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ECOSYSTEMS

Effects of an Experimental Increase of Temperature and Drought on the Photosynthetic Performance of Two Ericaceous Shrub Species Along a North–South European Gradient

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ABSTRACT

Plant ecophysiological changes in response to climatic change may be different in northern and southern European countries because different abiotic factors constrain plant physiological activity. We studied the effects of experimental warming and drought on the photosynthetic performance of two ericaceous shrubs (*Erica multiflora* and *Calluna vulgaris*) along a European gradient of temperature and precipitation (UK, Denmark, The Netherlands, and Spain). At each site, a passive warming treatment was applied during the night throughout the whole year, whereas the drought treatment excluded rain events over 6–10 weeks during the growing season. We measured leaf gas exchange, chlorophyll *a* fluorescence, and leaf carbon isotope ratio ($\delta^{13}C$) during the growing seasons of 1999 and 2000. Leaf net photosynthetic rates clearly followed a gradient from northern to southern countries in agreement with the geographical gradient in water availability. Accordingly, there was a strong correlation between net photosynthetic rates and the accumulated rainfall over the growing season. Droughted plants showed lower leaf gas exchange rates than control plants in the four sites. Interestingly, although leaf photosynthetic rates decreased along the precipitation gradient and in response to drought treatment, droughted plants were able to maintain higher leaf photosynthetic rates than control plants in relation to the accumulated rainfall over the months previous to the measurements. Droughted plants also showed higher values of potential photochemical efficiency (F_v/F_m) in relation to controls, mainly at midday. The warming treatment did not affect significantly any of the studied instantaneous ecophysiological variables.

Key words: *Key words*: *Calluna vulgaris*; chlorophyll fluorescence; climatic change; drought; *Erica multiflora*; European transect; leaf gas exchange; stable isotopes; warming.

INTRODUCTION

Considerable evidence is now available showing that the global average surface temperature has increased by 0.6°C (±0.2°C) since the late 19th

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century (IPCC 2001). Indeed, most of Europe has experienced increases of about 0.8°C on average. Global climatic models predict that over the period 1990–2100 the globally averaged surface temperature will rise by 1.4–5.8°C (IPCC 2001). Although future precipitation patterns are more uncertain, global climate change is likely to increase the risk of summer drought in central and southern Europe (IPCC 2001). These climatic changes may induce ecophysiological changes in plants which could affect their long-term performance (Michelsen and others 1996).

Over the last decade, a growing number of field experiments simulating some of the predicted climatic changes were initiated around the world (for example, see Shaver and others 2000 for a review of warming experiments). However, until now most of the techniques used to experimentally warm ecosystems alter the environmental conditions (for example, light, humidity, soil structure), and, moreover, experiments usually simulate a diurnal increase in temperature rather than the predicted stronger increase in T_{min} (nighttime temperature) (IPCC 2001). Furthermore, whereas experimental manipulations provide short-term responses to climate change (annual to decadal scale), studies along natural climatic gradients are thought to mirror longer-term changes (decades to centuries) (Shaver and others 2000). To date, the majority of the climate experiments have been conducted in north temperate, boreal, and arctic ecosystems. However, climate-change responses of vegetation could be different in northern and southern countries of Europe because different abiotic factors constrain plant physiological activity. Therefore, a clear need exists to also explore the responses of warmer and/or drier ecosystems, such as those of the Mediterranean, to climate change.

In Mediterranean-type regions, drought proves to be the climatic factor essentially responsible for the restriction of growth and survival of evergreen woody plants (Larcher 2000). The predicted extension of the drought period and the indirect effects of future warming, acting through changes in evapotranspiration and soil dryness, may severely constrain the physiological activity of Mediterranean plants. In addition, warming may increase the likelihood of heat stress in Mediterranean plants during summer. Conversely, European northern ecosystems may be particularly sensitive to climate change due to low-temperature constraints on biological activity (Körner and Larcher 1988). Then, increases in minimum temperatures are expected to impact these ecosystems by alleviating such temperature constraints (Rustad and others 2001).

The aim of the present study was to test how future warming and drought events will affect the photosynthetic performance of two ericaceous shrubs (*Erica multiflora* and *Calluna vulgaris*). *E. multiflora* was studied in northern Spain, whereas *C. vulgaris* was studied in Denmark, the UK, and The Netherlands. The four studied sites represent a European gradient in temperature and precipitation. In addition, climatic manipulations were performed in each site to simulate the expected increase in drought and temperature. We extended the drought period by removing rain during the growing season and we warmed plots by avoiding IR dissipation at night over the whole year. We assessed photosynthetic performance of plants by means of leaf gas exchange and chlorophyll *a* fluorescence measurements during the growing season and through the analyses of the leaf carbon isotope ratio (δ^{13}C) at the end of the growing season.

Leaf gas exchange measurements (stomatal conductance and net photosynthetic and transpiration rates) are among the classical instantaneous methods used to detect functional limitations in plants imposed by environmental factors. It is now well established that the rate of CO_2 assimilation in the leaves is depressed at moderate leaf water deficits or even before leaf water status is changed in response to a drop in soil water potential, mostly as a consequence of stomatal closure (see reviews Chaves 1991; Yordanov and others 2000). On the other hand, photosynthesis is temperature dependent and exhibits a temperature optimum. In response to small and short-term shifts in ambient temperature away from this optimum, photosynthetic activity decreases (Battaglia and others 1996).

Chlorophyll *a* fluorescence is one of the most important noninvasive methods for monitoring changes in the functioning and the regulation of the photosynthetic apparatus (Schreiber and others 1994). The variation in the maximum fluorescence ratio ($F_{\mathrm{v}}/F_{\mathrm{m}}$) of a dark-adapted leaf is a reliable measure of the *potential* photochemical efficiency of photosystem II PSII and has been used as an estimate of the functional state of the photosynthetic apparatus at a given environmental situation (see Maxwell and Johnson 2000 for a recent review). Exposure of leaves to light levels in excess of what can be utilized in photosynthesis results in photoinhibition (Krause 1988), which is reflected by a reduction in the photochemical efficiency of [PSII] (Long and others 1994). 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 the long term, chronic photoinhibition results in photon damage to the photosystem II reaction center, the end point of which is the destruction of the functioning photosynthetic apparatus and the oxidation of chlorophyll (Osmond 1994).

During photosynthesis, plants discriminate against the heavier isotope of carbon (^{13}C) in ways which reflect plant metabolism and environment (Farquhar and others 1989). Environmental conditions influence the plant carbon isotope composition by affecting the ratio of intercellular and atmospheric CO_2 concentrations (c_i/c_a), which is controlled by stomatal conductance and the rate of CO_2 assimilation (Farquhar and others 1989). Therefore, the fractionation of carbon isotopes integrates information about how a plant regulates CO_2 and water fluxes over long periods, being that $\delta^{13}C$ for C_3 plants is positively correlated with the ratio of carbon assimilation to transpiration (water-use efficiency, WUE) (Farquhar and others 1989). Along continental-scale gradients elsewhere in the world, it has been shown that, for herbaceous plants, increasing aridity can result in increasing stomatal closure and associated increases in $\delta^{13}C$ of leaves (Ehleringer and Cooper 1988).

We approached our aim of investigating climate-change effects on the physiological activity of ericaceous shrubs by testing the following hypotheses:

1. We expected to find decreasing photosynthetic performance of control plants with decreasing water availability, that is, from northern to southern countries.
2. We hypothesized that a reduction in water availability during the growing season would decrease leaf gas exchange rates mainly in the Spanish site. The reduction in photosynthetic energy conversion under drier conditions may result in enhanced photoinhibition if leaves are exposed to excessive levels of radiation. Moreover, the extension of the drought period would raise both instantaneous (A/E) and integrated water-use efficiency, the latter shown by increased leaf $\delta^{13}C$ values.
3. The warming treatment would have a stronger effect in the three northern sites than in the Mediterranean site. In northern vegetation, temperature increases may stimulate photosynthetic rates directly because of warmer mornings or indirectly through increases in decomposition and nutrient mineralization and thus nutrient availability (Rustad and others 2001). The stimulation of photosynthesis may decrease the incidence of photoinhibition. On the contrary, in Mediterranean vegetation, positive direct effects of warming on photosynthesis would be relatively smaller because temperatures are already near the optimum for photosynthesis (Shaver and others 2000).

METHODS

Study Site and Plant Species

The present study was carried out at four sites: Garraf (Spain), Oldebroek (The Netherlands), Clocaenog (UK), and Mols (Denmark), spanning a gradient in temperature and precipitation (see Beier and others 2004 for the description of the sites). The Garraf site is a Mediterranean shrubland with abundant *Erica multiflora* plants, whereas the three northern sites are *Calluna vulgaris* heathlands. *Erica multiflora* L., and *Calluna vulgaris* (L.) Hull are two perennial shrubs of the Ericaceae family. *E. multiflora* is a short-leafed sclerophyllous shrub that typically occurs on basic soils of the western Mediterranean Basin (Vilà and Terradas 1995). *C. vulgaris* is an upland slow-growing species which typically occupies nutrient-poor acidic soil types in open situations (Iason and Hester 1993).

Experimental System

Two types of climatic manipulations of ecosystems were performed using automatically sliding roofs:

- Ecosystem warming: The roofs (reflective curtains) covered the vegetation and soil during the night, reducing the loss of IR radiation, over the whole year. A rain sensor automatically removed the curtains during rain events.
- Extended summer drought: The roofs (transparent polyethylene plastics) covered the vegetation during all rain events over 6–10 weeks of the growing season in the spring and summer.

Nine plots (20 m^2 per plot) were established in each site: 3 untreated controls, 3 warming plots, and 3 drought plots. The warming treatment started in March–May 1999, whereas the drought treatment was applied for a 6–10-week period between March and August each year (Beier and others 2004). During the growth period, the warming treatment increased the average air temperatures by 0.3–1.4°C (mean 0.5°C) and the soil temperatures by 0.4–1.1°C (mean 0.8°C) at all sites. During the drought period, 60%–90% of the water was removed, amounting to approximately 30%–70% of the total yearly water input (Beier and others 2004). Drought treatment reduced the soil water content by 50%–80% at the peak of the drought (Beier and others 2004).

Measurements of leaf gas exchange rates and chlorophyll *a* fluorescence of *C. vulgaris* (in the three northern sites) and *E. multiflora* (in Spain) were conducted during the growing season, at least one month after starting the drought treatment, in 1999 and 2000. Measurements were performed in 3–4 randomly selected plants in each plot, except in 2000 in Denmark, where no plants were available in some plots because a heather beetle attack removed all the *C. vulgaris* resprouts.

Leaf Gas Exchange Measurements

Net photosynthetic (*A*) and transpiration rates (*E*) were determined on one sun-exposed shoot, from the current year, per plant. The tips of nonflowering heather shoots (approximately 2–3 cm) were used. Although we performed two rounds of measurements (one in the morning and the other in the afternoon) in each site each year, in the statistical analyses we used the average of the two rounds because there were no significant differences between them. Measurements were performed with a portable open-flow gas exchange system (ADC4, ADC Inc., Hoddesdon, Hertfordshire, England), which also monitored photosynthetically active radiation (PAR) and air temperatures in the leaf chamber. All results are expressed on area basis, which was measured using ImagePC (version α9 for Windows, Scion Co., Frederick, MD, USA) from photocopies of all the leaves of a measured shoot. Instantaneous water-use efficiency (WUE), defined as mmol of net CO_2 uptake per mol of H_2O transpired, was calculated by dividing instantaneous values of *A* by *E*.

Chlorophyll a Fluorescence Measurements

Components of chlorophyll fluorescence were quantified with a portable modulated fluorometer PAM-2000 (Heinz Walz GmbH, Effeltrich, Germany). One sun-exposed shoot from the current year per plant was measured in situ. 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 $F_v = F_m - F_0$ (see Schreiber and others 1994 for methodology description). F_v/F_m reflects the potential (maximum) efficiency of photosynthetic energy conversion of PSII. We measured F_v/F_m in the UK, The Netherlands, and Spain in 1999 and in the four studied countries in 2000. Measurements were performed at predawn and midday.

The actual photochemical efficiency of PSII in the light-adapted state was estimated as $\phi_{PS2} = (F_m' - F)/F_m'$, 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 (Genty and others 1989). The theoretical maximum ϕ_{PS2} is 1 electron/photon absorbed. In practice, the maximum values obtained experimentally from fluorescence analysis are around 0.85 (Krall and Edwards 1992). We measured the ϕ_{PS2} at midday in Denmark, The Netherlands, and Spain in 1999 and in the four sites in 2000.

Leaf Carbon Isotope Analyses

Leaf carbon isotope analyses were performed on leaf material collected in August– September in 1999 and 2000. Samples from each plot were combined, dried, and ground. All analyses were carried out with an elemental analyzer EA1108 (Carlo Erba, Milano, Italy) attached to a Delta C isotope mass spectrometer and using a CONFLO II interface (Thermo Finnigan MAT, Bremen, Germany).

The isotopic composition of a sample ($\delta^{13}C$) was calculated as the ratio (‰): 1

$$\delta^{13}C_p = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where R_{sample} and $R_{standard}$ are the ^{13}C:^{12}C ratios of the sample and the PeeDee Belemnite (PDB) standard, respectively.

Statistical Analyses

All the statistical analyses were performed using one value per plot, obtained from averaging the 3–4 plants measured per plot in the case of leaf gas exchange and Chl *a* fluorescence measurements and from *combining* material from different plants in the case of leaf $\delta^{13}C$. In the analyses of the variables sensitive to the instantaneous light (leaf gas exchange rates and ϕ_{PS2}), we used PAR as a covariable in the ANOVAs. Differences were considered statistically significant at $p < 0.05$.

To study the variability among countries, we performed ANOVAs with country (UK, Denmark, The Netherlands, Spain) and year (1999, 2000) as fixed factors. We used only control leaves to avoid the confounding effect of potential interactions between treatment and country. When the interaction between country and year was significant, we performed one-way ANOVAs for each year separately. When the effect of country was significant, we compared values of the four countries by means of post-hoc Tukey tests.

To analyze treatment effects on the studied variables, we used ANOVA procedures, comparing

Table 1. Accumulated Precipitation (mm) in Control (C) and Drought (D) Plots and Mean Temperature (°C) over the Three Months Previous to the Measurements in 1999 and 2000

	Accumulated precipitation (mm)				Temperature (°C)	
	1999		2000			
	C	D	C	D	1999	2000
UK	209	177	268	208	11.3	11
Denmark	240	30	179	38	15.1	12.5
Netherlands	144	28	268	109	16.7^{a}	16.1
Spain	97	24	97	64	20.4	20.8

[a] *Average only for May and July since June data were not available.*

drought and warming leaves with control leaves separately. Treatment (control − drought or control − warming), country, round (predawn and midday in the analysis of the Fv/Fm values) and year were considered fixed factors in the Anovas.

Pearson's correlations were performed between mean values of the studied variables for control leaves and the accumulated rainfall during the growing season (the three months previous to the measurements) for each country and year. On the other hand, to test whether treatments affected differentially the studied variables in relation to the accumulated rainfall over the growing season, we performed analyses of covariance (ANCOVAs) comparing separately drought and warming leaves with control leaves and using the rainfall as the covariable.

RESULTS

European Transect

Climatological Data. The gradient of accumulated rainfall over the growing season (the three months before the measurements) among the four studied countries was from the highest to the lowest values: DK, UK, NL and SP in 1999 and UK equal to NL which was more than DK which was more than SP in 2000 (Table 1). In addition, the gradient of mean temperatures for the same period (May to July) for both years was in order from highest to lowest; SP, NL, DK, UK (Table 1).

Leaf Gas Exchange Rates. Differences among sites in leaf net photosynthetic rates of control plants (Table 2) followed the gradient of accumulated rainfall over the growing season experienced each

Table 2. Net Photosynthetic Rate (A), Transpiration Rate (E), WUE, and Potential Photochemical Efficiency (F_v/F_m) at Predawn and Midday for Control Plants in the UK, Denmark (DK), The Netherlands (NL), and Spain (SP) sites in 1999 and 2000

	1999				2000			
	UK	DK	NL	SP	UK	DK	NL	SP
A (Mmol CO_2 m^{-2} s^{-1})	4.93±0.5^{a}	5.07±0.8^{a}	2.84±0.3^{ab}	1.41±0.3^{b}	8.02±0.8^{A}	3.63±0.8^{BC}	5.84±0.5^{AB}	1.48±0.7^{C}
E (mmol HO_2 m^{-2} s^{-1})	4.63±0.4^{a}	4.03±1.1^{a}	2.74±0.2^{ab}	0.92±0.2^{b}	1.90±0.2^{B}	5.47±0.4^{A}	3.20±0.3^{B}	2.90±0.1^{B}
WUE (A/E)	1.13±0.02^{a}	1.72±0.7^{a}	1.12±0.2^{a}	1.89±0.2^{a}	4.79±0.5^{A}	0.87±0.2^{C}	2.34±0.2^{B}	0.80±0.05^{C}
F_v/F_m (predawn)	0.80±0.003^{a}		0.80±0.01^{a}	0.75±0.005^{b}	0.78±0.008^{A}	0.80±0.002^{A}	0.78±0.001^{A}	0.77±0.008^{A}
F_v/F_m (midday)	0.78±0.006^{ab}	0.81±0.003^{a}	0.77±0.006^{bc}	0.74±0.01^{c}	0.75±0.005^{A}	0.76±0.002^{A}	0.74±0.001^{A}	0.74±0.001^{A}

Values are mean ± SE ($n = 3$, except for Denmark in 2000, where $n = 2$). Significant differences ($p < 0.05$) among countries are indicated by different letters.

Table 3. Actual Photochemical Efficiency ($\varnothing_{\mathrm{PS2}}$) for Control, Drought, and Warming Plants in the Study Sites

	Control	Drought	Warming
UK†	0.51±0.01	0.39±0.05	0.38±0.04
Denmark	0.53±0.07^{a}	0.37±0.05*	0.37±0.05$^{(*)}$
Netherlands	0.39±0.02^{b}	0.44±0.03	0.41±0.04
Spain	0.30±0.03^{b}	0.27±0.02	0.26±0.03

In Denmark, The Netherlands, and Spain, both years were pooled together since there were no significant interactions between treatment and year.
Values are means ± SE (n = 6, except for control and warming treatment in Denmark, where n = 5 and for the UK, where n = 3). Significant differences among countries for control plants are indicated by different letters. Asterisks indicate significant differences between treatments and controls $^{*}p < 0.05$, $^{(*)}p < 0.1$.
†*In the UK, only data for 2000 were available.*

Table 4. Leaf δ^{13}C Values in the UK, Denmark, The Netherlands, and Spain Sites for Control, Drought, and Warming Plants in 1999 and 2000

	Control	Drought	Warming
UK	−28.53±0.09^{a}	−28.43±0.09	−28.88±0.18
Denmark	−28.57±0.30^{a}	−28.79±0.41	−28.40±0.37
Netherlands	−28.06±0.19^{a}	−27.98±0.12	−28.32±0.11
Spain	−25.23±0.36^{b}	−25.78±0.31	−25.75±0.23

Both years were pooled because the interactions between treatment and year were not significant. Values are means ± SE (n = 6, except for warming treatment in Denmark, where n = 5). Significant differences among countries for control plants are indicated by different letters. Differences between control and treatments were not significant within each site, although there was a marginally significant global effect of warming (p = 0.08).

year (Table 1). Differences among sites in leaf transpiration rates of control plants (Table 2) were also in accordance with the precipitation gradient in 1999. However, in 2000, control plants in Denmark showed the highest transpiration rates ($p < 0.01$), whereas there were no significant differences in leaf transpiration rates among the other three countries. Values of instantaneous WUE were similar among countries in 1999 (Table 2). In 2000, control plants in the UK showed the highest WUE values ($p < 0.01$), whereas control plants in The Netherlands had higher values than in Denmark ($p = 0.03$) and in Spain ($p = 0.02$).

Chlorophyll a Fluorescence. Control plants in Spain had the lowest F_v/F_m values in 1999, at predawn and midday, whereas there were no significant differences between F_v/F_m values of control plants in the UK and The Netherlands (Table 2). F_v/F_m values of control plants in Denmark in 1999 at midday were similar to those in the UK and were higher than F_v/F_m values of control plants in The Netherlands or Spain ($p < 0.05$). In 2000, there were no significant differences among the four sites in the F_v/F_m values of control plants, either at predawn or midday (Table 2).

When we pooled the data for the two study years, control plants in Denmark showed the highest actual photochemical efficiency of PSII (ϕ_{PS2}), whereas there were no significant differences between the ϕ_{PS2} values of control plants in The Netherlands and Spain (Table 3). However, when we used data only for the year 2000, in order to include UK data in the analysis, control plants in the UK showed the highest ϕ_{PS2} values ($p < 0.01$) (Table 3).

Leaf Carbon Isotope Ratio (δ^{13} C). In both years, foliar δ^{13}C values of control plants were more positive in Spain than in the other three countries ($p < 0.001$), whereas there were no significant differences among the three northern countries in the leaf δ^{13}C values of control plants (Table 4).

Relationship Between the Studied Physiological Variables and the Precipitation Gradient. Leaf net photosynthetic rates of control plants were highly correlated with the amount of rainfall during the growth period (that is, the three months previous to the measurements) within and across sites ($r = 0.95$, $p < 0.001$) (Figure 1). This correlation was still significant when we considered only data from the three northern countries ($r = 0.90$, $p = 0.01$).

Neither the potential nor the actual photochemical efficiencies of PSII were significantly correlated with the accumulated precipitation over the growing season when we considered data for the control plants of the four sites. However, when we considered data only for the control plants of the three northern countries, the correlation between the

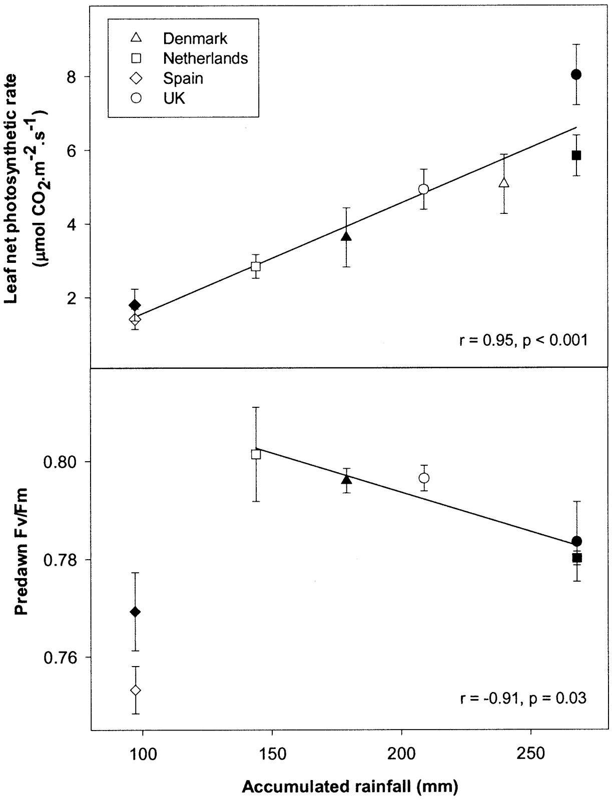

Figure 1. Leaf net photosynthetic rates and potential photochemical efficiency (F_v/F_m) for control plants versus accumulated rainfall (mm) over the three months previous to the measurements for the UK, Denmark, The Netherlands, and Spain sites and in 1999 (open symbols) and 2000 (filled symbols). Error bars indicate the standard errors of the mean of 3 plots' values.

predawn potential photochemical efficiency (F_v/F_m) and the accumulated rainfall was significant ($r = -0.91$, $p = 0.03$) (Figure 1).

Treatment Effects

Leaf Gas Exchange Rates. Droughted plants showed lower leaf net photosynthetic ($F_{1,31} = 6.6$, $p = 0.01$) and transpiration rates ($F_{1,31} = 21.0$, $p < 0.001$) than control plants (Figure 2). In contrast, we did not find any significant effect of warming on leaf gas exchange rates. Neither drought nor warming treatments affected significantly the leaf WUE of plants (Figure 2).

Chlorophyll a Fluorescence. Midday values of potential photochemical efficiency (F_v/F_m) were consistently lower than predawn values ($F_{1,72} = 121.8$, $p < 0.001$) in all the countries, treatments, and years (Table 2 and Figure 3). Droughted plants showed slightly higher F_v/F_m values than control plants ($F_{1,48} = 4.4$, $p = 0.04$), mainly at midday

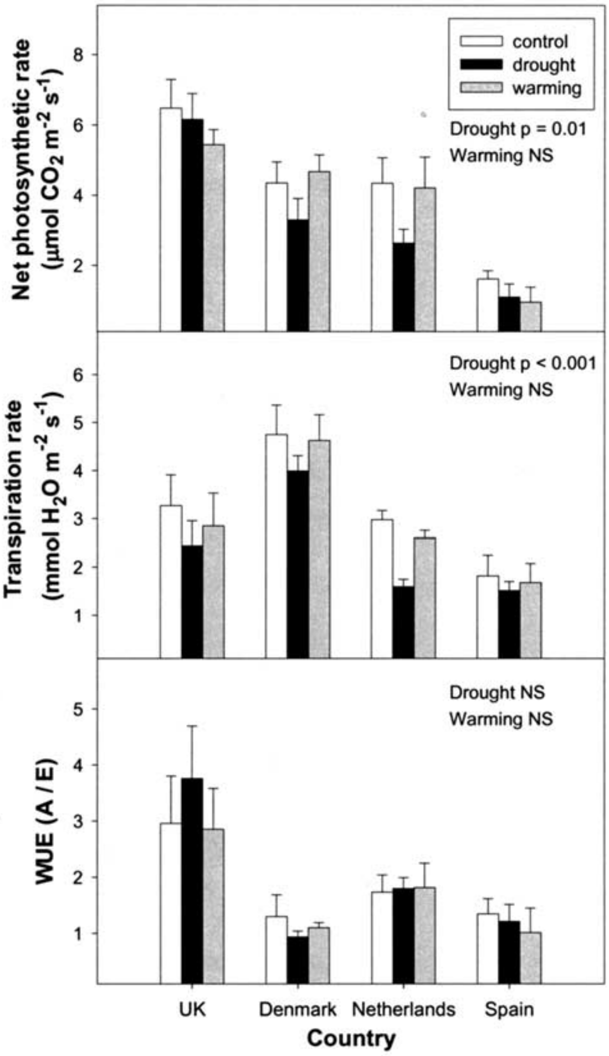

Figure 2. Net photosynthetic rate, transpiration rate, and WUE measured in 1999 and 2000 in control, drought, and warming plants. Years were pooled because the interactions between treatment and year were not significant. Error bars indicate the standard errors of the mean ($n = 6$, except for warming treatment in Denmark, where $n = 5$).

(predawn: $F_{1,26} = 0.1$, $p = 0.7$; midday: $F_{1,26} = 6.6$, $p = 0.02$). In contrast, the warming treatment did not affect the potential photochemical efficiency of plants (Figure 3).

Drought and warming plants showed lower ϕ_{PS2} than control plants in Denmark ($F_{1,6} = 6.3$, $p = 0.046$ and $F_{1,5} = 5.6$, $p = 0.06$, respectively) (Table 3). In contrast, treatments had no significant effects on the ϕ_{PS2} of plants in the UK (only values for 2000), The Netherlands, and Spain.

Leaf Carbon Isotope Ratio ($\delta^{13}C$). Drought treatment did not affect significantly the leaf δ^{13}C values of plants. Conversely, plants in the warming treat-

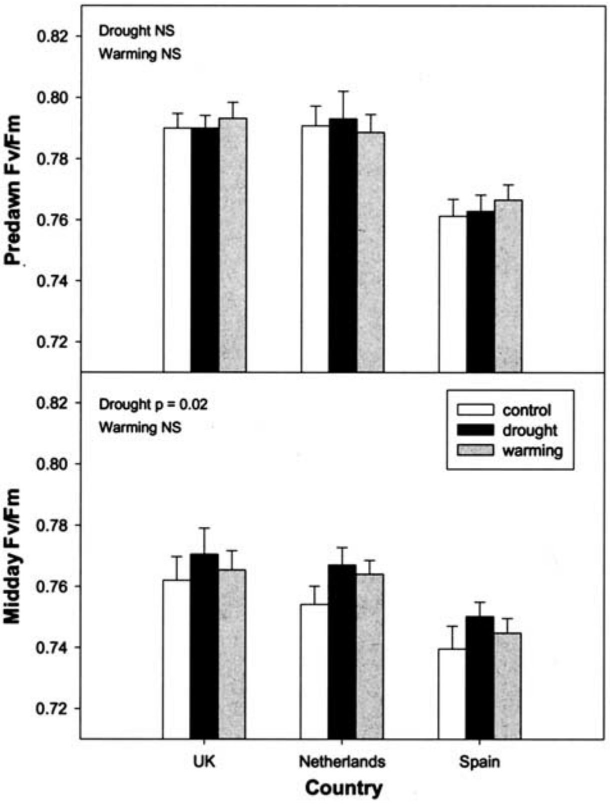

Figure 3. Potential photochemical efficiency (F_v/F_m) at predawn and midday for control, drought, and warming plants in the UK, The Netherlands, and Spain sites in 1999 and 2000. Years were pooled because the interactions between treatment and year were not significant. Error bars indicate the standard errors of the mean ($n = 6$).

ment showed more negative leaf $\delta^{13}C$ values than control plants, although differences were only marginally significant ($F_{1,31} = 3.3$, $p = 0.08$) (Table 4).

Treatment Effects on the Relationship Between Leaf Net Photosynthetic Rates and Accumulated Rainfall Over the Growing Season. Leaf net photosynthetic rates of control, drought, and warming plants were significantly correlated with the amount of rainfall over the growing season (that is, the three months previous to the measurements) (Figure 4). Using the rainfall as a covariable in the analyses, droughted plants showed higher leaf net photosynthetic rates than control plants ($F_{1,13} = 9.9$, $p = 0.008$), whereas there were no significant differences between warming and control plants (Figure 4).

DISCUSSION

European Transect

We examined the photosynthetic performance of Ericaceae shrubs along a European transect spanning a range in mean annual precipitation from 450 to 1700 mm and in mean annual temperature from 7 to 15°C. Excluding the Spanish site (where we studied a different species), the range in mean annual precipitation for the three northern countries with *Calluna vulgaris* was 700–1700 mm and the range in mean annual temperature was 7–11°C.

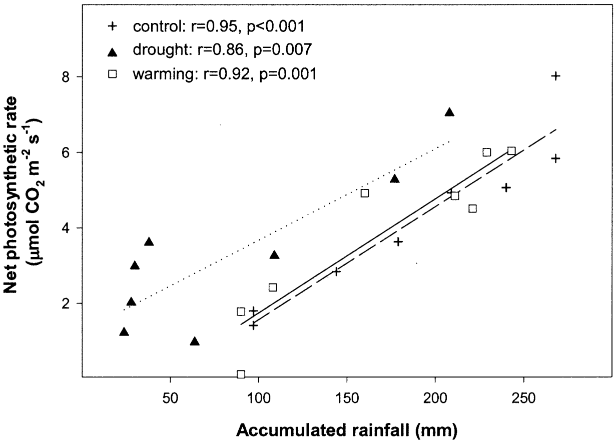

Figure 4. Net photosynthetic rates versus accumulated rainfall for the three months previous to the measurements for control, drought, and warming treatments in the UK, Denmark, The Netherlands, and Spain sites and in 1999 and 2000 ($n = 8$ for each treatment).

Leaf net photosynthetic rates of control plants followed a gradient from northern to southern countries, closely related to the accumulated rainfall over the growth period (Table 2 and Figure 1). This result suggests that the amount of precipitation during the growing season was the main factor controlling differences in the photosynthetic rates among countries, regardless of other site characteristics and in agreement with the observed general effect of drought treatment on photosynthetic rates (see below). In contrast, there was not a consistent gradient in the leaf transpiration rates or WUE of plants in both years (Table 2), because these two parameters were more affected by the vapor pressure deficit of the measuring day than net photosynthetic rates.

Our results also suggest that the accumulated rainfall over the growing season is an important factor controlling the differences in the predawn potential photochemical efficiency (F_v/F_m) of *C. vulgaris* plants within and across the three northern sites (Figure 1). Interestingly, lower amounts of rainfall during the growing season in these three northern sites were correlated with higher predawn F_v/F_m values, which is in accordance with the higher F_v/F_m values that we found in droughted plants compared with controls (see below). The fact that in Spain we studied a different species may explain the lack of correlation between predawn F_v/F_m values and accumulated rainfall when we used the data from the Spanish site.

Our results on leaf $\delta^{13}C$ (Table 4) are in accordance with the abundant literature reports showing that foliage of species from xeric habitats has more positive values of $\delta^{13}C$ than that from mesic habitats (for example, see Ehleringer and Cooper 1988; Garten and Taylor 1992; Meinzer and others 1992; Stewart and others 1995; Peñuelas and others 1999). Because theory predicts that values of leaf $\delta^{13}C$ are positively related to the integrated water-use efficiency over the growing season (WUE_i) (Farquhar and others 1989), the more positive values of leaf $\delta^{13}C$ of control plants in Spain would imply that these plants had higher WUE_i than control plants in the other three countries. However, this result disagrees with the results that we obtained on instantaneous WUE (Table 2). Discrepancies between gas exchange and isotopic data have been reported in previous studies (for example, see Vitousek and others 1990; Picon and others 1996) and may arise from instantaneous WUE being highly dependent on meteorological conditions on the date of measurements, whereas leaf $\delta^{13}C$ is largely based on the long-term integration of WUE.

Drought Effects

Photosynthesis at leaf level is one of the physiological processes more strongly affected by water shortage (Epron and Dreyer 1993). Accordingly, we found a general reduction in the net photosynthetic (A) and transpiration rates (E) in the droughted plants compared with controls (Figure 2), in agreement with previous studies on plants suffering from water stress (reviews in Schulze 1986; Chaves 1991; Yordanov and others 2000). However, we did not find any significant effect of drought treatment on the instantaneous WUE of plants, which means that photosynthetic and transpiration rates changed proportionally. In agreement with the results on instantaneous WUE, we did not find significant effects of drought treatment on $\delta^{13}C$ (Table 4) and, thus, on integrated WUE. Because drought did not significantly decrease the leaf nitrogen concentration (Peñuelas and others 2004) or the potential photochemical efficiency (F_v/F_m) of photosystem II (PSII), it appears that the reduction in leaf assimilation rates was mainly produced by stomatal closure.

Stomatal closure, and the consequent reduction in the assimilation rate, may result in damage to the photosystem, since the absorption of light energy may be in excess of that required for carbon fixation (Jefferies 1994). Accordingly, an increase in drought has been related in many cases to a drop in potential photochemical efficiency of PSII (for example, see Björkman and Powles 1984; Epron and others 1992; Jagtap and others 1998). Nevertheless, some authors raise doubts about PSII sensitivity to water shortage, citing studies showing a remarkable resistance of the photosynthetic apparatus to dehydration (for example, see Gamon and Pearcy 1990; Havaux 1992; Epron and Dreyer 1993; Jefferies 1994; Epron 1997). In addition, some authors demonstrated that a water deficit may enhance the resistance of PSII photochemistry to superimposed constraints, namely, high-temperature stress combined or not with photoinhibitory light (Havaux 1992; Epron 1997; Yordanov and others 1999). The results of our study support the latter idea, because we found higher values of potential photochemical efficiency (F_v/F_m) in droughted plants compared with controls, mainly at midday (Figure 3). This pattern held across sites and years (see also Figure 1), suggesting that it is a consistent result. Demonstration of a general increase in the stability of PSII to superimposed constraints caused by drought is of great ecophysiological significance because it is very likely that climate change will increase the frequency of hot and dry days, particularly in the Mediterranean countries.

According to the observed high stability of PSII in droughted leaves, as shown by F_v/F_m values, we did not find significant effects of drought on ϕ_{PS2} values in the UK, The Netherlands, and Spain (Table 3), in spite of the general decrease in leaf photosynthetic rates. This result suggests that droughted plants from these countries may have higher photorespiration rates than controls. Higher photorespiration rates in droughted plants would consume the excess of energy and would maintain high rates of electron transport, protecting the photosystem from photodamage (Krall and Edwards 1992; Kozaki and Takeba 1996).

Warming Effects

Our warming treatment did not affect the leaf gas exchange rates of plants (Figure 2). Previous studies of the effects of warming on leaf gas exchange rates have reported contrasting results. Some authors found no effect of warming manipulations on leaf gas exchange rates (for example, Wookey and others 1994; Nijs and others 1996; Loik and others 2000; Starr and others 2000), other authors found stimulated rates (for example, Chapin and Shaver 1996; Huxman and others 1998; Apple and others 2000), whereas others found decreases (Callaway and others 1994; Roden and Ball 1996). However, comparisons among studies are difficult given the different characteristics of warming treatments among the experiments and the different temperature sensitivities and optima for photosynthesis between species and ecotypes (Chapin and others 1995; Shaw and others 2000). Moreover, leaf gas exchange responses to temperature are often strongly influenced by other interacting factors, such as the internal plant water status and the water vapor pressure difference between the leaf and the surrounding air (Chaves 1991).

In our experiment, the lack of direct warming effects on leaf gas exchange rates is probably due to the fact that the increase in temperature during the day in the warming plots was too weak to produce significant differences in leaf gas exchange rates between warming and control plants. However, we did not detect indirect effects of the warming treatment on leaf gas exchange rates via increased foliar nitrogen either, since leaf nitrogen concentrations were not significantly affected by the warming treatment (Peñuelas and others 2004).

In agreement with the lack of warming effects on leaf gas exchange rates, the warming treatment did not affect the instantaneous WUE (Figure 2) or the chlorophyll fluorescence parameters, that is, the efficiency of PSII (Table 3 and Figure 3). Warming plants tended to show more negative values of $\delta^{13}C$ than control plants (Table 4), which would imply lower WUE_i.

Water Availability Control of Photosynthetic Performance

The lower leaf net photosynthetic rates (A) of plants in the drought treatment compared with controls (Figure 2) is consistent with the decrease in A of control plants associated with the reduction in the precipitation experienced along the geographical transect (Figure 1). This result indicates that a consequence of the extension of the drought period will probably be a decrease in the annual productivity of these ericaceous shrubs in the short term, as well as in the long term. Previous studies found approximately linear decreases in forest productivity with decreases in annual precipitation below 1500 mm (O'Neill and DeAngelis 1981). A close positive correlation between net primary productivity and precipitation was also reported across precipitation gradients in temperate grasslands (Lauenroth and Sala 1992) or among sites and years in deserts (Gutierrez and Whitford 1987).

Leaf net photosynthetic rates (A) were significantly correlated with the accumulated rainfall over the growing season for plants in the drought and warming treatments (Figure 4), in spite of the differences across sites and years in the intensity of these treatments. These results support the idea that precipitation over the growing season is really a major factor controlling A.

Interestingly, although A decreased in response to drought treatment, droughted plants showed higher photosynthetic rates than control plants in relation to the amount of water received over the previous months (Figure 4). This could be related to the higher potential photochemical efficiency (F_v/F_m) that we found in droughted plants in relation to controls (Figure 3). Moreover, droughted plants could have developed more and/or deeper roots, which might allow them to extract soil water more efficiently than control plants and/or to access deeper and probably more permanent water sources.

FINAL CONCLUSIONS AND REMARKS

We found a decrease in the leaf net photosynthetic rates of plants from northern to southern countries, in agreement with the geographical gradient in water availability.

Drought treatment decreased the leaf gas exchange rates of plants in the four sites. However, droughted plants had higher photosynthetic rates than controls in relation to the accumulated rainfall over the previous months. Moreover, plants in the drought plots showed higher potential photochemical efficiency than controls mainly at midday, suggesting an enhanced ability to cope with superimposed constraints, which, however, did not avoid a decrease in growth (Peñuelas and others 2004).

The warming treatment had no significant effects on the instantaneous photosynthetic performance of plants during the growing season in any of the four sites, which does not preclude possible significant differences when considering the integrated whole-year plant productivity (Peñuelas and others 2004).

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