Drought changes nutrient sources, content and stoichiometry in the bryophyte *Hypnum cupressiforme* Hedw. growing in a Mediterranean forest

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**SUMMARY**

We conducted a 6 year field experiment in an evergreen *Quercus ilex* forest where we simulated the increased drought projected by Global Circulation Models (GCM) and ecophysiological models for the immediate decades. We tested the hypothesis that enhanced drought will change C, N, P, K, Ca, Fe, Mg, Mo and S concentrations of the widespread moss *Hypnum cupressiforme* Hedw. and its capacity to absorb nutrients of soil-borne or airborne origin. During the period of study, from 1999 to 2005, the soil moisture in the drought treatment was on average 9% lower than the soil moisture in the control plots. Drought increased the K concentration by 10% and the C concentration by 6%, and decreased the Fe and Mo concentrations by 33% and 18%, respectively, and the N/P content ratio by 15%. A principal component analysis showed that 69% of the variation in moss elemental concentrations is explained by the drought treatment. Drought increased the enrichment factors of several elements, mainly of P, K, Ca, Mg, S and Mo, relative to bedrock extracts, thus showing that the proportion of these elements absorbed from the atmosphere had been increased by drought. The results show that drought increased the concentration of elements linked to drought resistance such as C and K, and decreased the contents of others important for plant productivity such as Mo and Fe. Drought thereby changed moss stoichiometry, and this could also affect the palatability and quite probably, the moss–herbivore relationships and decomposition rates.

**KEYWORDS:** Drought, *Hypnum cupressiforme*, Spain, magnesium, moss, nitrogen, phosphorus, potassium.

**INTRODUCTION**

Water is the most important limiting factor in Mediterranean ecosystems. In these ecosystems, aridity has increased in recent decades (Pinol, Terradas & Lloret, 1998; Peñuelas, Filella & Comas, 2002) and both Global Circulation (IPCC, 2007) and ecophysiological models predict greater levels of drought in the near future. For example, GOTILWA predicts a decrease in 25% in soil moisture between 2000 and 2040 (Sabaté, Gracia & Sánchez, 2002; Peñuelas et al., 2005). Furthermore, more intense and more frequent dry periods are expected to occur along with even heavier torrential rainfall (Houghton et al., 2001). This will probably affect the cycling of nutrients in these Mediterranean ecosystems where nutrients such as N and P are often limiting factors (Kruger, 1979; Henkin et al., 1998; Sardans, Rodà & Peñuelas, 2004, 2005; Sardans, Peñuelas & Rodà, 2006), despite increasing human inputs (Peñuelas & Filella, 2001; Peñuelas et al., unpublished results).

The effects of increasing drought on nutrient levels are especially critical for mosses since they generally need moist environments. Together with the direct effects of drought reducing the environmental water availability, the indirect effects through nutrient changes could be critical for the survival of mosses in Mediterranean forest. The capacity to maintain internal nutrient homeostasis is important: first, because nutrients such as N, P or Mg are needed to maintain adequate levels of water use efficiency, and second, because some elements such as K are important to control water losses (Beckett & Hoddingott, 1997; Beckett, 1999). Drought can alter the nutrient concentrations in the moss by changing the soil moisture, soil enzyme activity, soil nutrient availability and moss growth. On the other hand, the capacity of mosses to increase the synthesis of structural compounds rich in C in order to increase their
capacity to resist water stress is another factor that might account for elemental concentration changes in response to drought.

Total atmospheric deposition is another very important source of nutrients for mosses. The total N atmospheric deposition was estimated to be 15–22 kg ha⁻¹ in a Western Mediterranean Quercus ilex forest located in the Montseny Mountains (Catalonia, N.E. Spain) (Rodà, Avila & Rodrigo, 2002). The total depositions of some other elements were also unusually elevated including P, K, Ca, Mg and S (Rodrigo & Avila, 2002). As mosses are able to absorb significant amounts of nutrients from air (Bargagli, 1995), the possibility that the proportion of nutrient absorbed from air relative to that absorbed from soil may change in response to drought warrants investigation.

As far as we are aware, there have been no studies on the effects of drought on the concentrations of nutrients such as N, P, K, Ca, Fe, Mg, Mo and S in mosses of Mediterranean ecosystems nor on the air/soil origin of those nutrients. Here, we studied the effects of 6 years of continuous experimental drought, representing a 9% average decrease in soil moisture, on the concentration, stoichiometry and enrichment factors (soil/airborne origin) in Hypnum cupressiforme of the most important elements for plant nutrition, C, N, P, K, Ca, Mg, Fe, S and Mo. This treatment is within the range of drought scenarios predicted as possible in the immediate future (Sabaté et al., 2002; Peñuelas et al., 2005; IPCC, 2007). Hypnum cupressiforme is the most widespread moss species in the Mediterranean Holm oak forest that we studied.

**Material and Methods**

**Study site**

The study was carried out on a south-facing slope (25%) in a native Quercus ilex L. forest in the Prades Mountains in southern Catalonia (N.E. Spain) (41°13’N 0°55’E). The soil consists of a stony Dystric Xericrept lying on a bedrock of metamorphic sandstone of 35–100 cm depth, with the depth of the A horizon ranging between 25 and 30 cm. The average annual temperature is 12°C and average annual rainfall 658 mm. Summer drought is pronounced and usually lasts for 3 months. The vegetation consists of a dense forest dominated by Quercus ilex (20.8 m² ha⁻¹ trunk basal area, measured at a height of 50 cm) accompanied by abundant Phillyrea latifolia L. (7.7 m² ha⁻¹ trunk basal area, measured at a height of 50 cm), Arbutus unedo L., a number of other evergreen species well-adapted to drought conditions such as Erica arborea L., Juniperus oxycedrus L. and Cistus albidus L., and occasional individuals of deciduous trees such as Sorbus torminalis (L.) Crantz and Acer monspessulanum L. In winter 1999, the above-ground biomass of Quercus ilex represented 77.1% of the total biomass, Phillyrea latifolia was 12.6%, and that of Arbutus unedo, 7.8%, these three species accounting for 97.6% of the above-ground tree biomass of the ecosystem. In winter 2005, the figures for the same three species were 75.6, 13.3 and 8.7%, respectively, again collectively representing 97.6% of the total above-ground biomass.

**Experimental design and moss, soil and bedrock sampling**

Eight 15 × 10 m plots were established at the same altitude (930 m a.s.l.) on a mountainside. Four of the plots received the drought treatment and four were left as control plots. The drought treatment consisted of a 0.8–1 m deep ditch that was dug along the entire top edge of the upper part of the treatment plots to intercept any run-off water. The water intercepted by the ditches was channeled to the bottom edge of the plots. The drought treatment began in March 1999 (Ogaya et al., 2003). Soil moisture content was measured every 2 weeks throughout the experiment by time domain reflectometry using a Tektronix 1502 C instrument (Beaverton, OR, USA: Zegelin, White & Jenkins, 1989). Three stainless steel cylindrical rods, 25 cm long, were fully driven into the soil at randomly-selected places in each plot. The time domain reflectometer was connected to the ends of the rods to determine the soil moisture content.

In January 2005, 6 years from the beginning of the experiment, six different patches of Hypnum cupressiforme were collected in each plot. Five soil cores of 4 cm diameter and 15 cm height were collected from each plot. Two bedrock samples were collected in each plot; in all plots there were zones where bedrock reaches the surface.

**Sample preparation**

Samples were stored at 4°C until analyzed. In order to ensure that only the trace elements in mosses tissues were analyzed, mosses were washed with distilled water only to clean the encrusted soil particles in some zones and dried with air using a hair-dryer as described by Porter (1986). Soil samples were passed through a 2 mm sieve and litter was removed. After all moss samples had been washed and soil samples had been sieved, they were dried to constant weight in an oven at 60°C and then ground in a Cyclotec 1093 (Foss Tecator, Höganäs, Sweden) (in the case of mosses), in an agate grinder (Pulverisette 6, Rudolstadt, Germany) (in the case of soils) and in a Fritsch Pulverisette (Rudolstadt, Germany) (in the case of the rocks). In all cases, the grinding system was cleaned with bi-distilled water between samples.

**Soil and bedrock extracts**

Total concentrations and the extractable fractions of C, N, P, K, Ca, S, Fe and Mo were analyzed in each soil and bedrock sample. The soil extracts were obtained by shaking 2 g soil (or pulverized bedrock) with 12 ml solvent (0.01M NaNO₃) following Yin et al. (2002) and van Elteren &
Budic (2004). The soil and the 0.01M NaNO₃ 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/min for 5 h, a technique based on batch extraction studies by Gupta & Mackay (1986). After equilibrium, soil solids were separated from the solution by centrifugation and then by filtration through a 0.45 μm pore-size membrane filter.

Chemical analyses

The concentration of Mo was measured in all moss and soil samples using ICP-MS (mass spectroscopy with inductively-coupled plasma) using a Elan-6000 instrument (Perkin Elmer Corp. Inc. Norwalk, CO, USA), and those of P, K, Ca, Mg, Fe and S by ICP-OES (optic emission spectroscopy with inductively-coupled plasma) using a Jobin Iyon JY 38 instrument (Longjumeau, HORIBA. Jobin Ibon S.A.S., France). Before the moss ICP-MS and ICP-OES analyses, an acid digestion of the samples was carried out with an acid mixture of HNO₃ (60%) (143255, purissimum, PANREAC, Barcelona, Spain) and HClO₄ (60%) (141054, purissimum, PANREAC, Barcelona, Spain) (2:1) in a microwave oven (SAMSUNG, TDS, Seoul, 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 dry biomass for each moss sample. The digested solutions were diluted to 10 ml final volume. During the acid digestion process, two blank solutions (2 ml 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) was used. For the soil and bedrock samples, the digestion was carried out with 0.25 g ground sample in 9 ml HNO₃ (65%) and 4 ml HF (40%) in a microwave oven at 120°C for 8 h (Bargagli, Brown & Nelli, 1995). The digested solutions were diluted to 50 ml final volume, filtered with a Millex 0.45 μm filter, and then stored at 4°C until their determination.

For C and N concentration determination in mosses and for N determination in soils, 1-2 mg finely-sieved moss sample plus 2 mg V₂O₅ (as oxidant) were used. Moss 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). For both biomass and soil analyses, the analytical precision was better than 5% in all samples as verified by parallel analyses to the international certified standards DC73351 (leaf poplar, purchased from China National Analysis Center for Iron & Steel) and GSR-6 (Carbonate rock purchased from Institute of Geophysical and Geochemical Prospecting of China) for biomass and soil respectively.

Enrichment factor determination and statistical analyses

The enrichment factor (EF) recently proposed by Sardans & Pe˜nuelas (2006) was calculated for each element

\[ EF = \frac{C_{\text{moss}}}{C_{\text{Al}}} \frac{C_{\text{Al}}}{C_{\text{bedrock extracts}}} \]

where \( C_i \) is the concentration of the element of concern, \( C_{\text{Al}} \) is the concentration of aluminum (reference element), and Al was determined by IPC-MS in a manner similar to Mo.

However, in the case of N, due to the low concentration of N in rock extracts, and since the natural inputs in soil of this element are not from bedrock, we used N and Al soil extracts instead of bedrock extracts.

The effects of the drought treatment on each variable were investigated by one-way ANOVAs conducted with the Statview 5.01 statistical package (Abacus Concepts, SAS Institute Inc., Berkeley, CA, USA). We also conducted a principal component analysis (PCA) using a correlation matrix with standardization of the concentrations of the analyzed elements and plot sites (Loska & Wiechula, 2003). We used the concentration values of each element as variables and the different plots (four control plus four drought treatment plots) as different cases. The aim was to detect similarities in the spatial patterns of elements among the plots, and to evaluate the importance of the drought treatment on element concentrations (Loska & Wiechula, 2003). The Statistica 6.0 statistical program package from StaSoft Inc. (Tulsa, OK, USA, 2001) was used to conduct the PCA analysis.

Results

During the period 1999–2005, the droughted plots (D) had an average soil moisture content of 17.5 ± 0.5% (n=100), i.e. 9% lower than the control (C) plots (soil moisture 19.2 ± 0.5% (n=100; p<0.05).

In the moss, drought increased K concentration by 10% and C by 6%, and decreased Fe, Mo and S concentrations by 33, 18 and 11%, respectively (Fig. 1). Drought also decreased N/P content ratio by 15% (Fig. 1). The PCA analysis shows that the moss concentrations in the control plots are separated from those of drought plots because C, P and K concentrations are higher in the drought plots whereas Mo, S and N concentrations tended to be higher in the control plots (Fig. 2). The first two components explained 40 and 29% of the variance (Fig. 2). The coefficients of the two first components of the loading factors were respectively: C (0.50 and 0.09), N (–0.14 and 0.45), Mg (0.12 and 0.53), Ca (–0.37 and –0.35), P (0.25 and –0.25), K (0.33 and –0.25), Fe (–0.46 and –0.20), S (–0.17 and 0.44) and Mg (–0.41 and 0.09). Block 3, and therefore control 3 and drought 3, were separated from the other control and drought plots because they had shallower soils than the other plots.
The enrichment factors (EF) for P, K, Ca, Mg, S and Mo increased significantly in drought plots (Table 1). In contrast, the EF of Fe decreased in drought plots. The EF of N, calculated using soil extracts instead of bedrock extracts, increased marginally significantly ($p=0.08$) in the drought plots (Table 1).

Figure 1. Nutrient concentrations and concentration ratios in Hypnum cupressiforme growing in the control and drought treatment plots. Different letters indicate significant differences between treatments ($p<0.05$). When within brackets, $p<0.10$. 

The enrichment factors (EF) for P, K, Ca, Mg, S and Mo increased significantly in drought plots (Table 1). In contrast, the EF of Fe decreased in drought plots. The EF of N, calculated using soil extracts instead of bedrock extracts, increased marginally significantly ($p=0.08$) in the drought plots (Table 1).
PCA was carried out based on the inter-element correlation matrix. Enrichment factors (mean ± SE) of the different elements calculated as $EF = \frac{(C_{moss})_{obs} - (C_{Almoss})_{bedrock extracts}}{(C_{Cx}/C_{Al})_{bedrock extracts}}$ (Sardans & Peñuelas, 2006) in the control (C) and the drought (D) treatments.

<table>
<thead>
<tr>
<th>Element</th>
<th>Treatment</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>C</td>
<td>-25.89 ± 8.4(b)*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>-5.9 ± 4.2(a)</td>
</tr>
<tr>
<td>P</td>
<td>C</td>
<td>-0.90 ± 0.452(b)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.675 ± 0.546(a)</td>
</tr>
<tr>
<td>K</td>
<td>C</td>
<td>-560 ± 51b</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>-367 ± 39a</td>
</tr>
<tr>
<td>Ca</td>
<td>C</td>
<td>-1900 ± 173b</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>-1252 ± 134a</td>
</tr>
<tr>
<td>Mg</td>
<td>C</td>
<td>-487 ± 44b</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>-322 ± 34a</td>
</tr>
<tr>
<td>Fe</td>
<td>C</td>
<td>7.77 ± 0.30a</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>5.20 ± 0.90b</td>
</tr>
<tr>
<td>S</td>
<td>C</td>
<td>-2480 ± 223b</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>-1642 ± 172a</td>
</tr>
<tr>
<td>Mo</td>
<td>C</td>
<td>-2671 ± 240b</td>
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<tr>
<td></td>
<td>D</td>
<td>-1770 ± 185a</td>
</tr>
</tbody>
</table>

Different letters indicate statistically significant differences (p<0.05). Different letters between brackets indicate marginally significant differences between treatments (p<0.1). * In the case of EF for nitrogen, soil extracts were used instead of bedrock extracts.

DISCUSSION

Drought increased C and K concentrations and decreased Fe, S and Mo concentrations. The increased concentration of C is an indication of increased sclerophyll and the increases in K concentration are related to mechanisms of preventing desiccation in water-stress situations (Beckett, 1999) and for controlling turgor pressure (Beckett & Hoddinott, 1997). The decreases in Mo concentrations are probably more related to a reduction in the moss's absorption capacity for the element. The exact mechanisms of plant capture of Mo remain unclear, but an active absorption capacity for the element. The exact mechanisms probably became more available in the soils, thus favoring Fe uptake and reducing its enrichment factor. On the other hand, over 6 years, drought reduced soil P availability (Sardans & Peñuelas, 2005). The lower concentration of soil soluble phosphate in the drought treatments permitted a greater soil Fe capture and thus a decrease of moss Fe EF factor. On the other hand, drought reduced soil P availability and capture enhanced the moss's rewetting capacity when water availability increased. Bates (1997) observed that intracellular K⁺ leaked from the cells during the desiccation treatment, was retained by cation exchange on the negatively-charged cell walls in Brachythecium rutabulum. This can contribute by increasing the K⁺ absorption into the cell in the rewetting periods. On the other hand, an additional explanation for K increase in moss from drought plots is that reduced water availability could lead to a lower leakage and a consequent lower loss from the cells. K is present mainly as a free ion in the cytoplasm and hence is presumably particularly liable to uncontrolled leakage from stressed cells (Bates 1997).

Drought increased the enrichment factors of most elements, except Fe. Atmospheric inputs of N, P, Mg, Ca and S in the western part of the Mediterranean Basin are significant (Rodrigo & Avila, 2002) and may constitute an additional source of these elements for mosses. The Fe EF decreased presumably because soil lowered water content increased Fe oxidation in soil increasing Fe³⁺/Fe²⁺ ratio and thus Fe soil solubility when soil was rewetting. In fact, an increase in soil Fe solubility without a significant change in total Fe soil content was observed under drought. This permitted a greater soil Fe capture and thus a decrease of moss Fe EF factor. On the other hand, over 6 years, drought reduced soil P availability (Sardans & Peñuelas, 2004), partly as a result of a decrease in soil phosphatase activity (25–40%) and probably also as a result of a reduction in the activities of other soil enzymes such as β-glucosidase (15–80%), urease (40–60%) and protease (30–60%) (Sardans & Peñuelas, 2005). The lower concentration of soil soluble phosphate in the drought treatments decreased the probability of fixing Fe, and as a result, Fe became more available in the soils, thus favoring Fe uptake and reducing its enrichment factor.

Mosses are an important source of food for vertebrate (Ili & Barboza, 2007) and invertebrate (Smith, Young & Marquis, 2001) herbivores, and since their elemental composition is important in their palatability (Bragazza...
et al., 2007), the changes in elemental composition and stoichiometry (for example, the decrease in N/P ratio) may affect moss–herbivore relationships. The nutrient changes may also alter decomposition rates and thereby, trophic relationships (Makino et al., 2003; Ngai & Jefferies, 2004).

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