hoc test in an ANOVA

wood of *Arbutus unedo* and the leaves of *Phillyrea latifolia*, however drought increased the root Ca concentrations of *Quercus ilex*. There is a high



**Fig. 9** Concentration of Mg in soil (mg g<sup>-1</sup>) and in soil extracts (mg ml<sup>-1</sup>) (0.01 M NaNO<sub>3</sub>), and the ratio between concentration in soil extract/concentration in soil (mean  $\pm$  SE) of control plots and of drought plots at 0–15 cm and at 15–30 cm. Different letters indicate statistically significant differences at  $P < 0.05$ ; Bonferroni post-hoc test in an ANOVA

correlation between Ca absorption capacity and transpiration rates (Marchner 1995), which may account for the decreased Ca uptake of aboveground biomass



**Fig. 10** Concentration of S in soil (mg g<sup>-1</sup>) and in soil extracts (mg ml<sup>-1</sup>) (0.01 M NaNO<sub>3</sub>), and the ratio between concentration in soil extract/concentration in soil (mean ± SE) of control plots and of drought plots at 0–15 cm and at 15–30 cm. Different letters indicate statistically significant differences at  $P < 0.05$ ; Bonferroni post-hoc test in an ANOVA

mainly in *Phillyrea latifolia*. It has been frequently observed that increases in root Ca concentrations contribute to improve the root capacity to absorb

ammonium and thus plant growth (Fenn and Feagley 1999). Although drought decreased the foliar Ca concentrations, the observed values in drought plots (10–11 mg g<sup>-1</sup>) were in the range observed for this species or similar species in other sites (Sardans et al. 2005b, 2006a, b). On the other hand, the soil extractable Ca concentration in this study (0.15–0.20 mg g<sup>-1</sup>) was higher than in soils considered poor in soil extractable Ca with reported values of 0.055 mg g<sup>-1</sup> by Arocena and Glowa (2000) in a Canadian forest. Drought had a minor impact on soil extractable Ca, Ca concentration in the soil exchangeable complex and total soil Ca and in biomass contents. Since the observed soil Ca concentrations are not limiting in comparison with bibliographic data, we conclude that 6 years of drought had weak effects on Ca status in this Mediterranean forest ecosystem. They only reduced the Ca concentration in some species and tissues due to the reduction in transpiration fluxes.

### Iron

Drought increased Fe concentrations in roots (140% in the dominant tree species *Quercus ilex*) but decreased Fe concentrations in all fractions of aboveground biomass of *Quercus ilex*, and drought did not change the total Fe accumulation in aboveground biomass of either species. Thus, the increases in soil soluble Fe observed in drought plots did not increase the absorption of this nutrient in drought soils probably due to the reduction in soil moisture, soil diffusion capacity and water capture.

Drought increased soil soluble Fe without changing the total soil Fe concentration. In these 6 years, drought reduced soil P availability (Sardans and Peñuelas 2004), an effect related to the decrease of soil phosphatase activity (25–40%) and probably indirectly related to a reduction in the activities of other soil enzymes such as  $\beta$ -glucosidase (15–80%), urease (40–60%) and protease (30–60%) (Sardans and Peñuelas 2005). Less soluble phosphate in drought treatments decreased the probability of Fe fixing, and Fe became more available in soil. No effects of drought were observed in total soil Fe concentrations, and the values observed in this experiment (30–35 mg g<sup>-1</sup>) were in the range for unpolluted soils (23–67 mg g<sup>-1</sup>) reported by Abolli-no et al. (2002) in Italy and similar to those reported



**Table 4** Exchangeable cations (Ca, K, Mg and Na) in 1 M NH<sub>4</sub>OAc soil extracts (meq/100 g soil, mean  $\pm$  SE,  $n = 4$ ), cation exchangeable capacity (CEC) and percent base saturation (%) in control soil (C) and in soils submitted to runoff exclusion (D) and to runoff exclusion plus rainfall exclusion (DD)

| Treatments | meq/100 g soil |                         |                                      |                   |                  |
|------------|----------------|-------------------------|--------------------------------------|-------------------|------------------|
|            | Ca             | K                       | Mg                                   | Na                | Ca + K + Mg + Na |
| C          | 8.6 $\pm$ 0.7  | 0.416 $\pm$ 0.068       | <b>0.867 <math>\pm</math> 0.053b</b> | 0.090 $\pm$ 0.003 | 10.0 $\pm$ 0.8   |
| D          | 10.1 $\pm$ 2.1 | 0.452 $\pm$ 0.088       | <b>0.944 <math>\pm</math> 0.153b</b> | 0.086 $\pm$ 0.003 | 11.6 $\pm$ 2.3   |
| DD         | 9.5 $\pm$ 1.1  | 0.395 $\pm$ 0.090       | <b>1.106 <math>\pm</math> 0.064a</b> | 0.088 $\pm$ 0.002 | 11.1 $\pm$ 1.3   |
| CEC        |                | Percent base saturation |                                      |                   |                  |
| C          | 14.5 $\pm$ 0.9 | 70.0 $\pm$ 7.1          |                                      |                   |                  |
| D          | 13.5 $\pm$ 1.2 | 82.4 $\pm$ 12.9         |                                      |                   |                  |
| DD         | 14.7 $\pm$ 1.6 | 78.0 $\pm$ 1.9          |                                      |                   |                  |

Different letters indicate significant differences between control and drought concentrations at  $P < 0.05$  (Bonferroni post-hoc test). They are highlighted in bold type

**Table 5** Ca, Fe, Mg, Mo and S biomass concentrations (mg g<sup>-1</sup>, dw) reported in the literature for related Mediterranean species

| Species   | Biomass fraction | Element         |                   |                   |                     |                   |
|---|------------------|-----------------|-------------------|-------------------|---------------------|-------------------|
|   |                  | Ca              | Fe                | Mg                | Mo                  | S                 |
| <i>Quercus coccifera</i> (Papatheodorou and Stamou 2004, Mountains Hortiatitis, Greece) | Leaves           | 22–25           | 0.14–0.22         | 2.5–3.8           | –                   | –                 |
|   | Wood             | 18–23           | 0.13–0.23         | 1.6–1.9           | –                   | –                 |
| <i>Pinus halepensis</i> (López-Serrano et al. 2005, Yeste, Spain)                       | Needles          | 4–8.2           | –                 | 1.8–3.8           |                     |                   |
| <i>Quercus ilex</i> (present study, Prades mountains Catalonia, Spain)                  | Leaves           | 11.9 $\pm$ 0.7  | 0.284 $\pm$ 0.016 | 1.66 $\pm$ 0.11   | 0.120 $\pm$ 0.009   | 1.41 $\pm$ 0.06   |
|   | Wood             | 19.5 $\pm$ 1.8  | 0.242 $\pm$ 0.015 | 1.35 $\pm$ 0.02   | 0.053 $\pm$ 0.006   | 0.635 $\pm$ 0.036 |
|   | Leaf litter      | 16.1 $\pm$ 0.9  | 1.073 $\pm$ 0.161 | 1.78 $\pm$ 0.11   | 0.096 $\pm$ 0.013   | 1.31 $\pm$ 0.08   |
|   | Roots            | 12.5 $\pm$ 0.6  | 1.098 $\pm$ 0.105 | 1.68 $\pm$ 0.16   | 0.0004 $\pm$ 0.0001 | 0.619 $\pm$ 0.029 |
| <i>Phillyrea latifolia</i> (present study, Prades mountains Catalonia, Spain)           | Leaves           | 13.0 $\pm$ 0.7  | 0.138 $\pm$ 0.011 | 2.08 $\pm$ 0.09   | 0.191 $\pm$ 0.033   | 1.96 $\pm$ 0.08   |
|   | Wood             | 4.54 $\pm$ 0.21 | 0.191 $\pm$ 0.027 | 0.743 $\pm$ 0.059 | 0.051 $\pm$ 0.011   | 0.853 $\pm$ 0.038 |
|   | Leaf litter      | 20.0 $\pm$ 1.1  | 0.807 $\pm$ 0.156 | 2.29 $\pm$ 0.10   | 0.146 $\pm$ 0.019   | 0.150 $\pm$ 0.070 |
| <i>Arbutus unedo</i> (present study, Prades mountains Catalonia, Spain)                 | Leaves           | 10.2 $\pm$ 0.4  | 0.107 $\pm$ 0.007 | 2.49 $\pm$ 0.06   | 0.121 $\pm$ 0.029   | 1.53 $\pm$ 0.05   |
|   | Wood             | 9.5 $\pm$ 0.1   | 0.343 $\pm$ 0.105 | 0.813 $\pm$ 0.109 | 0.101 $\pm$ 0.011   | 0.934 $\pm$ 0.214 |
|   | Leaf litter      | 18.3 $\pm$ 1.1  | 0.414 $\pm$ 0.072 | 2.4 $\pm$ 0.2     | 0.095 $\pm$ 0.012   | 0.914 $\pm$ 0.076 |
| <i>Quercus ilex</i> (Sardans et al. 2006b, Bages Catalonia, Spain)                      | Leaves           | 5–13            | 0.100–0.022       | 1.0–1.7           |                     | 0.57–0.90         |
|   | Wood             | 1.3–1.4         | 0.180–0.019       | 1.8–2.0           |                     | 0.56–0.60         |
| <i>Pinus halepensis</i> (Sardans et al. 2005b, Bages Catalonia, Spain)                  | Leaves           | 3.8–8.2         | 0.120–0.011       | 1.0–1.6           |                     | 1–1.2             |
|   | Wood             | 4.3–4.7         | 0.080–0.010       | 1.06–1.11         |                     | 0.42–0.70         |
| <i>Rosmarinus officinalis</i> (Sardans et al. 2005a, Bages Catalonia, Spain)            | Leaves           | 10–16           |                   |                   |                     | 1.3–1.5           |
|   | Wood             | 4.0–5.0         | 0.180–0.260       |                   |                     |                   |

The reference and the studied sites are depicted between parentheses

(25–35 mg g<sup>-1</sup>) by Vandecasteele et al. (2005) in Belgium. The values of soil extractable Fe concentrations observed in this experiment in the control soils (between 0.09 and 0.15 mg kg<sup>-1</sup>) are relatively low, lower than those observed as a limit for growth (0.5 and 2.2 mg kg<sup>-1</sup>) in a European forest (Dabkowska-Naskret 2000). The effects of drought increasing these values up to 0.30 mg kg<sup>-1</sup> in the soils with runoff exclusion plus partial rainfall exclusion can improve plant nutrition but can also make the soil more vulnerable to Fe losses during the torrential rainfalls. This increase in soil soluble Fe concentrations in drought plots might account for the increased (140%) root Fe concentrations in the dominant species *Quercus ilex*.

### Magnesium

No data about the limiting role of Mg leaf concentrations in Mediterranean ecosystems are available but leaf Mg concentrations of 0.5–1.1 mg g<sup>-1</sup> have been reported as limiting and causing chlorosis in *Pinus radiata* in New Zealand (Laing et al. 2000), leaf Mg concentration of 1.5 mg g<sup>-1</sup> was reported as limiting in European chestnut trees (Weber-Blaschke et al. 2002) and leaf Mg concentrations of 0.16–0.27 mg g<sup>-1</sup> were reported in a *Picea abies* forest heavily affected by Mg deficiency in Germany (Nechwatal and Obwald 2003). Therefore, the observed values of leaf Mg concentrations in this experiment in *Quercus ilex* in the control plots, 1.66–2.83 mg g<sup>-1</sup>, must be considered as low and near limiting, and thus, the increase of leaf Mg concentrations observed in drought plots in *Quercus ilex* and *Arbutus unedo*, that reached values of 2.0 mg g<sup>-1</sup>, might have positive effects for plant photosynthetic capacity. The increases in leaf Mg concentration in *Quercus ilex* and *Arbutus unedo* can be related to a concentration effect because drought reduced growth without changing the transpiration fluxes.

Mg was the element most affected by drought at soil level because drought increased soil soluble Mg and Mg concentration in the soil exchangeable complex whereas drought decreased total soil Mg. These effects in soil were accompanied by an increase in leaf Mg concentrations in the dominant tree, *Quercus ilex*, and an increase in Mg concentrations in leaves and a decrease in Mg concentration in

wood of *Arbutus unedo*, probably as a result of the concentration effect due to the decrease in leaf biomass growth that drought induced in these two species. The proportional increases in Mg in leaves observed in *Quercus ilex* and *Arbutus unedo*, and the soil soluble Mg increases under drought conditions could lead to better nutrient concentration in leaves for plant production and WUE efficiency, but also to a faster Mg cycling between plants and soil. However, in this case these increases in Mg under drought did not produce enhancements in photosynthetic capacity and in WUE (Martínez-Vilalta et al. 2003; Ogaya and Peñuelas 2003).

On the other hand, from a global point of view, drought reduced total Mg contents in the ecosystem because it reduced total soil content and tended to reduced Mg content in aboveground biomass, while increasing the potential Mg losses due to increased soil availability of water exportable Mg. The reduction in Mg content in aboveground biomass was mainly due to the Mg reduction in the wood biomass of *Arbutus unedo*, the species most affected by drought. Since Mg absorption by roots is strongly dependent on the transpiration fluxes (Marchsner 1995), the tendency to maintain transpiration in *Quercus ilex* and *Arbutus unedo*, together with the reduction in soil diffusion capacity by soil moisture reduction in drought plots, can explain the tendency to decrease total Mg accumulation in aboveground biomass.

The increase in soil exchangeable Mg in drought soils was related to this decrease of Mg contents observed in aboveground biomass. Increases in soil available Mg together with decreases in total soil Mg in drought events have also been observed in other ecosystem of the world. In a Mexican tropical dry forest, Campo et al. (2000) observed that in dry years Mg was accumulated in the ecosystem but in a form that lead to net loss during the next wet year after the dry period. A faster Mg plant–soil cycle together with water soluble Mg soil increases are likely to make the ecosystem more vulnerable to Mg losses. Mg can be leached out of the ecosystem by the torrential rainfalls typical of this climate. The absolute decreases in Mg contents in aboveground biomass and in soils suggest that Mg losses out of the ecosystem may have occurred in these 6 years of experimental drought. Since Mg is an important source for plant productivity (Hermans and

Verbruggen 2005) its reduction can have a negative synergic effect on plant fitness. Output fluxes of Mg are on average higher than through fall input fluxes, which lead to an average net loss of Mg from most European forest ecosystems of  $0.5\text{--}1.5\text{ kg ha}^{-1}\text{ year}^{-1}$  (Ambruster et al. 2002). But up to now the concentration values in soils and biomasses of this experiment are far higher than the reported values in Mg deficient forest ecosystems which are widespread in Europe (Ambruster et al. 2002; Nechwatal and Obwald 2003).

### Molybdenum

Leaf Mo concentrations observed in this experiment ( $0.07\text{--}0.38\text{ mg kg}^{-1}$ ) are in a range not considered limiting for the growth of most crop plants, but they are close to the observed limiting concentrations in some reports ( $0.04\text{--}0.27\text{ mg kg}^{-1}$ ) (Gupta et al. 1990). On the other hand, the observed values fall below the limiting values that plants must reach to have sufficient Mo amounts for animal diet (about  $0.5\text{ mg kg}^{-1}$ ) and are much lower than the plant biomass concentration values that can be toxic for most mammals ( $10\text{ mg kg}^{-1}$ ) (Albasel and Pratt 1989; Neunhäuserer et al. 2001). At the ecosystem level, Mo was the only element analyzed that had no tendency to decrease its biomass accumulation in total aboveground biomass in response to drought, probably because the capture of this nutrient is more independent of water than the others. However, when we analyzed the results at the species level, drought had a specific asymmetrical effect on the accumulation of this element. In one of the two dominant shrubs, *Arbutus unedo*, drought decreased the Mo accumulation because drought decreased aboveground growth and wood Mo concentration in this species, whereas in the other dominant shrub, *Phillyrea latifolia*, drought increased the Mo accumulation. This effect in *Phillyrea latifolia* was produced by an increase of Mo concentration in wood biomass without a decrease in wood biomass growth. In *Arbutus unedo* the decrease was produced as a consequence of both a decrease in Mo wood concentration and a decrease in growth. *Phillyrea latifolia* was the species less affected by drought, and the species that better adapted its metabolism to drought. In summer, when the soil moisture reached

the minimum values, *Phillyrea latifolia* was the only species that was able to increase WUE (56%), calculated by net photosynthetic rate/transpiration rate, in drought plots mainly by reducing transpiration losses (20% in sunlit leaves) during midday (Ogaya and Peñuelas 2003). *Phillyrea latifolia* was also able to maintain water transport at much lower water potentials than the other two species (Martínez-Vilalta et al. 2003). In this species, the tendency to increase WUE and reduce transpiration rates in some year periods together with the absence of effects on growth and photosynthetic capacity explain the absence of changes in Mg, Fe and S uptake and concentrations, in spite of their great soil solubility. The increase in the Mo uptake in this species can improve N anabolism and protein and enzyme production, contributing to a better metabolic capacity to adapt to drought. An enhancement of N productivity has been observed when N fertilization was accompanied by great Mo uptake (Fullin et al. 1999).

The effects of drought on soil Mo and on biomass Mo concentrations were in general lower than those observed for Mg and S concentrations. Thus, regarding Mo, drought did not change the quality of plant tissues as a food source for animals. In soil, drought did not increase total soil Fe concentration and therefore drought did not affect the Mo immobilizing capacity by increasing Mo fixation on iron oxides, a process that has been observed several times in natural environments (see Lang and Kaupenjohan 2003).

### Sulfur

Drought increased leaf and root S concentrations in the dominant tree *Quercus ilex*. Some previous studies (Schulte et al. 1998) have also reported an increase in leaf S concentrations under drought and attributed it to the inhibition of S export out of the leaves towards apical tissues due to a reduction of growth in these tissues such as the one observed in this experiment. These increases in S concentrations in some biomass fractions in drought plots were accompanied by increased total S soil S concentrations and soil soluble S concentrations. The increase of soil soluble S can be related to the increase of foliar S concentrations observed in *Quercus ilex*, the species which provides the greatest part of the leaf

biomass and leaf litter of the ecosystem. Some reports have shown that soil organic matter rich in S is degraded by soil microorganisms faster than soil organic matter poor in S content (see Ichinose et al. 2002). This was not observed; on the contrary, in this experiment we observed a decrease by 42–60%, 35–45%, 35–45% and 35–83% of urease, protease,  $\beta$ -glucosidase and phosphatase activities in drought plots (see Sardans and Peñuelas 2005). This decrease in soil enzyme activity together with the tendency to decrease S accumulation in above-ground biomass explains the increase of total soil S, since the main source of S in this soil is the organic matter.

The increase in S concentration in leaves together with the increase in soil oxidation capacity in drought soils would stimulate the available soil S and total soil S by enhancing sulfur oxidation. This would permit a greater soil accumulation of both total soil S content and soil extractable S content. Similarly to the case of Mg, these results indicate that in the short term, drought improved soil S availability and permitted greater foliar S concentration, but it generated a faster plant–soil cycling, increasing the potential S losses out of the ecosystem by torrential rainfalls.

#### General remarks and some implications

Drought decreased soil moisture, thus decreasing the soil diffusion capacity of the nutrients studied. This effect decreased plant uptake capacity, but was counterbalanced by two processes: an increase in soil soluble Fe, Mg and S, and of total soil Mg and S concentration, and a trend to increase the percent saturation of the soil bases exchangeable complex, mainly through the significant Mg increase in the complex. On the other hand, reduction in soil water content decreases soil enzyme activities (Sardans and Peñuelas 2005), which slows down nutrient release mainly from soil organic S.

There was a decrease in plant accumulation of the elements related to water uptake such as Mg, Fe and S in *Quercus ilex* and *Arbutus unedo*. Drought reduced all nutrient concentrations in *Arbutus unedo* stems and increased leaf Mg concentrations in *Arbutus unedo* leaves, increasing leaf/stem Mg concentrations in this species. Drought increased Mg and

S leaf concentrations in *Quercus ilex*. In fact, in this species there was a general tendency to increase leaf nutrient concentrations as a result of a concentration effect by leaf biomass reduction under drought. Nutrient accumulation was not related to WUE increase since these two species were not able to increase their WUE under drought, but it was related to a decrease of growth, photosynthetic capacity and sap flow, showing a tendency to decrease total plant uptake under drought.

Regarding the elements related to water uptake, *Phillyrea latifolia* was the species less affected by drought; only a decrease in leaf Ca concentrations was observed, an effect related to an increase in WUE resulting from the reduction of transpiration losses under drought. This species also showed the greatest capacity to adapt to drier conditions with no effects on growth and with an increase of Mo uptake capacity under drought. Thus, the weak effects of drought on Ca, Fe, Mg and S accumulation in aboveground biomass were due to the fact that the increases of soil availability were compensated by the reduction in soil diffusion and growth in *Quercus ilex* and *Arbutus unedo*. In the case of *Phillyrea latifolia* which did not reduce its growth under drought, the decreases in transpiration could account for the absence of accumulation in aboveground biomass of these elements in spite of their soil availability increase.

The asymmetrical response of nutrient status in the different dominant species of this community in response to drought might have several ecological implications, affecting the competitive interspecific plant relations and the stoichiometry between the different elements in the different species and in soil. These changes can also affect plant–herbivore relationships and thus trophic chains (Makino et al. 2003; Ngai and Jefferies 2004; Diehl et al. 2005). Drought will favor species with more flexible body compositions. This could negatively affect the resistance of the ecosystem to drought, since species with flexible body composition have low physiological functioning efficiencies (Jaenike and Markov 2003).

At the ecosystem level, drought tended to reduce Ca, Fe, Mg and S and to increase Mo accumulation in aboveground biomass probably because of the different plant capture mechanism of Mo with respect to Ca, Fe, Mg and Fe. The tendency to decrease the contents in aboveground biomass together with the

increase in soil soluble forms makes the ecosystem vulnerable to net losses of Fe, S and overall Mg, at mid and long term under a scenario of increasing drought with an increased number of torrential rainfalls such as is predicted by several climatic models for the Mediterranean basin (IPCC 2001, 2007). The increase of Fe and S concentrations in *Quercus ilex* roots observed in drought plots might counterbalance the losses through leaching of these two elements.

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