

Warming and drought alter C and N concentration, allocation and accumulation in a Mediterranean shrubland

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Abstract

We investigated the effects of warming and drought on C and N concentrations, nitrogen use efficiency (NUE), and C and N accumulation in different ecosystem compartments. We conducted a 6-year (1999–2005) field experiment to simulate the climate conditions projected by IPCC models for the coming decades in a Mediterranean shrubland. We studied the two dominant species, *Globularia alypum* and *Erica multiflora*, and an N-fixing species, *Dorycnium pentaphyllum*, also abundant in this shrubland. Warming (1 °C) decreased N leaf concentrations by 25% and increased N stem concentrations by 40% in *G. alypum*. Although warming changed the available ammonium in soil in some seasons, it did not increase total soil N contents. Drought (19% average reduction in soil moisture) decreased leaf N concentrations in the two dominant shrub species, *E. multiflora* and *G. alypum* by 16% and 19%, respectively, and increased stem N concentrations by 56% and 40%, respectively. Neither warming nor drought changed the leaf N concentrations in the N-fixing species *D. pentaphyllum*, although warming increased stem N concentration by 9%. In *G. alypum*, the increase of stem N concentrations contributed to the observed increase of N accumulation in stem biomass in drought treatments with respect to control plots (8 kg N ha⁻¹). Neither warming nor drought changed NUE in the period 1999–2005. Warming increased soil organic C relative to drought. The effects of warming and drought on C and N concentrations, on N accumulation and on leaf/stem N distribution were not the result of dilution or concentration effects produced by changes in biomass accumulation. Other factors such as the changes in soil N availability, photosynthetic capacity, and plant internal C and N remobilization must be involved. These changes which differed depending on the species and the plant tissue show that the climate change projected for the coming decades will have significant effects on the C and N cycle and stoichiometry, with probable implications for ecosystem structure and function, such as changes in plant–herbivore relationships, decomposition rates or community species composition.

Keywords: C/N, climate change, *Dorycnium pentaphyllum*, drought, *Erica multiflora*, *Globularia alypum*, Mediterranean shrubland, N, NUE, warming

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Introduction

Climate change generated by elevated atmospheric greenhouse gases has increased the Earth's surface temperature by 0.74 °C in the past century (IPCC, 2007). Temperatures in the Mediterranean region have also shown warming trends (Peñuelas *et al.*, 2002, 2005;

Peñuelas & Boada, 2003). Precipitation has begun to exhibit either a long-term downward trend, mainly in the dry season (Esteban-Parra *et al.*, 1998), or no significant change (Piñol *et al.*, 1998; Peñuelas *et al.*, 2002, 2005), although in all cases a rise in potential evapotranspiration has led to increased aridity (Piñol *et al.*, 1998; Peñuelas *et al.*, 2005). Current global circulation models (GCM) and ecophysiological models such as Gotilwa (Sabaté *et al.*, 2002; Peñuelas *et al.*, 2005) predict further increases in warming and drought in the

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Mediterranean region in the coming decades. Moreover, the main predictions of the most general circulation models (GCMs) indicate that over this century the mean global surface temperature will increase by 1.1–6.4 °C depending on socioeconomic scenarios and the resulting emissions of greenhouse gases (IPCC, 2007).

Such changes in climate are expected to influence C cycling (Cox *et al.*, 2000). In addition to C changes, N availability and its dynamics and stocks in ecosystems could also be affected. The effect of climate change on N stocks in different ecosystem compartments could have significant impacts both on ecosystem performance and on long-term C sequestration in terrestrial ecosystems (Hungate *et al.*, 2003; Luo *et al.*, 2004). This is because leaf N concentration is one of the most important plant traits that determine photosynthetic C fixation and biomass accumulation capacity.

Warming and/or drought can change N plant concentration mainly by changing biomass accumulation and N soil availability, thereby also affecting C/N concentration ratios, N use efficiency (NUE), N mineral masses and N relative allocation to different tissues as observed in temperate non-Mediterranean ecosystems (An *et al.*, 2005) and in some arctic ecosystems (Tolvanen & Henry, 2001; Weintraub & Schimel, 2005). The magnitude of these effects depends on the functional group (Lilley *et al.*, 2001) and on the ecosystem climate type (Lal, 2003; Luomala *et al.*, 2003), and may be different among different species in the same ecosystem (Read & Morgan, 1996), leading to shifts in plant community structure (Lilley *et al.*, 2001). The changes in C and N stoichiometry at plant and ecosystem levels have been shown to play an important role in trophic chains and finally in the species composition structure of ecosystems (Ngai & Jefferies, 2004; Diehl *et al.*, 2005).

Less is known about the impact of climate change on C and N contents in Mediterranean ecosystems. Nutrients are a frequent limiting factor in Mediterranean ecosystems (Hanley & Fenner, 2001; Sardans *et al.*, 2004, 2005a). Several studies have reported N limiting photosynthetic capacity (De Lillis & Federici, 1993) and biomass accumulation capacity (Rodríguez-Echeverría & Pérez-Fernández, 2003; Villar-Salvador *et al.*, 2004). By analyzing herbaria specimens and tree rings, Peñuelas & Matamala (1990) and Peñuelas & Estiarte (1997) have reported that foliar N concentrations have decreased in recent decades in the Mediterranean Basin. N availability may offset positive plant growth responses to elevated CO₂ in Mediterranean ecosystems (Cruz *et al.*, 2003), thus having an important role in C sequestration capacity.

Warming can contribute to CO₂ fixation in temperate and high latitude ecosystems during the growth seasons (Keeling *et al.*, 1996) because of its positive effects

on biological processes in environments where temperatures are currently far from optimum. In Mediterranean ecosystems, however, it is unclear as to whether such a warming effect would occur. In Mediterranean environments, the effects of warming are instead strongly dependent on warming's influence on water availability and the efficiency of its use. Drought, on the other hand, can limit photosynthetic capacity by decreasing soil moisture. Growth decrease can contribute to concentrating N in plant tissues and to changes in leaf photosynthetic capacity, in transpiration fluxes and in water use efficiency (WUE), which has consequences for C sequestration capacity. These effects can change the C and N leaf-stem allocation. Drought limits plant growth capacity in Mediterranean ecosystems (Ogaya *et al.*, 2003) and reduces soil microbial activity (Sardans & Peñuelas, 2005) and, therefore, can lead to a decrease in the ecosystem's capacity to accumulate C and N in plant tissues. Subsequently, there are changes in the amounts and chemical forms of C and N in soil.

It is, thus important to gain knowledge about the response of plant and soil N to warming and drought, particularly in Mediterranean plants which are described to have a great capacity for resource remobilization through the year (Lloret *et al.*, 1999; Filella & Peñuelas, 2003; Mediavilla & Escudero, 2003). The response of the C and N contents of Mediterranean ecosystems to climate change is especially important for additional reasons. Some climatic models have projected a partial loss of C from ecosystems to the atmosphere in southern European forests (White *et al.*, 1999) and on the contrary, others forecast an accumulation in soil C stocks (Wessel *et al.*, 2004). Moreover, there is a lack of experimental data on the direct and indirect effects of warming and drought on C and N concentrations and contents in the different compartments in Mediterranean ecosystems.

In Mediterranean regions, a reduction in mineralization rates together with a decrease in plant biomass accumulation and N capture would increase N accumulation in soil. Furthermore, as a result of the decrease in soil water availability, there would be an increase in oxidation of the soil environment that can increase soil nitrification. The increase in water solubility of soil N could result in greater N losses through leaching after more frequent torrential rainfalls, such as those projected by most models (IPCC, 2007). This scenario leads to an increase in the eutrophication of the continental waters and to a reduction in the N content of the ecosystem, thus compromising the future growth capacity and WUE of the ecosystem and reducing the capacity of carbon sequestration. Finally, human activity has increased the nutrient inputs in natural ecosystems in wide areas of the Mediterranean basin in recent

decades (Peñuelas & Filella, 2001; Rodrigo & Avila, 2002). Changes in N accumulation capacity in terrestrial ecosystems in a new climate scenario in the coming decades would affect the future N balance.

To test the hypothesis that there will be significant changes in C and N content in biomass and soils in response to the warming and drought projected by GCM and ecophysiological models such as GOTILWA in the Mediterranean basin (Sabaté *et al.*, 2002; Peñuelas *et al.*, 2005; IPCC, 2007), we conducted a field experiment simulating this predicted warming and drought in a Mediterranean shrubland. We investigated the effects of 6 years of experimental warming and drought on (1) C and N concentration and accumulation in the biomass of the two dominant shrub species, *Globularia alypum* and *Erica multiflora*, and of an abundant N-fixing species *Dorycnium pentaphyllum*, and (2) C and N concentrations in litter and soil.

Materials and methods

Study site

The study was carried out in a natural Mediterranean calcareous shrubland on a south-facing slope in the Garraf mountains in Catalonia (NE Spain) (41°18'N, 1°49'E). The site is located on formerly cultivated terraces – abandoned approximately a century ago – with a *Petrocalcic calcixerept* soil lying on a bedrock of sedimentary limestone. During the study period (1999–2005), the average annual temperature was 15.1 °C (7.4 °C in January and 22.5 °C in July) and the average annual rainfall was 580 mm. The summer drought is pronounced and usually lasts for 3 months. The total vegetation cover is 75% and consists of a calcareous shrubland with plants about 1 m high dominated by the shrubs *G. alypum* (L.), *E. multiflora* (L.), *D. pentaphyllum* (Vill.), *Rosmarinus officinalis* (L.), *Ulex parviflorus* (Pourr.), and *Pistacia lentiscus* (L.). Aleppo pines *Pinus halepensis* (Mill.) were air-seeded after the last forest fire in 1994 and are today gaining ground. The undergrowth is dominated by small shrubs such as *Fumana ericoides* (Cav.) and *Fumana thymifolia* (L.).

Experimental design

We conducted field-scale night-time warming and drought treatments (Beier *et al.*, 2004; Peñuelas *et al.*, 2007) and compared treatment plots with control plots. Each of the three treatments (control, warming and drought) was applied in three plots. Each plot occupied an area of $4 \times 5 \text{ m}^2$, although to avoid the effect of edge disturbance, samples were only taken from an internal

area of $3 \times 4 \text{ m}^2$. Manipulation started in March 1999 and has continued till present.

The warming treatment consisted in increasing night-time temperatures by covering the vegetation with an aluminum screen coiled on a beam and connected to a motor controlled by light sensors that automatically covered the vegetation at night. This screen reflected long-wave infrared radiation back into the vegetation, resulting in a temperature increase in relation to untreated plots. In order to avoid interfering with the hydrological cycle, this cover was automatically removed when it rained. The warming screen was removed if the wind speed exceeded 10 m s^{-1} in order to avoid structural damage.

The drought treatment reduced spring and autumn rainfall input. This was achieved by automatically covering vegetation with a transparent plastic curtain during rain events by means of automatic rain sensors. Once the rain stopped, the screen was automatically removed. As in the warming treatment, the cover was also removed if wind speed exceeded 10 m s^{-1} . In summer and winter (outside the drought period), the treatment was not applied and drought plots were allowed to develop under the same conditions as control plots.

The control plots were equipped with the same scaffolding as the treatment plots but without the screen roof.

Environmental conditions were monitored in all plots. Soil moisture was measured biweekly by the TDR (time domain reflectometry) model Tektronix 1502C (Tektronix, Beaverton, OR, USA), using three probes installed in each plot. The air and soil temperatures were recorded for every plot by temperature sensors (RTD Pt 100 1/3 DIN; Desin Instruments, Barcelona, Spain) located at depths of –10 and –5 cm in the soil and at 20 cm above the soil. Air and soil temperatures, as well as the correct functioning of the motorized screen (magnetic sensors were installed at the end of the screen track), were recorded by a data-logger (Campbell Scientific Inc., Logan, UT, USA). Precipitation was measured by standard rain gauges and all water entering the plots was collected biweekly.

Biomass growth

In 1999 and in 2005, the aboveground biomass was estimated by means of the pin point method (Peñuelas *et al.*, 2004, 2007). In each plot, five permanent 3 m long transects separated by 80 cm distance were established. A vertical metal rod was lowered every 5 cm within each transect and contacts between the rod and plants were recorded for 305 points per plot. This allowed us to obtain a value of contacts and maximum height per pin that was used to estimate aerial biomass.

Pin-point measurements were also conducted in additional plots external to the experiment. After pin-point measurements, the aerial biomass of these external plots was cut and brought to the laboratory where it was oven-dried at 75 °C to constant weight. Regression analysis between aerial biomass (AB, g m⁻²) and maximum height (MH, cm) measured by point transect resulted in the following equations: *E. multiflora* AB = 0 + 35.587 MH ($R^2 = 0.91$, $P < 0.001$); *G. alypum* AB = 44.38 + 33.61 MH ($R^2 = 0.98$, $P < 0.001$). These regression equations were used to estimate the aerial biomass of experimental plots.

From these plants that were external to the experiment, we also obtained allometric relationships between leaf and stem biomass and plant height for both *E. multiflora* and *G. alypum* plant species. Equations used were the following: for *E. multiflora* plant leaf biomass ($y = 2.58x - 5.89$, $R^2 = 0.82$) and plant stem biomass ($y = 3.95x - 11.40$, $R^2 = 0.87$), and for *G. alypum* plant leaf biomass ($y = 3.40x - 10.68$, $R^2 = 0.69$) and plant stem biomass ($y = 5.33x - 17.55$, $R^2 = 0.82$), where y is the ln of biomass (g) and x the ln of stem length (cm). Plants within experimental plots were measured (a total of 235 *E. multiflora* and 268 *G. alypum* individuals) and were used to obtain a leaf to stem biomass ratio for 1999 and 2005. This ratio was used to separate the biomass per plot, obtained by the pin-point method, into a leaf and stem biomass for each species.

Sampling process

Biomass. To analyze C and N concentrations, we sampled the plant biomass twice, once at the beginning of the experiment in January 1999 and then again 6 years later in January 2005. Each time we sampled 15 individual plants per plot, five pertaining to each one of the two dominant shrub species, *G. alypum* and *E. multiflora*, and to the N-fixing species *D. pentaphyllum* (this latter species only in 2005). Five branches were sampled from each plant. Leaf life is longer in *E. multiflora* than in the other two species and the leaf population consisted mostly of current-year leaves and some 1 year old leaves. Therefore, for this species we analyzed the two different leaf-year cohorts (current year and 1 year old). In the other two species, only current-year leaves were present during the sampling campaign and, therefore, only one cohort was considered. Thus, two fractions of aboveground biomass were considered in *G. alypum* and *D. pentaphyllum* (stem and current-year leaves) and three (stem, current-year leaves and 1 year old leaves) in *E. multiflora*. For the root biomass, we took three sample cores (30 cm deep, $\phi = 5$ cm) from each plot. Because of the difficulty of distinguishing between the

roots of different species, we sampled near *G. alypum* and only collected roots of this species. In each core, we selected the roots of $\phi < 1$ mm and the roots of $\phi > 1$ mm and analyzed these two fractions separately.

Litterfall. The litterfall of four to eight plants of *E. multiflora* and 9–12 plants of *G. alypum* per plot was monitored during the whole year in 1999 and 2004. Plant litterfall was collected bimonthly by means of 19 open collectors (4.4 cm diameter) in each plot located under each selected plant. Samples were dried to constant weight and afterwards the leaf litterfall was selected from the rest, weighed and analyzed (leaf litterfall represented 70% of the total litter production).

Soil. Three sample cores (10 cm deep, $\phi = 5$ cm) from each plot were taken in January 2005 for soil analysis. Additionally, for soil KCl-extractable-ammonium and soil KCl-extractable-nitrate determination, we repeated the soil sampling in July and October 2004 and in May 2005 in order to detect the seasonal changes of these soil variables throughout the year.

Chemical analyses

Sample preparation. All the samples were taken to the laboratory and stored at 4 °C until analysis began. Soil samples were sieved and the fraction $\phi < 2$ mm was analyzed. In order to analyze only C and N in the foliar tissue, the leaves were washed with distilled water as described by Porter (1986). After all samples had been washed, they were dried in an oven at 60 °C to a constant weight and then ground in a CYCLOTEC 1093 (Foss Tecator, Höganäs, Sweden) in the case of the biomasses, or in a FRITSCH Pulverisette (Rudolstadt, Germany) in the case of the soils.

Total C, N and P in biomass and total N in soil. C and N were analyzed in all biomass and soil samples. For C and N concentration determination in biomasses and litter and for N determination in soils, 1–2 mg of fine-sieved sample of biomasses and litter or 10–12 mg of soil plus 2 mg of V₂O₅ (as oxidant) were analyzed by combustion coupled to gas chromatography (Sabaté & Gracia, 1994). We used a Thermo Electron Gas Chromatograph model NA 2100 (C.E. Instruments-Thermo Electron, Milano, Italy). In order to assess the accuracy of the biomass digestion and analytical procedures, we used standard certified biomass (DC73351). For soil analyses, the analytical precision – as verified by parallel analyses to an international (GSR-6) standard – was better than 5%.

P was analyzed in all samples of current-year leaves, stems and litter of *E. multiflora* and *G. alypum*, in leaf

and stem of *D. pentaphyllum* and in roots of *G. alypum*, in order to calculate the treatment effects on N/P concentration rates of these plant tissues. The P concentrations were measured by ICP-OES (optical emission spectroscopy with inductively coupled plasma) by using a model JOBIN IBON JY 38 (Longjumeau, HORIBA Jobin Ibon S.A.S., France). Before the biomass ICP-OES analyses, an acid digestion of the samples was carried out with an acid mixture of HNO₃ (60%) and HClO₄ (60%) (2:1) in a microwave oven (Samsung, TDS, Seoul, South Korea). Two milliliters of the mixed acid solution were added to 100 mg of dry biomass for each sample. The digested solutions were made up to 10 mL final volume. During the acid digestion process, two blank solutions (2 mL of acid mixture without any sample biomass) were also analyzed (Mateo & Sabaté, 1993). In order to assess the accuracy of biomass digestion and analytical procedures, we used standard certified biomass (DC73351).

Soil organic carbon. To determine total soil organic C in soil samples we used the Walkley–Black method (Walkley & Black, 1934). Briefly, 10 mL of 1 N potassium dichromate + 20 mL of concentrated H₂SO₄ solution were added to 0.1 g of sieved and dried soil, mixed by gentle rotation for 1 min and heated at 150 °C for 10 min and afterwards cooled at room temperature. Then the mixture was diluted to 200 mL with deionized water and 10 mL of phosphoric acid, 0.2 g of ammonium fluoride and 10 drops of diphenylamine indicator were added. Finally, the excess dichromate was titrated with Mohr salt solution (0.5 N SO₄FeNH₄ and 0.1 N H₂SO₄).

Soil KCl-extractable-ammonium and soil KCl-extractable-nitrate determination. The available ammonium and nitrate was analyzed in all soil samples. We extracted ammonium and nitrate with a 2 M KCl extracting water solution. Both ammonium and nitrate were assessed by colorimetric analyses using a spectrophotometer Spectronic 20 Genesys (Spectronic Instruments Inc., Rochester, NY, USA) against the reagent blank. We analyzed ammonium in water extracts by a modified Berthelot reaction (Schinner *et al.*, 1996) and nitrate by a cadmium reduction method (U.S. EPA, 1979).

Data analyses

The plot C and N contents (PCC and PNC, respectively) in stems, leaves and total aboveground biomass were calculated by multiplying the concentration in the biomass fraction by its corresponding biomass per plot. We obtained the absolute accumulation of C and N in the

aboveground content of each plot during the period 1999–2005 by the difference of the aboveground content in 2005 and in 1999 (i.e. in the case of N, $PNC_{2005} - PNC_{1999}$). The relative accumulation was then obtained, (i.e. in the case of N by $[(PNC_{2005} - PNC_{1999}) / PNC_{1999}] \times 100$). We also calculated the ratio of C and N contents in leaves with respect to the contents in stems of each species at the plot level. We estimated NUE in the two dominant species *E. multiflora* and *G. alypum* in the period 1999–2005 by the relationship between the increase in biomass during this period (biomass 2005–biomass 1999) and the mean nitrogen content during this period $[(PNC_{2005} + PNC_{1999}) / 2]$ (Sardans *et al.*, 2005b).

To analyze the results on concentrations and accumulations, we used the STATVIEW 5.1 package (Abacus concepts Inc., Berkeley, CA, USA). We conducted one-way ANOVAS using the mean of each plot of the measured variables, with the different treatments as factors. We used the Bonferroni–Dunn test for the *post hoc* comparisons. In the case of C/N, N/P and C- and N-leaf/stem ratios that did not follow the assumptions of ANOVA, we used a nonparametric Kruskal Wallis test. If significant differences were detected, we conducted comparisons between control and drought and between control and warming treatments using the Wilcoxon Rank Sum Test.

Results

Soil moisture and temperature

During the 6 years of study (1999–2005), the average T increase in the warming treatment was 1 °C at 20 cm height and 0.95 °C at a soil depth of –5 cm. The drought treatment led to a mean reduction in soil moisture of 19% with respect to the control treatment and of 17.9% with respect to the warming treatment. The warming treatment increased the average soil and air T during the 6 years of study but did not significantly change the soil moisture. Therefore, the warming effects were mainly due to the direct effects of temperature increase, more than to possible indirect effects through decrease in moisture. A significant decrease in soil moisture occurred in the drought plots in spring and autumn rainy seasons with the drought treatment running (Fig. 1). Regarding warming, winter was the period with greater effect (Fig. 2). In winter, the warming plots had soil temperatures 1.20 ± 0.09 °C higher than in control soils whereas in summer they were 0.77 ± 0.08 °C higher (Fig. 2).

Aboveground biomass accumulation (1999–2005)

Neither warming nor drought had a statistically significant effect on total accumulated aboveground biomass

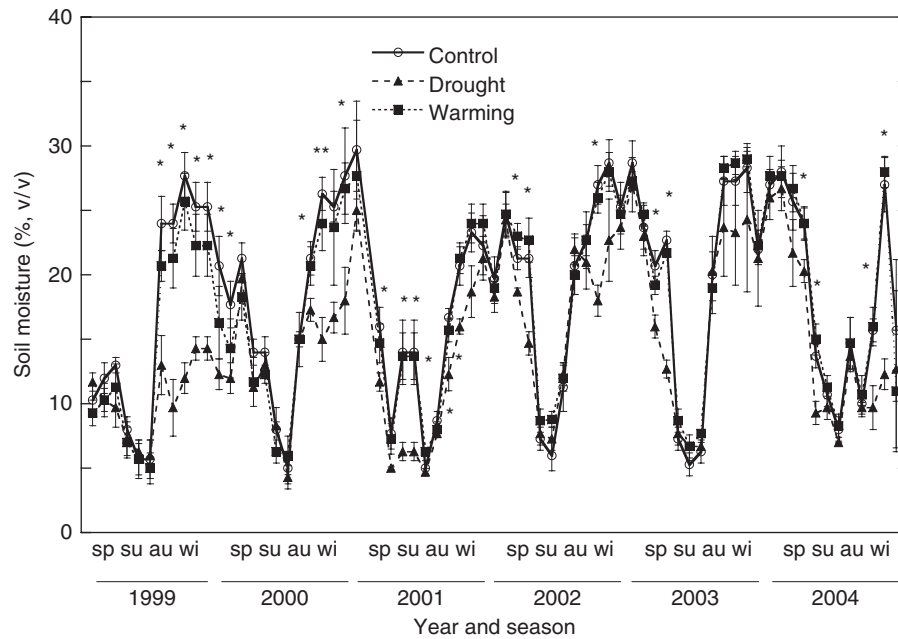


Fig. 1 Soil moisture (% v/v) (mean \pm SE) during the period March 1999–January 2005 for the different treatments. ($n = 3$ means of $n =$ three replicates per plot). Asterisk (*) indicates significant differences at $P < 0.05$ between control and drought treatment.

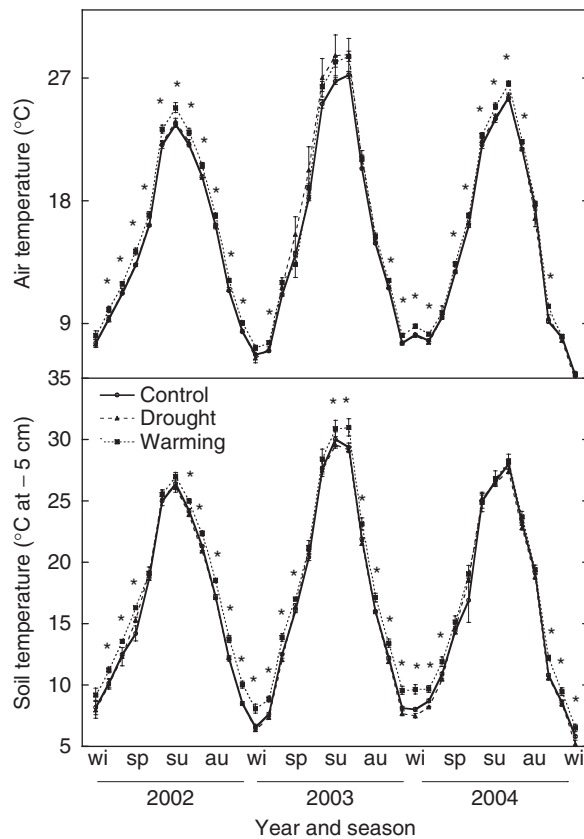


Fig. 2 Soil and air temperatures ($^{\circ}\text{C}$) (mean \pm SE) during the period January 2002–January 2005 for the different treatments ($n = 3$ plots). Asterisk (*) indicates significant differences at $P < 0.05$ between control and drought treatment.

Table 1 Aboveground biomass accumulation (kg ha^{-1} , mean SE, $n = 3$ plots) of *Erica multiflora* and *Globularia alypum* in control, drought and warming plots during the period 1999–2005

Species	Treatment	Leaves	Stems	Above-ground
<i>E. multiflora</i>	Control	290 \pm 197	668 \pm 358	958 \pm 551
	Drought	143 \pm 47	292 \pm 87	435 \pm 129
	Warming	381 \pm 236	1066 \pm 563	1447 \pm 799
<i>G. alypum</i>	Control	382 \pm 249	643 \pm 153	1025 \pm 173
	Drought	263 \pm 28	1712 \pm 490	1975 \pm 477
	Warming	61 \pm 164	1023 \pm 446	1083 \pm 610

There were no significant differences between treatments.

in the period 1999–2005 in *E. multiflora*. However, there was a trend to decrease biomass accumulation of *E. multiflora* in drought plots and to increase biomass accumulation of *G. alypum* in drought plots (Table 1).

C and N concentrations in biomass and litter

C and N concentrations and C/N concentration ratios in the *E. multiflora* and *G. alypum* plants of different plot treatments did not present differences in January 1999 just before treatments began (data not shown).

In *E. multiflora* after 6 years of treatment application, in January 2005, warming had not changed N concentration in current-year leaves and stems (Fig. 3) but had increased the N/P ratio in leaf litter (230%) (Fig. 3), a

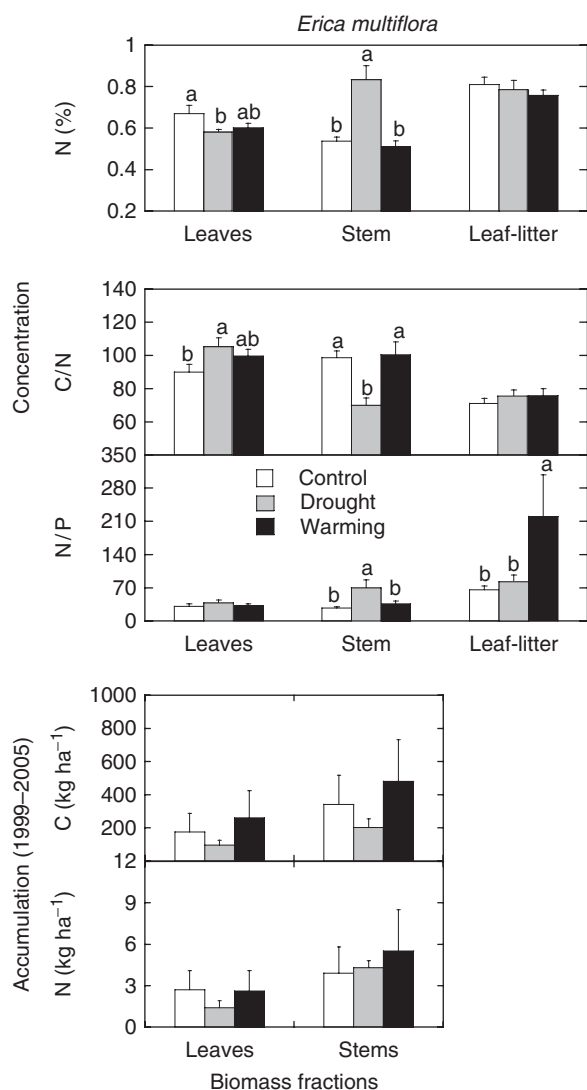


Fig. 3 N concentrations (% dw) and C/N and N/P concentrations ratio in leaves, stems and leaf litter, and absolute accumulation (kg ha⁻¹) in leaves and stems (period 1999–2005) of *Erica multiflora* (mean \pm SE) under control, drought or warming treatments. Different letters indicate significant statistical differences between treatments ($P < 0.05$, *post hoc* Bonferroni–Dunn test, ANOVA) ($n = 3$ means of $n =$ five replicates per plot).

change that was related to the increase in leaf P concentrations (Table 2). Drought decreased N concentrations by 16% in current-year leaves, increasing the corresponding C/N ratio by 17% (Fig. 3). On the contrary, drought increased N concentrations in stems by 56% and as a result, it decreased the C/N concentration ratio in stems by 25% (Fig. 3). Drought increased the N/P ratio in stems by 130% (Fig. 3), because of the increase in N concentration and the decrease in P concentrations (Table 2). Neither warming nor drought changed significantly C and N concentrations

in 1 year old leaves (data not shown). The N concentration values in 1 year old leaves ($0.6 \pm 0.03\%$ in control plants) were similar to those of current-year leaves.

In *G. alypum* after 6 years of treatment application, in January 2005, warming and drought reduced N concentration in leaves by 25% and 19%, respectively, and increased N concentrations in stems by 40% (Fig. 4). As a result, warming and drought increased the C/N concentration ratio in leaves by 31% and 33%, respectively, and decreased the C/N concentration ratio in stems by 29% and 27%, respectively (Fig. 4). Warming increased N concentrations by 100% and decreased the C/N concentration ratio by 52% and 47% in roots (fine and coarse, respectively) (data not shown). Warming increased the N/P concentration ratio in roots by 165% and 125% (fine and coarse, respectively) and in leaf litter by 45%. However, with warming, the N/P ratio was decreased in leaves by 43% (Fig. 4), in a change that was related to the decrease in P concentrations in the leaf litter (Table 2).

In *D. pentaphyllum* after 6 years of treatment application, in January 2005, warming had increased N concentrations in stems by 9% (Fig. 5) and had increased the leaf N/P ratio with respect to drought plots by 110% (Fig. 5).

C and N accumulation in aboveground biomass and litter (period 1999–2005)

Neither warming nor drought had statistically significant effects on the absolute accumulation of C and N in *E. multiflora* although drought tended to decrease C and N accumulation in this species (Fig. 3).

In *G. alypum*, warming decreased 3.3 kg ha^{-1} the N accumulation in leaves (Fig. 4), but increased C and N accumulation in stems (by 120 and 4 kg ha^{-1} , respectively) (i.e. by 92% and 49%), relative to the respective initial values for *G. alypum* in 1999. Drought increased C accumulation (563 kg ha^{-1} , 56% relative to initial values) and N accumulation (8.1 kg ha^{-1} , 87% relative to initial values) in stems of *G. alypum* (Fig. 4).

Warming and drought did not significantly change the leaf and total aboveground biomass NUE both in *E. multiflora* and in *G. alypum* (data not shown).

C and N in soil

Warming increased ($P = 0.067$) the concentration of extractable ammonium in soil in winter 2004–2005, whereas it reduced it in spring 2004 (Table 3). No effects of treatments were observed in soil extractable ammonium in autumn and summer 2004 (Table 3). Warming increased soil extractable nitrate in summer and in autumn 2004, whereas no effects of treatments were

Table 2 P concentrations (mg g^{-1}) in current-year leaves and leaf-litter measured in January 2005 in *Erica multiflora* and *Globularia alypum* for different experimental treatments

Species	Fraction	Treatments		
		Control	Drought	Warming
<i>E. multiflora</i>	Current-year leaves	$0.125 \pm 0.020b$	$0.129 \pm 0.019ab$	$0.178 \pm 0.023a$
	Stems	$0.238 \pm 0.041a$	$0.165 \pm 0.017b$	$0.175 \pm 0.018ab$
	Leaf litter	$0.126 \pm 0.014a$	$0.099 \pm 0.011a$	$0.045 \pm 0.014b$
<i>G. alypum</i>	Current-year leaves	$0.418 \pm 0.032a$	$0.331 \pm 0.031b$	$0.429 \pm 0.023a$
	Stems	0.256 ± 0.006	0.281 ± 0.011	0.270 ± 0.017
	Leaf litter	$0.336 \pm 0.044a$	$0.261 \pm 0.023ab$	$0.225 \pm 0.023b$
	Roots	0.476 ± 0.070	0.502 ± 0.236	0.392 ± 0.078

Different letters indicate significant ($P < 0.05$) differences between treatments. They are italic type. ($n = 3$ means of $n =$ five replicates per plot).

observed in winter 2004 and in spring 2005 (Table 3). Neither warming nor drought had any significant effect on total soil N content (Table 3).

Warming decreased total organic C in soil with respect to drought plots (2.9 vs. 2.1% [w/w], $P = 0.04$) (Table 3).

Discussion

N allocation to stems

Foliar N concentrations and total leaf N content decreased in drought conditions in both dominant species, *G. alypum* and *E. multiflora*. It also decreased in *G. alypum* in response to warming. These decreases occurred in parallel to the increases in N allocation to stems, and under warming in *G. alypum* also to roots. The N allocation to stems in the drought plots, observed mainly in *G. alypum*, can be related to a mechanism of drought avoidance. The accumulation of osmoprotectors rich in N such as nitrate, proline and other α -amino N compounds is typical of shoots in plants adapted to drought while non-N osmoprotectors are more frequent in leaves (Chiang & Dandekar, 1995; Bloch & Hoffmann, 2005). Villar-Salvador *et al.* (1999) also reported that moderate drought resulted in more N concentrating in the shoots of *P. halepensis*. The decrease in foliar N concentrations due to warming has also been observed in different temperate-type non-Mediterranean ecosystems (Tolvanen & Henry, 2001; An *et al.*, 2005). In

contrast, in colder regions some studies have reported increases in N concentrations in leaves under warming conditions due to the increase of physiological and

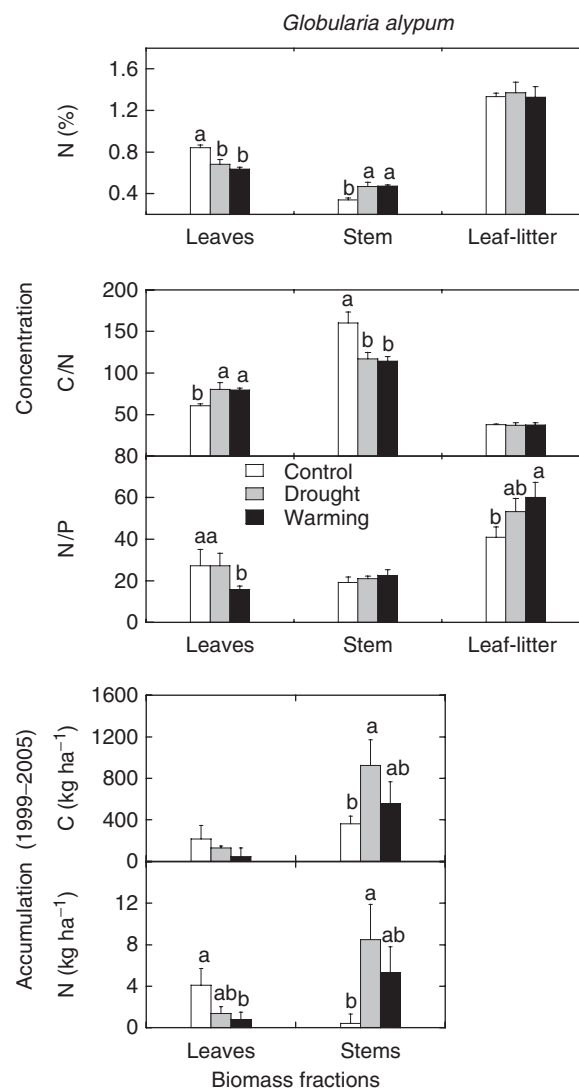


Fig. 4 N concentrations (% dw) and C/N and N/P concentration ratios in leaves, stems and leaf litter, and absolute accumulation (kg ha^{-1}) in leaves and stems (period 1999–2005) of *Globularia alypum* (mean \pm SE) under control, drought or warming treatments. Different letters indicate significant statistical differences between treatments ($P < 0.05$, *post hoc* Bonferroni–Dunn test, ANOVA) ($n = 3$ means of $n =$ five replicates per plot).

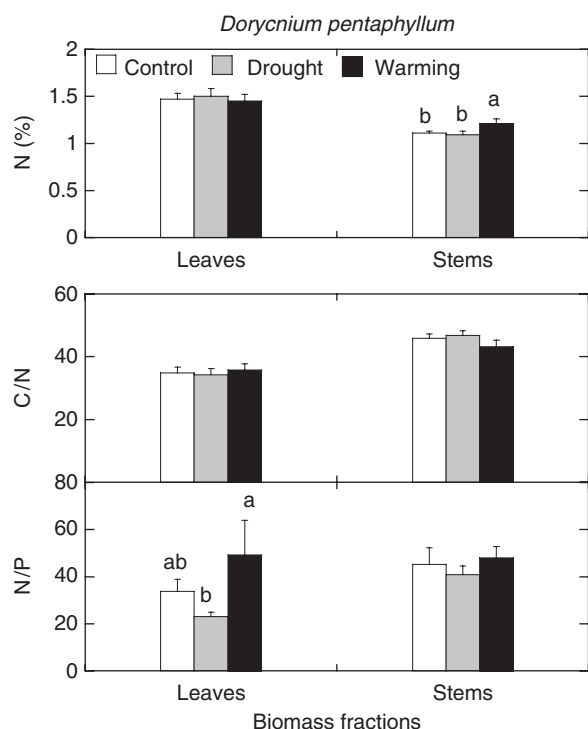


Fig. 5 N concentrations (% dw) and C/N and N/P concentration ratios in leaves and stems of *Dorycnium pentaphyllum* (mean SE) under control, drought or warming treatments. Different letters indicate significant statistical differences between treatments ($P < 0.05$, *post hoc* Bonferroni–Dunn test, ANOVA) ($n = 3$ means of $n =$ five replicates per plot).

Table 3 Carbon and nitrogen concentrations in the soil: total C (% w/w), total N (% w/w), C/N ratio, total extractable (0.1 M KCl) ammonium and nitrate ($\mu\text{g g}^{-1}$)

	Treatment		
	Control	Drought	Warming
Total organic Carbon	2.5 ± 0.3 ^{ab}	2.9 ± 0.2 ^a	2.1 ± 0.2 ^b
Total nitrogen	0.154 ± 0.021	0.173 ± 0.023	0.163 ± 0.018
C/N ratio	17.6 ± 1.4	20.6 ± 4.1	14.9 ± 2.3
Soil NH ₄ ⁺			
Spring	1.04 ± 0.10 ^a	0.94 ± 0.17 ^{ab}	0.73 ± 0.19 ^b
Summer	1.50 ± 0.35	1.20 ± 0.21	1.64 ± 0.20
Autumn	1.25 ± 0.10	1.14 ± 0.14	1.37 ± 0.17
Winter	1.28 ± 0.09 ^(b)	1.51 ± 0.10 ^(ab)	1.56 ± 0.12 ^(a)
Soil NO ₃ ⁻			
Spring	2.5 ± 1.8	6.2 ± 3.9	3.3 ± 1.4
Summer	7.5 ± 4.3 ^b	11.1 ± 3.9 ^{ab}	22.1 ± 6.8 ^a
Autumn	8.5 ± 2.3 ^b	4.1 ± 1.7 ^b	18.4 ± 4.2 ^a
Winter	17.8 ± 3.5	25.9 ± 6.1	24.8 ± 2.9

Different letters indicate significant statistical differences between treatments (they are highlighted in italics) ($P < 0.05$, *post hoc* Bonferroni–Dunn test, ANOVA). (When between brackets $P < 0.10$). ($n = 3$ means of $n =$ five replicates per plot).

photosynthetic activity and of plant N uptake capacity (Soussana *et al.*, 1996; Luomala *et al.*, 2003).

Neither warming nor drought had significant effects on the leaf and stem biomass accumulation in the period 1999–2005; therefore, the decreases in N leaf concentrations and the increases in N stem concentrations observed mainly in the drought plots seem not to be due to a dilution or concentration effect. Other factors such as changes in photosynthetic capacity, the different C and N allocation between leaf and stems or the synthesis and accumulation of osmoprotectors are the most likely causes.

The increase of N in stems with respect to leaves in the two dominant non-N-fixing species under drought and of *G. alypum* under warming may slow down the N turn-over, because stem turn-over is slower than leaf turn-over. This may favor the accumulation of more N in total plant biomass. Moreover, this increase of N allocation to stems instead of to leaves can affect the production capacity and plant survival of non-N-fixing dominant species under long-term warming and/or drought conditions.

In contrast with the leaf N concentration decrease observed in the other two shrub species, warming did not decrease leaf N concentrations in *D. pentaphyllum*. This species is a shrub legume and warming could have enhanced its N fixation, thus increasing N capture more than in the other two species.

N vs. P limitation

We observed similar values in leaf N concentrations in current-year and 1 year old leaves in *E. multiflora* and higher values of N concentration in litterfall in both species. Foliar N concentration decreased in warming conditions without decreasing the plant production capacity, that even tended to increase in both species (Llorens *et al.*, 2003a, b). All these results indicate that N is probably not limiting in this ecosystem, and that other scarce nutrients, such as P, are probably more limiting than N. In *G. alypum*, warming decreased the N/P concentration ratio in leaves and increased the N/P concentration ratio in litterfall indicating a greater accumulation of P than N in leaves and a greater reabsorption of P than N before leaf fall under warming conditions. Moreover, *E. multiflora* also presented a higher N/P concentration ratio in leaf litter. These results confirm that P is probably the limiting nutrient in this ecosystem with low soil pH and P availability (Sardans *et al.*, 2006b). Thus, the increase in photosynthetic capacity in *G. alypum* (Llorens *et al.*, 2004) was associated with an increase in P retranslocation and P uptake under warming (Sardans *et al.*, 2008b) and to a decrease in N/P ratio in leaves, but not to an increase in

either N retranslocation nor leaf N concentrations. Phosphorus concentration is related to plant photosynthetic capacity and is frequently a limiting factor for growth (Delzon *et al.*, 2005), particularly in Mediterranean shrublands (Hanley & Fenner, 2001; Sardans *et al.*, 2004, 2005b, 2006a). Thus, it seems that in these calcareous Mediterranean shrublands the ecosystem response to climate change can be mediated by P availability more than by N availability.

Increasing C/N ratio

The increase in the C/N leaf concentration ratio formed in the dominant non-N-fixing species in response to warming and drought is a widely observed phenomenon (Groom & Lamont, 1999; Bussotti *et al.*, 2000). These effects were due to a decrease in N concentrations under warming and to an increase in C concentrations and a decrease in N concentrations under drought. This usually results from an increase in structural compounds rich in carbon and poor in N, such as lignin, in order to protect leaf tissues from water deficit (Bussotti *et al.*, 2000). With further drought, an increase in leaf C concentrations and stem N concentrations and content can be expected in non-N-fixing shrubs in order to improve the plant's capacity to withstand drought.

By increasing the leaf C/N ratio, warming and drought contributed to decreasing the nutritional quality of the plants, but these changes were different among plant tissues and shrub species of this community. These C/N stoichiometric changes can affect trophic chains and species community composition (Ngai & Jefferies, 2004; Diehl *et al.*, 2005). The increases in the leaf C/N concentration ratio may, for example, have implications in plant–herbivore relationships because grazing pressure is correlated with foliar N concentrations (Estiarte *et al.*, 1994; Papatheodorou & Stamou, 2004).

Soil C and N

Whereas, in warming plots there was a tendency to reduce soil C and increase N availability, in drought plots there was a tendency to increase soil C concentrations. These results are related to an increased soil microbe enzyme activity observed in warming plots and to a decrease of soil microbe activity in drought plots (Sardans *et al.*, 2006b, 2008a). The increases in soil enzyme activity in warming plots are directly related to the increase in soil temperatures during winter and spring and the decreases in soil enzyme activities in drought plots were a direct consequence of the decrease in soil moisture throughout the year (Sardans *et al.*, 2006b, 2008a).

Warming increased soil soluble NH_4^+ in winter and soil extractable NO_3^- in summer and autumn in accordance with the observed increase in soil enzyme activity. In winter, urease was particularly active and in general there was a greater soil biological activity observed in warming plots (Sardans *et al.*, 2008a). The decreases in soil extractable NH_4^+ in spring observed in warming plots are related to higher N plant capture. This was due to the enhancement of growth observed in warming plants in the growing season (spring) of some years during the period 1999–2005 (Llorens *et al.*, 2004; Prieto, 2007). The increases in soil extractable NO_3^- concentrations in warming plots in summer and in autumn were expected, because temperature is positively related to nitrification in temperate non-Mediterranean ecosystems (Ryan *et al.*, 1998; Wang *et al.*, 2006).

C and N biomass accumulation 1999–2005: differences between species

Neither warming nor drought had significant effects on the NUE of *E. multiflora* and *G. alypum* in the period 1999–2005. The observed results were not consistent with those observed in some non-Mediterranean ecosystems. For example, An *et al.* (2005) in a warming experiment in a North American grassland observed that warming decreased the NUE. The different patterns of growth between temperate grasses and Mediterranean shrubs, the different experimental warming conditions (achieved by infrared heaters) and the different climate described by An *et al.* (2005) can account for the disparity.

Neither warming nor drought changed the C and N accumulation in the aboveground biomass of *E. multiflora* as a result of the counterbalance between changes in foliar N concentration and in biomass growth. In contrast, in *G. alypum*, warming decreased N accumulation in leaves by a substantial decrease in leaf N concentration, whereas drought increased C and N stem accumulation by an increase in N concentration in stems and by a tendency to increase stem biomass accumulation. In *G. alypum*, the effects of warming on N concentrations were associated to a greater plasticity to allocate N to different plant tissues and not to an absolute increase in N uptake and accumulation in aboveground biomass, because the sum of N accumulated in leaf biomass plus that accumulated in stem biomass was similar in the control as in drought and warming plots. These different effects of warming on C and N accumulation capacity in the two dominant non-N-fixing shrub species were related to the previously observed differences in growth capacity, leaf net photosynthetic rates and retranslocation in response to the treatments (Llorens *et al.*, 2003a, b).

Regarding drought plots, *G. alypum* accumulated more N in stems and less N in leaves in drought than in control plots; however, these changes were not observed in *E. multiflora*. *E. multiflora* was the most affected species, because drought reduced its photosynthetic capacity and stem length growth more when compared with *G. alypum* (Llorens *et al.*, 2004; Prieto, 2007). *G. alypum* has a greater capacity to resist lower water potentials than *E. multiflora* when water is scarce, and presents greater water absorption, transpiration, photosynthetic rates and growth when water is available (Llorens *et al.*, 2003b).

Thus, these different species have a different capacity to respond to warming and drought. This has been observed in similar experiments conducted in other ecosystems, mainly in colder temperate climates or in boreal climate (Tolvanen & Henry, 2001; Kudo & Suzuki, 2003). Given the global warming scenario, this capacity to respond may end up changing species community composition and structure in the long term. In fact, changes in the community species diversity in this Mediterranean shrubland are already starting to occur (Peñuelas *et al.*, 2007; Prieto, 2007).

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