

## Ecophysiological responses of two Mediterranean shrubs, *Erica multiflora* and *Globularia alypum*, to experimentally drier and warmer conditions

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A new approach was used to experimentally dry and warm a Mediterranean shrubland. By means of automatically sliding curtains, the drought period was extended by excluding rain over the two growing seasons (spring and autumn), and passive warming was created by avoiding infra-red dissipation at night over the whole year. The aim of the study was to test how a future extended drought period and an increase in temperatures could affect the photosynthetic and water use strategies of two co-occurring Mediterranean shrubs, *Erica multiflora* and *Globularia alypum*, which are common species of the dry coastal shrublands. The shoot water potential, leaf gas exchange rates and chlorophyll *a* fluorescence of plants was monitored seasonally during two years (1999–2001). In addition we measured the photosynthetic response curves to light and CO<sub>2</sub> in autumn 2001 and the foliar N concentration and leaf C and N stable isotopes in summer 1999 and 2000. Droughted plants of both shrub species showed lower shoot water potentials, transpiration rates and stomatal conductances than control plants, although there was a high seasonal variability. Drought treatment reduced significantly the overall leaf net photosynthetic rates of *E. multiflora*, but not of

*G. alypum*. Droughted plants of *E. multiflora* also showed lower leaf net photosynthetic rates in response to light and CO<sub>2</sub> and lower carboxylation efficiency than controls, but there was no significant effect of drought on its overall photosystem II (PSII) photochemical efficiency. Although warming treatment did not affect the leaf net photosynthetic rates of the two species overall the study, it increased significantly the carboxylation efficiency and leaf net photosynthetic rates of *G. alypum* plants in response to CO<sub>2</sub> levels in autumn 2001. In addition, warming treatment increased the potential photochemical efficiency of PSII ( $F_v/F_m$ ) of both species (but especially of *G. alypum*) at predawn or midday and mainly in autumn and winter. Thus, the results suggest that drier conditions might decrease the annual productivity of these Mediterranean shrubs, particularly of *E. multiflora*, and that future warming could alleviate the present low temperature constraints of the photosynthetic performance of the two studied species, but especially of *G. alypum*, during the colder seasons. Ultimately, drier and warmer conditions in the near future may change the competitive relationship among these species in such Mediterranean ecosystems.

### Introduction

Human activities are increasing atmospheric concentrations of greenhouse gases, which are projected to lead to regional and global changes in climate (IPCC 2001). Over the last decade, a growing number of field experiments simulating some of the predicted climatic changes were initiated around the world (e.g. see Shaver et al. 2000 for a review of warming experiments). However, to

date, the majority of these climate experiments have been conducted in north temperate, boreal and arctic ecosystems. Thus, a clear need exists to explore the responses of warmer and drier ecosystems to climate change.

Current climate projections predict drier and warmer conditions for the Mediterranean basin in future decades (IPCC 2001). Until now, most of the techniques used to

Abbreviations – *A*, net photosynthetic rate; CE, carboxylation efficiency; *E*, transpiration rate;  $g_s$ , stomatal conductance; PPFD, photosynthetic photon flux density; TDR, time domain reflectometry.

experimentally warm ecosystems alter other environmental factors (e.g. light, humidity, soil structure) and most of the experiments usually simulate a diurnal increase in temperature rather than the predicted stronger increase in  $T_{\min}$  (night-time temperature) (IPCC 2001). Thus, we used a new approach for the experimental drought and warming of a dry Mediterranean shrubland (Beier et al. 2003). On one hand, we extended the drought period by excluding rain over the two growing seasons (spring and autumn). On the other hand, we created passive warming by avoiding infra-red dissipation at night over the whole year, which simulates the way climate change alters the heat balance of ecosystems.

Mediterranean summer drought is generally considered the primary constraint to the productivity and distribution of the Mediterranean vegetation (Larcher 2000). Indeed, many studies have described reductions in photochemical efficiency and low photosynthetic rates during summer drought (e.g. Harley et al. 1987, Tenhunen et al. 1990, Damesin and Rambal 1995, Valladares and Percy 1997, Gratani et al. 2000, Llorens et al. 2003b). In addition, temperature during Mediterranean summer may reach potentially damaging thresholds for physiological processes (Epron 1997), especially when drought-induced stomatal closure limits the ability of plants to avoid heat stress by transpirational cooling (Ladjal et al. 2000). A marked decline in maximal rate of photosynthesis at saturating irradiance and high internal  $\text{CO}_2$  concentration has been observed at temperatures ranging from 35 to 40°C (Tenhunen et al. 1984, Niinemets et al. 1999, Gratani et al. 2000). Maximum summer air temperatures of 35–40°C frequently occur in the Mediterranean maquis (Larcher 2000). Therefore, the increase in the duration and severity of drought, together with the indirect effects of future warming on evapotranspiration and soil dryness, may constrain the physiological activity of Mediterranean plants severely. Moreover, higher temperatures may significantly enhance leaf heat stress in summer, limiting growth and survival of plants due to severe restrictions on photosynthesis.

Conversely, a future warming may alleviate the physiological constraints that Mediterranean plants experience in winter. Mediterranean winters may be relatively cold, and often associated with long rainless and cloudless periods. Indeed, low photosynthetic rates (reviewed by Larcher 2000) and reductions of the efficiency of photosystem II (PSII) have been reported during winter (García-Plazaola et al. 1999, Karavatas and Manetas 1999, Larcher 2000, Oliveira and Peñuelas 2001, Llorens et al. 2003b). Some authors have suggested that winter cold stress also plays a relevant role in the development and distribution of Mediterranean evergreen species (Mitrakos 1980, Tretiach 1993).

Since co-occurring Mediterranean species often have different climatic constraints, each species will likely respond differently to the climate change. *Erica multiflora* and *Globularia alypum* are two co-occurring Mediterranean shrubs that differ in their photosynthetic and water use strategies to cope with seasonal variance in

water availability and temperature (Llorens et al. 2003b). Whereas *E. multiflora* is a more water-conservative species, *G. alypum* follows a prodigal or non-conservative water use strategy. The aim of our study was to test how future warming and extended drought events could affect the physiological performance and the photosynthetic and water use strategies of these two co-occurring Mediterranean shrubs. Particularly, we tested whether future warmer and drier conditions could have a different effect on the photosynthetic performance and water use of these shrubs depending on the season. Our hypotheses were the following:

- (1) Drought would reduce leaf gas exchange rates and might result in enhanced photoinhibition (i.e. reduced photochemical efficiency of PSII). In addition, the decrease in water availability would raise both instantaneous ( $A/E$ ) and integrated water-use efficiency, the latter shown by increased values of  $\delta^{13}\text{C}$  of leaves. Plants in the drought treatment might reduce their foliar N concentrations (Heckathorn et al. 1997) and might show more positive foliar  $\delta^{15}\text{N}$  values (Peñuelas et al. 2000).
- (2) The effect of the warming treatment could be different depending on the season. In summer, warming treatment might decrease leaf gas exchange rates and thus, increase photoinhibition due to leaf overheating. Conversely, in winter, warming might stimulate leaf gas exchange rates, decreasing the risk of suffering photoinhibition. Instantaneous and integrated water-use efficiency might be expected to decrease through an increase in evaporative demand and larger water losses through transpiration. However, if warming reduces soil humidity, then, the reduction of transpiration rates could lead to increases in water-use efficiency. The nutrient availability (and the  $\delta^{15}\text{N}$  values) will depend on the balance between the warming positive effect on mineralization and the negative effect of reduced soil moisture.

## Materials and methods

### Study site and species description

The study was carried out in a dry shrubland (*Rosmarino-Ericion*) in the Garraf Natural Park, Barcelona, NE Spain (41°18' N, 1°49' E), at 210 m above sea level and on a south-south-east slope (13°). The climate is typically Mediterranean, with mild temperatures and few but torrential rains during spring and autumn, cool winters and hot and dry summers. The site, which is located on terraces from abandoned vineyards, suffered large fires in the summers of 1982 and 1994. The soil is a petrocalcic calcixercept (SSS 1998), thin (12–37 cm), with a loamy texture and abundant calcareous nodules. Currently the regenerating vegetation covers 50–60% with a maximum height of 70 cm.

*Erica multiflora* L. (Ericaceae) and *Globularia alypum* L. (Globulariaceae) are evergreen, sclerophyllous shrubs

that typically occur in basic soils of the western Mediterranean Basin, where they are common components of the coastal shrubland. Both species re-sprout from underground organs after above-ground biomass removal. Vegetative growth occurs twice a year: in spring (from March to June) and autumn (from September to November). Flowering starts in August–September

### Experimental system

Two types of climatic manipulations were performed using automatically sliding covers (Beier et al. 2003):

- (1) Extended summer drought was induced by covering the plots with transparent and waterproof plastic curtains during all rain events over the two annual growing seasons, starting in March–April in spring and in September–October in autumn.
- (2) Passive night-time warming was achieved by covering the vegetation and soil during night with curtains made of a material refractory to the infra-red radiation. Curtains drew over the vegetation at sunset and they were removed at sunrise (below and above 200 lux, respectively). Thus, they retained a portion of the energy accumulated in the ecosystem during the light period, simulating the mechanism of global warming. Warming treatment started on 16 March 1999 and it was working all nights throughout the study, except during two periods (1–30 August 1999 and 1–26 January 2000), when the treatment was stopped due to mechanical problems or for calibration of system effects.

The covers were mounted on metal scaffolding 0.2 m above the vegetation (approximately 0.8–1 m above the ground). Nine plots of 20 m<sup>2</sup> (4 m × 5 m) were established: three untreated controls, three warming and three drought plots. Plots were organized in three blocks (each block with one control, one drought and one warming plot). Control plots had similar scaffolding to the warming and drought plots, but with no cover. The plots were open at the sides to allow free wind movement. Movement of the covers were controlled automatically by climatic conditions according to preset criteria (Beier et al. 2003). The automatic control of the covers minimized unintended side-effects on the light regime, hydrology and wind (Beier et al. 2003). For instance, at night, warming covers were automatically removed when it rained in order to avoid influencing the hydrological cycle. We assigned the outer 0.5 m of each study plot as a buffer zone with all measurements carried out in a central 12 m<sup>2</sup> area.

### Environmental data

Precipitation was registered at the study site with a standard rain gauge. Soil moisture was measured on three fixed sampling points per plot, every 1–2 weeks through the study period, using time domain reflectometry (TDR).

Air and soil temperatures (0, 2 and 10 cm depth) during the sampling period were obtained by means of temperature sensors RTD Pt100 1/3 DIN (Desin Instruments, Barcelona, Spain) located in the three plots (control, drought and warming) of one block. Temperatures were measured every 10 min, being recorded the average of three measurements of each sensor.

### Shoot water potential

Water potentials of apical shoots were measured seasonally with an Scholander-type pressure bomb (PMS Instruments, Corvallis, OR, USA). On each sampling date, one shoot of *E. multiflora* and *G. alypum* per plot was measured at predawn (0230–0430 h in spring and summer and 0430–0630 h in autumn and winter, solar time) and midday (1100–13.00 h, solar time).

### Leaf gas exchange rates and chlorophyll fluorescence

We measured leaf gas exchange rates and chlorophyll (Chl) fluorescence during four to six consecutive days in spring (May), summer (August), autumn (November) and winter (February) throughout two years (spring 1999 – winter 2001). Pre-treatment measurements (February 1999) were conducted in order to identify the variability between blocks, but they were not used in the statistical analyses. Measurements were taken on sun-exposed shoots grown in the spring of the current year.

Each season, leaf gas exchange rates were measured on three to four plants per plot (one shoot per plant) of *E. multiflora* and *G. alypum* in the morning (from 30 min after sunrise to 1130 h, solar time, at the latest) and in the afternoon (from midday to 1630 h, solar time, at the latest). Stomatal conductances ( $g_s$ ), net photosynthetic rates ( $A$ ) and transpiration rates ( $E$ ) were measured with a portable open-flow gas exchange system (ADC4; ADC Inc., Hoddesdon, Hertfordshire, UK), which also measured air temperatures in the leaf chamber (PLC4B; ADC Inc.) and incident photosynthetic photon flux density (PPFD) at the moment of the leaf gas exchange measurements. Branch tips with several leaves were introduced into the chamber of the system. All results are expressed on a leaf area basis, which was measured using IMAGEPC (v.  $\alpha 9$  for Windows, Scion Co., Frederick, MD, USA) from photocopies of all the leaves of a measured shoot. Water-use efficiency, defined as mmol of net CO<sub>2</sub> uptake per mol of H<sub>2</sub>O transpired, was calculated by dividing instantaneous values of  $A$  by  $E$ .

As complementary measurements, response curves of leaf net photosynthetic rates ( $A$ ) to PPFD and to CO<sub>2</sub> were conducted with a field-portable infra-red gas analysis system (model LI-6400; standard chamber 2 × 3 cm with 6400–02B light source and 9964–026 CO<sub>2</sub> source; Li-Cor Inc., Lincoln, NE, USA) during five consecutive days in November 2001. Light-response curves were carried out across the PPFD range 0–2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at 350 p.p.m. of CO<sub>2</sub>. CO<sub>2</sub>-response curves were constructed across the CO<sub>2</sub> range 50–800  $\mu\text{mol mol}^{-1}$  under

saturating PPFD ( $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Leaf temperatures were fixed at  $20^\circ\text{C}$ . Measurements were performed in one plant of each species per plot (i.e. three plants per species and treatment).

Components of Chl fluorescence were quantified with a portable modulated fluorometer PAM-2000 (Heinz Walz GmbH, Effeltrich, Germany). After a dark adaptation period of at least 30 min, we obtained minimum and maximum dark-adapted fluorescence ( $F_0$ ,  $F_m$ ) and  $F_v/F_m$ , where  $F_v = F_m - F_0$ .  $F_v/F_m$  has been used as a measure of the potential (or maximum) photochemical efficiency of PSII. Measurements were performed on three to five plants of *E. multiflora* and *G. alypum* per plot, at pre-dawn and midday.

The actual photochemical efficiency of PSII in the light-adapted state was estimated as:  $\Phi_{\text{PSII}} = \Delta F / F_m' = (F_m' - F) / F_m'$ , where  $F$  is the steady-state fluorescence yield under the given environmental conditions, and  $F_m'$  is the maximum level of fluorescence obtained during a saturating flash of light (when all the PSII traps are closed) under the same environmental conditions. From this index, we calculated the apparent electron transport rate (ETR) as:

$$\text{ETR} = \Delta F / F_m' \times \text{PPFD} \times 0.84 \times 0.5$$

where PPFD was the photosynthetic photon flux density incident on the leaf, 0.84 was the coefficient of absorption of the leaves, and 0.5 was the fraction of electron required to the absorption of one quanta, as two photosystems are involved (Krall and Edwards 1992). We measured the ETR on three to four plants of *E. multiflora* and *G. alypum* per plot, at morning (0700–1000 h, solar time) and midday (1100–1400 h, solar time).

### Isotope and elemental analyses

Leaf nitrogen (N) concentrations and foliar  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were determined on current year leaves collected in August 1999 and 2000. We sampled three plants of *E. multiflora* and *G. alypum* per plot each year. All analyses were carried out in an elemental analyser EA1108 (Carlo Erba, Milano, Italy) coupled to a Delta C isotope ratio mass spectrometer with a CONFLO II interface (Thermo Finnigan MAT, Bremen, Germany). The calibrations were performed using interspersed international isotopic standards of carbon and nitrogen (IAEA, Vienna, Austria). The elemental analysis calibration was performed using atropine (ThermoQuest Italia, Milan, Italy) as standard.

Values are expressed relative to PDB standard for  $\delta^{13}\text{C}$  and relative to atmospheric nitrogen for  $\delta^{15}\text{N}$ , according to the following equation:

$$\delta Z = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000$$

where  $Z$  is the heavy isotope of either nitrogen or carbon, and  $R$  is the ratio of heavier to lighter isotope for the sample and standard ( $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ ). The accuracy of the measurements was  $\pm 0.15\%$  for  $\delta^{13}\text{C}$  and  $\pm 0.3\%$  for  $\delta^{15}\text{N}$ .

### Statistical analyses

Drought and warming effects were analysed always separately. To test the effect of treatments on soil moisture throughout the study period, we performed repeated-measures analysis of variance (ANOVAR) using monthly means per plot and with treatment as the independent factor. For each species and time of the day, we conducted analysis of variance (ANOVA) with the mean of  $\psi$ ,  $g_s$ ,  $A$ ,  $E$ ,  $F_v/F_m$  and ETR in each plot as dependent variables and with year (first: March 1999–February 2000 and second: March 2000–February 2001), season (spring, summer, autumn and winter) and treatment (control-drought or control-warming) as independent factors. We did not conduct ANOVAR because we randomly sampled plants in the different seasons. Significant differences between means, as well as significant interactions between factors, were identified taking  $P \leq 0.05$  as the level of significance. When interactions were significant, we performed ANOVAS to analyse the effect of treatments within each year and/or season. In these analyses, since multiple tests were performed for the same variable, the significance level ( $P = 0.05$ ) was adjusted for the number of statistical tests using a sequential Bonferroni correction to prevent group-wide type errors (Rice 1989). To test the effect of treatments on the N concentration and the C and N isotope composition of the leaves, we performed ANOVAS for each species (using the mean values per plot), with treatment and year (1999, 2000) as independent factors.

To compare the photosynthetic response curves of *E. multiflora* and *G. alypum* to PPFD and  $\text{CO}_2$  levels we used the comparison of fitted curves method (Potvin et al. 1990). To linearize the curves we used the logarithmic transformation for PPFD values [ $x' = \log(x + 1)$ ] and the root square transformation for  $\text{CO}_2$  values [ $x' = (x + 0.5)^{1/2}$ ]. We compared drought and warming curves with control curves separately for each species. In the PPFD-response curves, we determined also the dark respiration ( $R_d$ ), as the mean of the values for the three plants per treatment recorded at PPFD = 0.  $A-C_i$  curves were also plotted to determine the carboxylation efficiency for each treatment, which was estimated as the slope of each curve obtained by linear regression, since saturation was not achieved (Long and Hällgren 1993).

## Results

### Environmental data

Rainfall in the first year of the study, from March 1999 to February 2000, was lower (370 mm) than in the second year, from March 2000 to February 2001 (511 mm) (Fig. 1). Drought treatment reduced significantly the soil moisture (% volume) throughout the study period by 23% on average in relation to control plots ( $F_{1,4} = 14.1$ ;  $P = 0.02$ ; Fig. 1), being the maximum reductions in autumn (up to 50% in October 1999). Warming treatment did not affect significantly the soil moisture throughout the study period, but it decreased it in

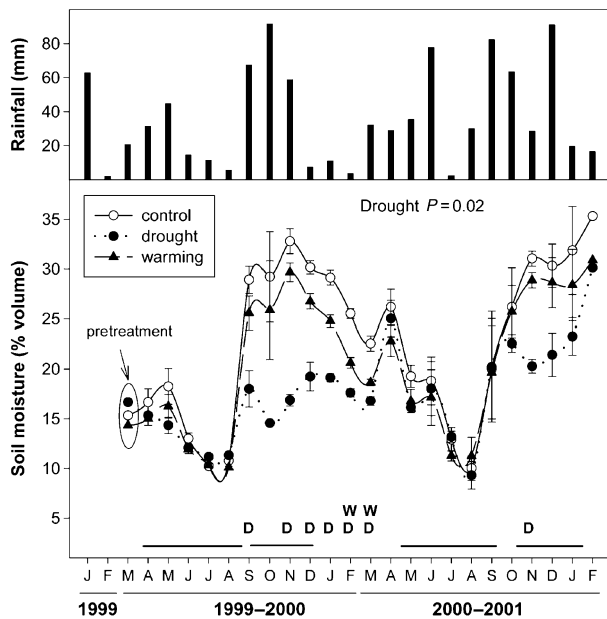


Fig. 1. Monthly rainfall (mm) and mean soil moisture (% volume/volume) of control, drought and warming treatments throughout the study period. Error bars indicate the standard error of the mean ( $n=2-6$  days; values are averages of three plots). Lines above the months indicate the periods when drought treatment was working on. Global effects of treatments are depicted when significant ( $P \leq 0.05$ ). D and W indicate significant differences (after sequential Bonferroni correction of the significance level  $P=0.05$ ) between control and drought or control and warming treatments within each month.

February–March 2000 (Fig. 1). The monthly significant effects of treatments (after sequential Bonferroni correction) are depicted in Fig. 1.

Warming treatment increased minimum temperatures on average  $0.7^\circ\text{C}$  at air (20 cm above ground),  $1.6^\circ\text{C}$  at 2 cm depth and  $1.15^\circ\text{C}$  at 10 cm depth. The reduced heat loss in the warming plots at night increased also the diurnal temperatures in the air and soil in comparison with the control plots, showing a diurnal pattern with a maximum difference after sunrise and a minimum difference in the late afternoon (Fig. 2).

### Shoot water potential ( $\psi$ )

Shoot water potential ( $\psi$ ) values of *E. multiflora* plants in the drought treatment were lower than those of controls at midday ( $F_{1,32} = 5.7$ ;  $P = 0.02$ ; Fig. 3). Conversely, droughted plants of *G. alypum* showed lower  $\psi$ -values than controls through all the study period at predawn ( $F_{1,28} = 13.0$ ;  $P = 0.001$ ) and only in autumn at midday ( $F_{1,8} = 22.2$ ;  $P = 0.002$ ; Fig. 3). Warming did not produce any significant effect on shoot water potential (data not shown).

### Leaf gas exchange rates

Droughted plants of *E. multiflora* showed on average a decrease of 41% in their overall net photosynthetic rates

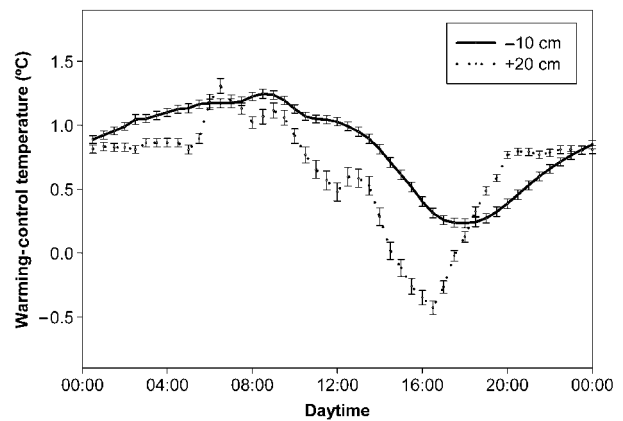


Fig. 2. Annual averages of air (20 cm above ground) and soil (10 cm depth) temperature ( $^\circ\text{C}$ ) differences between warming and control plots (measured in one of the blocks from April 2000 to April 2001).

(A) in the morning ( $F_{1,32} = 12.2$ ;  $P = 0.001$ ) and of 27% in the afternoon ( $F_{1,32} = 5.4$ ;  $P = 0.03$ ; Fig. 4). In contrast, drought treatment did not decrease significantly the overall A of *G. alypum* (Fig. 4).

There was also a global effect of drought treatment on leaf stomatal conductance of *E. multiflora* in the morning ( $F_{1,32} = 15.5$ ;  $P < 0.001$ ) and in the afternoon ( $F_{1,32} = 5.7$ ;  $P = 0.02$ ) and of *G. alypum* in the afternoon ( $F_{1,32} = 6.3$ ;  $P = 0.02$ ; Fig. 5). However, drought effect on stomatal conductance was not uniform throughout the study period since the interaction among treatment, year and season was always significant. Interactions were significant due to a stronger effect of drought treatment in autumn and spring (when the treatment was on).

Droughted plants of *E. multiflora* and *G. alypum* showed a decrease of 24% in their overall leaf transpiration rates (E) in the morning ( $F_{1,32} = 7.5$ ;  $P = 0.01$  and  $F_{1,32} = 8.2$ ;  $P = 0.007$ , respectively; Fig. 6). However, there was a significant interaction among treatment, year and season for *G. alypum* due to a stronger drought treatment in autumn 1999 (Fig. 1). In the afternoon, differences between control and droughted plants were not significant.

Warming treatment did not affect significantly the leaf gas exchange rates of either *E. multiflora* or *G. alypum* plants (data not shown). Treatments neither affected significantly the instantaneous water-use efficiency (A/E) of plants (data not shown).

In autumn 2001, at leaf temperatures of  $20^\circ\text{C}$  ( $\pm 0.05$ ) and at  $350 \pm 0.05$  p.p.m. of  $\text{CO}_2$ , a photon flux density of  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  was saturating, or very nearly so, for both study species (Fig. 7). Droughted plants of *E. multiflora* showed significantly lower leaf net photosynthetic rates in response to PPFD than control plants ( $F_{4,40} = 3.7$ ;  $P < 0.05$ ), whereas drought did not affect the net photosynthetic rates of *G. alypum*. Warming did not affect either *E. multiflora* or *G. alypum* PPFD-response curves, in agreement with the 2 years of data on leaf gas exchange rates. Dark respiration rates of *E. multiflora* plants ( $0.55 \pm 0.2$ ) were significantly lower

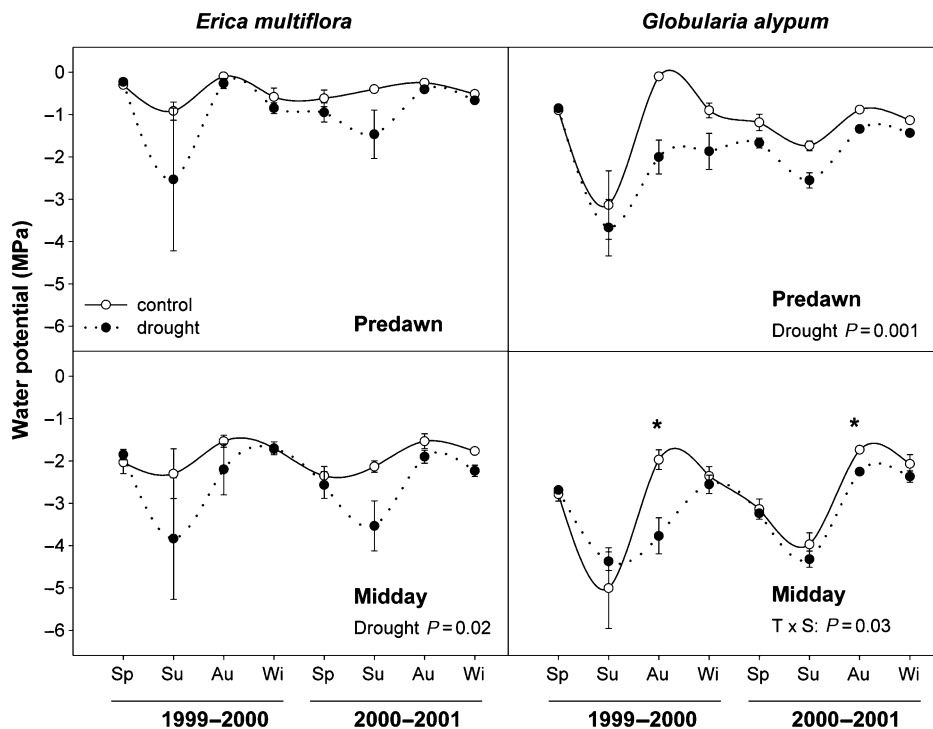


Fig. 3. Seasonal course of shoot water potential (MPa) of *E. multiflora* and *G. alypum* plants at predawn and midday for control and drought treatments. Error bars indicate the standard errors of the mean ( $n = 3$ ). Significant interactions or treatment effects are depicted (T, treatment; Y, year; S, season). Seasons are abbreviated as Sp, spring; Su, summer; Au, autumn; Wi, winter.

( $P = 0.01$ ) than those of *G. alypum* plants ( $1.96 \pm 0.3$ ). Whereas drought did not affect the dark respiration rates of *E. multiflora* plants, it slightly decreased the dark respiration rates of *G. alypum* plants (drought:  $1.34 \pm 0.03$ , control:  $1.96 \pm 0.3$ ;  $P = 0.08$ ).

Droughted plants of *E. multiflora* showed significantly lower net photosynthetic rates than control plants in response to  $\text{CO}_2$  ( $F_{4,34} = 24.6$ ;  $P < 0.001$ ; Fig. 8). Con-

versely, warming plants of *G. alypum* showed significantly higher net photosynthetic rates than controls ( $F_{4,34} = 10.7$ ;  $P < 0.001$ ).  $A-C_i$  curves (data not shown) showed the same response to treatments than  $A-CO_2$  curves, being the efficiency of carboxylation lower in droughted plants of *E. multiflora* ( $F_{1,38} = 10.2$ ;  $P = 0.003$ ) and higher in warming plants of *G. alypum* ( $F_{1,38} = 4.3$ ;  $P = 0.04$ ) compared to controls.

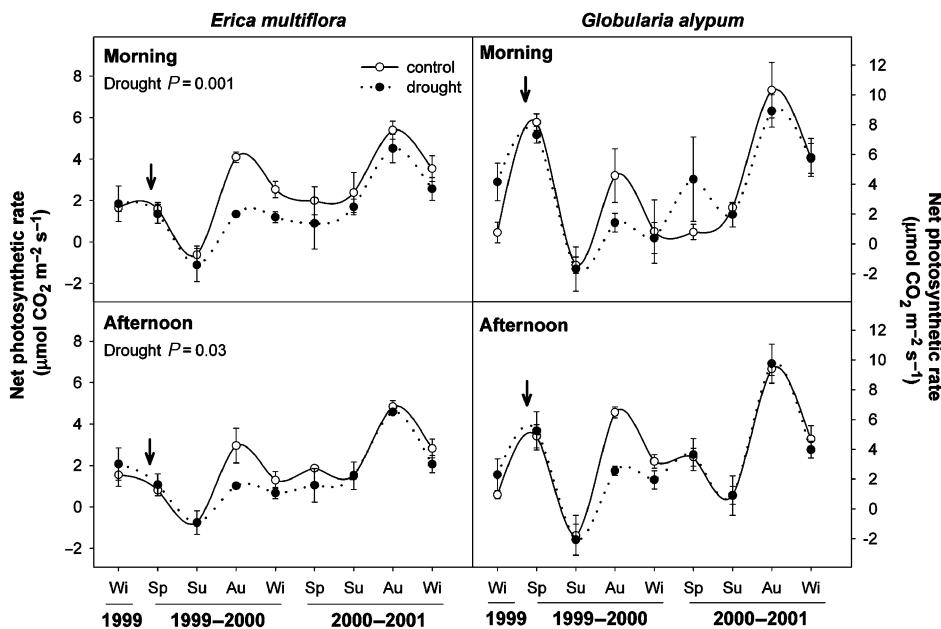


Fig. 4. Seasonal course of net photosynthetic rates ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) of *E. multiflora* and *G. alypum* plants, in the morning and in the afternoon, for control and drought treatments. Error bars indicate the standard errors of the mean ( $n = 3$ ). Arrows indicate the start of the treatments. Significant treatment effects are depicted. Seasons are abbreviated as in Fig. 3.

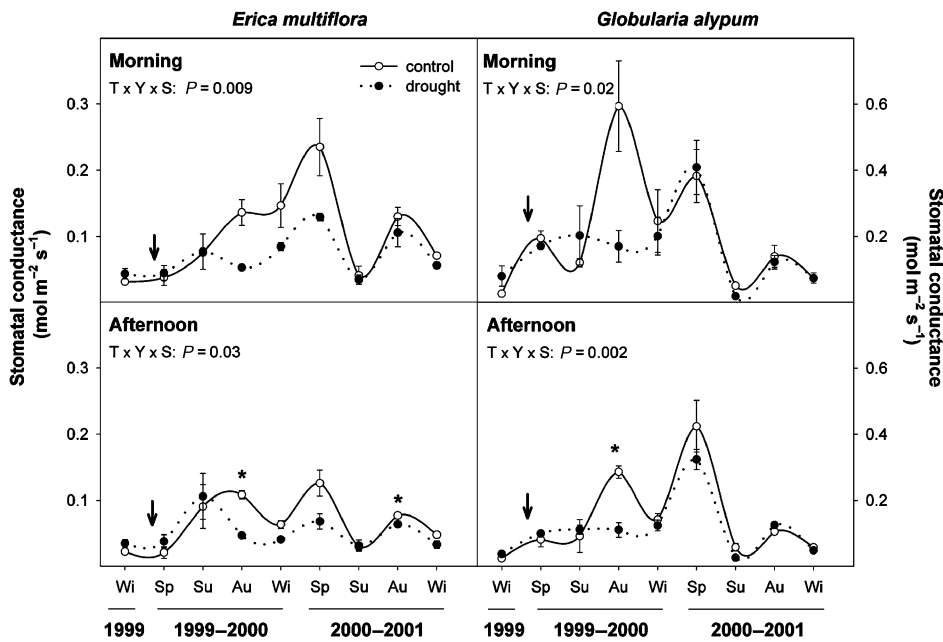


Fig. 5. Seasonal course of stomatal conductance ( $\text{mol m}^{-2} \text{s}^{-1}$ ) of *E. multiflora* and *G. alypum* plants, in the morning and in the afternoon, for control and drought treatments. Error bars indicate the standard errors of the mean ( $n=3$ ). Arrows indicate the start of the treatments. Significant interactions or treatment effects are depicted (T, treatment; Y, year; S, season). Seasons are abbreviated as in Fig. 3.

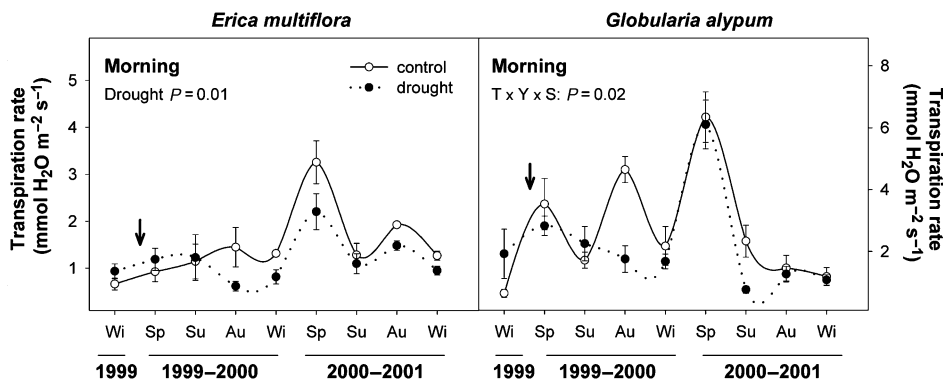


Fig. 6. Seasonal course of transpiration rates ( $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) of *E. multiflora* and *G. alypum* plants, in the morning, for control and drought treatments. Error bars indicate the standard errors of the mean ( $n=3$ ). Arrows indicate the start of the treatments. Significant interactions or treatment effects are depicted (T, treatment; Y, year; S, season). Seasons are abbreviated as in Fig. 3.

### Chl fluorescence

Droughted plants of *E. multiflora* tended to show slightly higher  $F_v/F_m$  values than control plants in the second year at midday ( $F_{1,16}=3.7$ ;  $P=0.07$ ) (Fig. 9). Conversely, droughted plants of *G. alypum* tended to present lower  $F_v/F_m$  values than control plants throughout

the study period at predawn ( $F_{1,28}=3.7$ ;  $P=0.06$ ) (Fig. 9). Warming plants showed slightly higher  $F_v/F_m$  values than controls through all the study period at predawn in the case of *E. multiflora* ( $F_{1,28}=4.0$ ;  $P=0.055$ ) and at midday in the case of *G. alypum* ( $F_{1,32}=5.8$ ;  $P=0.02$ ) (Fig. 10).

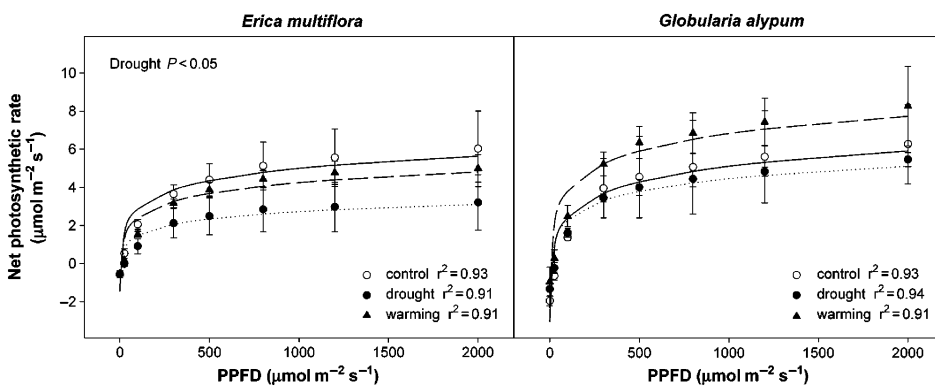


Fig. 7. Photosynthetic rate responses of *E. multiflora* and *G. alypum* shoots from control, drought and warming plots to different PPFD at ambient levels of  $\text{CO}_2$  (three plants per treatment). Differences in the light-response curves of drought and warming plants in relation to controls are depicted when significant ( $P \leq 0.05$ ).

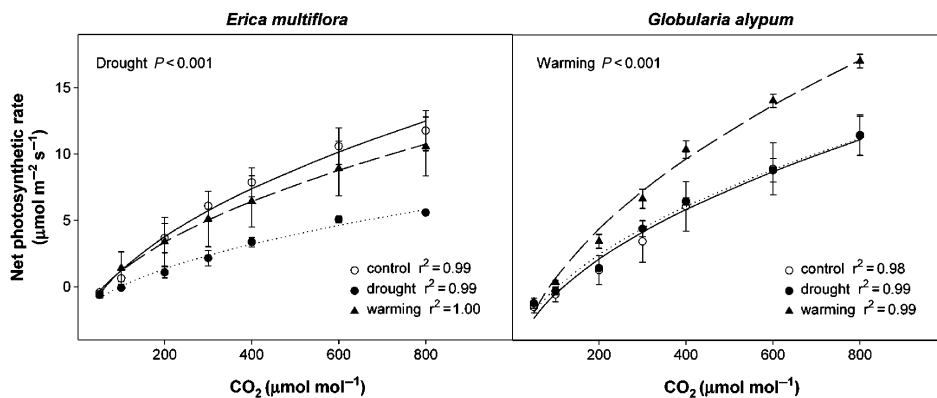


Fig. 8. Photosynthetic rate responses of *E. multiflora* and *G. alypum* shoots from control, drought and warming plots to different CO<sub>2</sub> concentrations at saturating PPFD (three plants per treatment). Differences in the CO<sub>2</sub>-response curves of drought and warming plants in relation to controls are depicted when significant ( $P \leq 0.05$ ).

Drought treatment did not affect significantly the apparent electron transport rates (ETR) of *E. multiflora* (data not shown), whereas the ETR of *G. alypum* plants in the drought treatment were lower (19%) than those of control plants only during the first year in the morning (drought:  $100.8 \pm 18.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; control:  $124.4 \pm 15.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ;  $F_{1,12} = 5.2$ ;  $P = 0.04$ ) (data not shown). Warming did not have any significant effect on the ETR values of these two species. Moreover, treatments did not affect the ETR of *E. multiflora* and *G. alypum* plants (after sequential Bonferroni correction) within each season.

controls, although differences were only marginally significant in both cases ( $F_{1,8} = 4.5$ ;  $P = 0.07$  and  $F_{1,8} = 5.0$ ;  $P = 0.06$ , respectively) (Table 1). Warming did not affect the leaf nitrogen concentration either of *E. multiflora* or *G. alypum* plants (Table 1).

#### Leaf stable isotopes

Neither drought nor warming treatment affected significantly the leaf  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values of *E. multiflora* and *G. alypum* plants (Table 1).

### Discussion

#### Drought treatment

*Erica multiflora* and *G. alypum* will experience lower shoot water potentials ( $\psi$ ) if a longer and stronger summer drought occurs (Fig. 3). Lower values of  $\psi$  would

#### Leaf nitrogen (N) concentration

Leaf N concentration of droughted plants was lower for *E. multiflora* and higher for *G. alypum* compared to con-

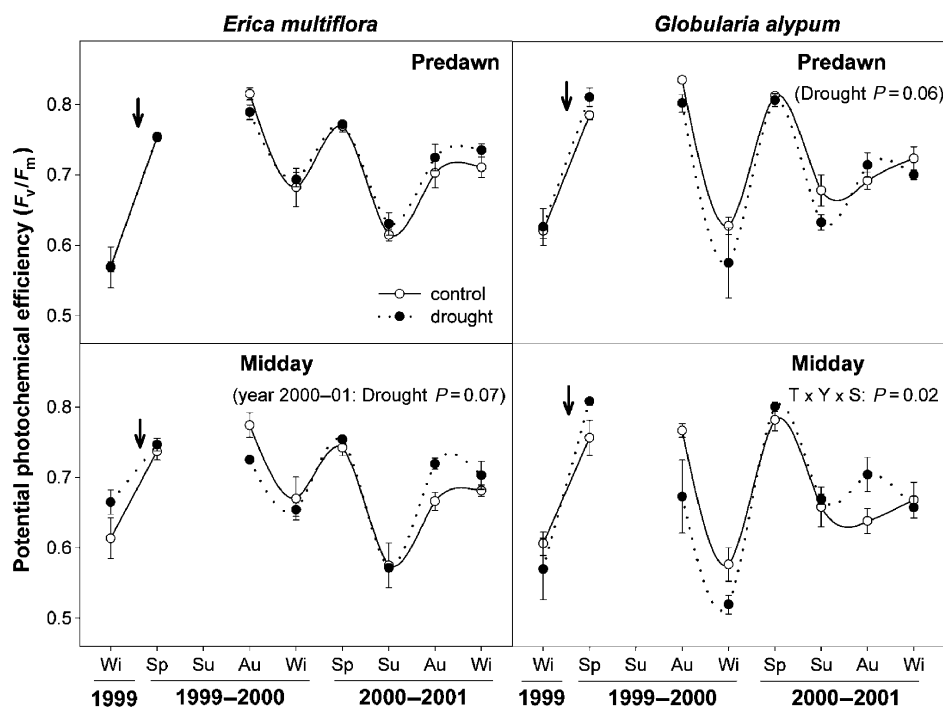


Fig. 9. Seasonal course of potential photochemical efficiency ( $F_v/F_m$ ) of *E. multiflora* and *G. alypum* plants, at predawn and midday, for control and drought treatments. Error bars indicate the standard errors of the mean ( $n = 3$ ). Arrows indicate the start of the treatments. Significant interactions or treatment effects are depicted (T, treatment; Y, year; S, season). Seasons are abbreviated as in Fig. 3.

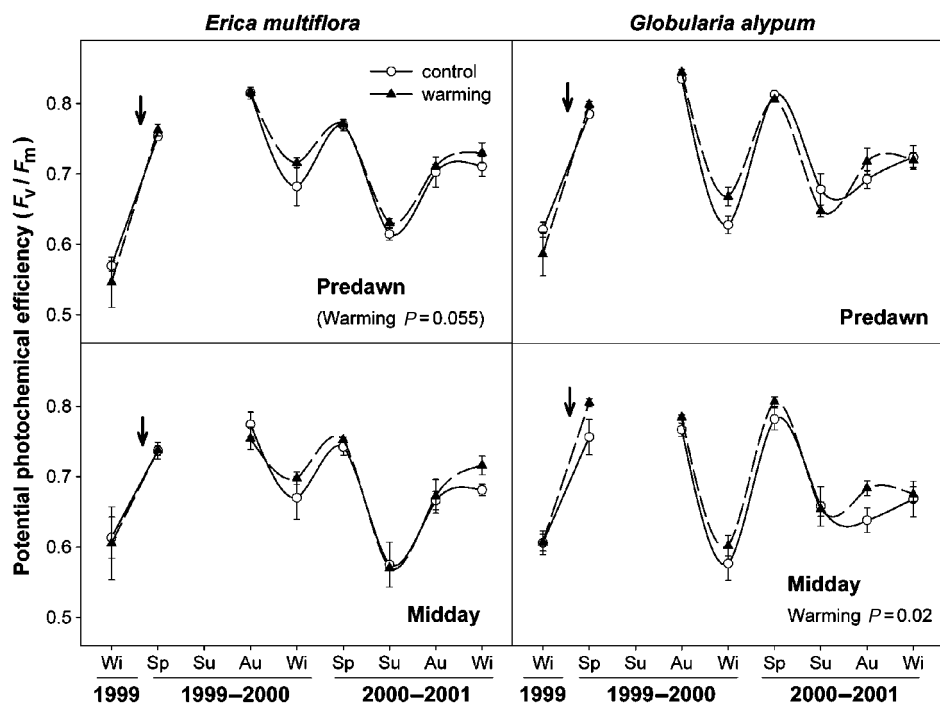


Fig. 10. Seasonal course of potential photochemical efficiency ( $F_v/F_m$ ) of *E. multiflora* and *G. alypum* plants, at predawn and midday, for control and warming treatments. Error bars indicate the standard errors of the mean ( $n = 3$ ). Arrows indicate the start of the treatments. Significant treatment effects are depicted. Seasons are abbreviated as in Fig. 3.

produce a stronger gradient of water potential between the soil and the plant, thus helping to maintain the flow of water into the plant (Dunn et al. 1976). However, decreases in plant water potential increase the risk of cavitation (Tyree and Sperry 1989), which can be responsible for the death of leaves and twigs, especially during summer (Correia and Catarino 1994). Stronger effects of drought on shoot water potentials of *G. alypum* compared to *E. multiflora* coincide with the more drought-avoiding strategy found in the latter (Llorens et al. 2003b).

Furthermore, in agreement with the more drought-avoiding strategy of *E. multiflora* (Llorens et al. 2003b), this species was more sensitive to soil drying than

*G. alypum*, experiencing higher reductions in their leaf gas exchange rates to avoid dehydration (Figs 4–6). Whereas drought treatment decreased the net photosynthetic rates of *E. multiflora* significantly, there was not a global effect of drought treatment on net photosynthetic rates of *G. alypum*. Despite the significant reduction in their photosynthetic rates, drought treatment did not change the electron transport rates of *E. multiflora* plants significantly. Higher photorespiration rates in droughted plants of *E. multiflora* compared to controls were probably one of the main drivers accounting for the maintenance of its electron flow, since, in  $C_3$  plants, PSII activity is mainly partitioned between photosynthesis and photorespiration (Krall and Edwards 1992).

Table 1. Nitrogen concentration (% dry mass),  $\delta^{13}C$  and  $\delta^{15}N$  of *E. multiflora* and *G. alypum* leaves from control, drought and warming treatments at the end of summer in 1999 and 2000. Values are means  $\pm$  SE ( $n = 3$ ). Leaf nitrogen concentration of droughted plants was slightly lower for *E. multiflora* ( $F_{1,8} = 4.5$ ;  $P = 0.07$ ) and slightly higher for *G. alypum* ( $F_{1,8} = 5.0$ ;  $P = 0.06$ ) compared to controls. Treatments did not affect leaf stable isotopes values significantly.

	<i>E. multiflora</i>		<i>G. alypum</i>	
	1999	2000	1999	2000
N (%)				
Control	0.68 $\pm$ 0.02	0.73 $\pm$ 0.03	0.76 $\pm$ 0.03	0.82 $\pm$ 0.06
Drought	0.59 $\pm$ 0.03	0.66 $\pm$ 0.06	0.85 $\pm$ 0.08	1.02 $\pm$ 0.09
Warming	0.63 $\pm$ 0.03	0.69 $\pm$ 0.04	0.80 $\pm$ 0.05	0.84 $\pm$ 0.03
$\delta^{13}C$				
Control	-24.9 $\pm$ 0.6	-25.5 $\pm$ 0.5	-27.6 $\pm$ 0.7	-28.1 $\pm$ 0.6
Drought	-25.7 $\pm$ 0.6	-25.8 $\pm$ 0.4	-27.0 $\pm$ 0.9	-26.9 $\pm$ 0.4
Warming	-25.7 $\pm$ 0.3	-25.8 $\pm$ 0.4	-27.6 $\pm$ 0.7	-28.0 $\pm$ 0.1
$\delta^{15}N$				
Control	-5.48 $\pm$ 0.6	-5.11 $\pm$ 0.8	-5.24 $\pm$ 0.8	-5.06 $\pm$ 0.5
Drought	-5.48 $\pm$ 0.6	-4.57 $\pm$ 1.0	-4.33 $\pm$ 0.5	-4.40 $\pm$ 0.5
Warming	-5.56 $\pm$ 0.7	-4.96 $\pm$ 0.6	-4.73 $\pm$ 0.7	-5.31 $\pm$ 0.4

Up-regulation of Mehler reaction may also help to dissipate excess excitation energy in water-stressed plants, protecting the photosystem from photodamage (Krall and Edwards 1992, Osmond and Grace 1995, Asada 1999). In accordance with the results obtained throughout the 2 years of the study, droughted plants of *E. multiflora* also showed significantly lower *A* than control plants in response to PPF and CO<sub>2</sub> in autumn 2001 (Figs 7 and 8).

A low rate of net photosynthesis in plants suffering from water stress has often been reported, usually as a consequence of stomatal closure (reviews in Chaves 1991, Yordanov et al. 2000). However, the lower carboxylation efficiency (CE), i.e. lower Rubisco activity, that we found in the droughted plants of *E. multiflora* compared to controls suggest that, in addition to a stomatal limitation, there was a non-stomatal limitation of photosynthesis. The decreases in Rubisco activity and net photosynthetic rates of *E. multiflora* plants as a response to drought were in agreement with the lower foliar N concentrations that we found in these plants compared to controls, although differences were only marginally significant (Table 1).

Decreases of foliar nitrogen concentration by drought have been attributed to three processes: (1) drought-induced retranslocation of shoot N to roots and rhizomes; (2) volatilization of foliar N; and (3) drought-related dilution of shoot N resulting from a greater impact of drought on soil N uptake than on growth (Heckathorn et al. 1997). Since we observed lower initial decomposition rates in the drought plots compared to controls (Emmett et al. 2003), decreases in the soil N availability and, thus, in the soil N uptake by droughted plants of *E. multiflora* could explain their slightly lower foliar N concentrations. However, droughted plants of *G. alypum* tended to have higher leaf nitrogen concentrations than control plants. Such unexpected result could be explained by a decrease in plant growth, which concentrated the nitrogen into less dry matter. Indeed, drought treatment decreased the growth of *G. alypum* significantly in relation to controls in spring 2000 (Peñuelas et al. 2003). Furthermore, droughted plants of *G. alypum* could have developed more and/or deeper roots. Greater biomass allocation to root versus shoot have been correlated with higher shoot N concentration (%) and δ<sup>15</sup>N values, suggesting that a larger root allocation allow plants to exploit more efficiently soil systems and to use more nitrogen from nitrogen-saturated soil sites (Lloret et al. 1999). Accordingly, droughted plants of *G. alypum* tended to have more positive leaf δ<sup>15</sup>N values than controls (Table 1).

Drought treatment did not either affect the instantaneous (*A/E*) or the integrated (deduced from δ<sup>13</sup>C values) water-use efficiency of *E. multiflora* or *G. alypum* plants, which indicates that leaf net photosynthesis and stomatal conductance (or leaf transpiration) changed proportionately. Although in many experimental and field studies drought has been shown to increase the instantaneous and/or the integrated water-use efficiency of woody plants (e.g. Ehleringer and Cooper 1988, Morecroft and Woodward 1990, Meinzer et al. 1992), proportional

decreases of leaf net photosynthesis and stomatal conductance (or leaf transpiration) have been also frequently reported (e.g. Schulze and Hall 1982, Epron and Dreyer 1993, Moriana et al. 2002, Llorens et al. 2003a).

An increase in drought has been related in many cases to a drop in potential photochemical efficiency of PSII (*F<sub>v</sub>/F<sub>m</sub>*), namely to an increase in photoinhibition (e.g. Björkman and Powles 1984, Jagtap et al. 1998). As far as photoinhibition is reversible within minutes to hours, it can be viewed as a protective mechanism that serves to dissipate excessive energy (Krause 1988, Osmond 1994). In our study, droughted plants of *G. alypum* showed slightly lower *F<sub>v</sub>/F<sub>m</sub>* values than control plants throughout the study period at predawn (Fig. 9), but not at midday. Predawn depressions in PSII efficiency have been related to the retention of de-epoxidized components of the xanthophyll cycle (zeaxanthin and antheraxanthin) overnight (Adams and Demmig-Adams 1994, García-Plazaola et al. 1999, Verhoeven et al. 1999, Kyparissis et al. 2000). The retention of zeaxanthin and antheraxanthin overnight would allow energy dissipation to take place at a fairly high level upon exposure to direct sunlight after sunrise, when temperatures are typically the coldest and the enzymatic conversion of violaxanthin to antheraxanthin and zeaxanthin would be largely inhibited (Adams and Demmig-Adams 1994). The retention of zeaxanthin and antheraxanthin overnight would be very sensitive to temperature, permitting reconversion of zeaxanthin to antheraxanthin and violaxanthin on warmer days when photosynthesis could presumably proceed at higher rates (Adams and Demmig-Adams 1994). Sustained reductions of *F<sub>v</sub>/F<sub>m</sub>* values may also result from accumulation of non-functional PSII reaction centres and partial photoinactivation of PSII (Niyogi 1999). However, the lack of differences in PSII efficiency between control and droughted plants of *G. alypum* at midday suggest that the predawn depression in the *F<sub>v</sub>/F<sub>m</sub>* values in droughted plants was related to energy dissipation activity rather than to damage to the photosystems.

A number of studies have reported a remarkable resistance of the photosynthetic apparatus to dehydration (e.g. Genty et al. 1987, Gamon and Pearcy 1990, Havaux 1992, Epron 1997) and several authors have demonstrated that water deficit may enhance the resistance of PSII photochemistry to superimposed constraints, such as high-temperature stress combined or not with photoinhibitory light (e.g. Havaux 1992, Epron 1997, Valladares and Pearcy 1997, Ladjal et al. 2000, Llorens et al. 2003a). Our results on *E. multiflora* support the latter two ideas, since drought did not affect or slightly enhanced (during the second year at midday) the *F<sub>v</sub>/F<sub>m</sub>* values of *E. multiflora* leaves (Fig. 9).

### Warming treatment

The slight warming treatment (approximately 1°C in the night and in diurnal hours until midday) did not affect

either the shoot water potential, nor the leaf gas exchange rates or the electron transport rates throughout the study period in either of the two studied species. Previous studies on the effects of warming on leaf gas exchange rates have reported contrasting results. Some authors did not find any effect of warming manipulations on leaf gas exchange rates (e.g. Wookey et al. 1994, Nijs et al. 1996, Loik et al. 2000, Starr et al. 2000), other authors found stimulated rates (e.g. Chapin and Shaver 1996, Apple et al. 2000), whereas others found decreases (Callaway et al. 1994). Most of these studies were performed in temperate and cold climates, where constraints of the physiological activity of plants are different to those in the Mediterranean region. Moreover, comparisons among studies are difficult given the different characteristics and magnitude of warming treatments and the different sensitivity to temperature and optima for photosynthesis of each species.

In accordance with the lack of effects of warming treatment on leaf gas exchange rates, warming did not affect significantly either the instantaneous water-use efficiency or the leaf  $\delta^{13}\text{C}$ , namely the integrated water-use efficiency, of *E. multiflora* and *G. alypum* plants (Table 1). Warming affected neither the leaf  $\delta^{15}\text{N}$  nor the leaf N concentration (Table 1), which also agrees with the absence of warming effects on decomposition and mineralization rates in the soil (Emmett et al. 2003).

In the present study, warming plants of *E. multiflora* and *G. alypum* showed higher overall potential photochemical efficiency values (Fv/Fm) than control plants at predawn and midday, respectively (Fig. 10). Differences were especially evident in winter, and also in autumn for *G. alypum* plants, suggesting that episodic freezing or frosting events during these seasons might affect severely the photosynthetic performance of these two Mediterranean shrubs. Accordingly, our warming treatment reduced the number of days with frost ( $T_{\min} < 0^\circ\text{C}$ ) by 50% for the period March 2000–2001 (22 days with frost in controls compared to 11 days in warming plots). Previous studies reported that low temperatures during winter constrain photosynthetic performance of some Mediterranean evergreen sclerophylls (e.g. Garcia-Plazaola et al. 1999, Karavatas and Manetas 1999, Larcher 2000, Oliveira and Peñuelas 2001).

In spite of the absence of warming effects on *A* throughout the study period, plants of *G. alypum* in the warming plots had higher photosynthetic rates than control plants in response to PPFD and  $\text{CO}_2$  in autumn 2001, although differences were significant only for the  $\text{CO}_2$ -response curves (Figs 7 and 8). During this season, *G. alypum* plants also showed higher CE, namely higher Rubisco activity, than control plants (data not shown, but see Fig. 8). Taking into account that warming did not alter the leaf N concentration, this result could be related to the higher photosynthetic performance that we found in the warming plants, mainly during the colder seasons.

The general absence of direct and indirect effects of the warming treatment on the studied instantaneous

ecophysiological variables is probably due to the low increase of temperature achieved, especially during the diurnal hours (Fig. 2). However, the lack of effects of the warming treatment on instantaneous variables does not preclude an effect of warming on integrative variables, such as growth and flowering (Peñuelas et al. 2003).

### Final remarks

Our results suggest that future drier conditions may decrease the annual productivity of these Mediterranean shrubs, although the magnitude of such decreases will be species-specific. However, leaf gas exchange responses (stimulation of photosynthesis, increase in water-use efficiency, decrease of transpiration) to the predicted rise in atmospheric  $\text{CO}_2$  concentration (Körner 2000) might compensate the effects of drier conditions. On the other hand, warmer conditions in the immediate decades could alleviate the low temperature constraints on the photosynthetic performance of these Mediterranean species during the colder seasons, possibly increasing the length of the growth period. During summer, temperature increases of approximately  $1^\circ\text{C}$  (at night and in the morning) will not alter significantly the already depressed photosynthetic activity and performance of such Mediterranean shrubs.

Ultimately, the drier and warmer conditions expected for the near future may change the competitive relationship among species of Mediterranean shrublands. Results of our study provide an example, since the two studied species, *E. multiflora* and *G. alypum*, showed different ecophysiological responses to our experimental drought and warming.

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