

Summer drought impedes beech seedling performance more in a sub-Mediterranean forest understory than in small gaps

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Summary Refugia of mixed beech forest persist in the central mountains of the Iberian Peninsula at the south-western limit of European beech (*Fagus sylvatica* L.) distribution. The lack of beech regeneration is a concern in this region that has experienced reduced rainfall and higher temperatures over the past 30 years. Beech is considered especially susceptible to climate change because of its conservative shade-tolerant growth strategy; hence seedling responses to drought stress in gaps and in the understory are of particular interest. During the summer of 2007, a watering treatment raised the soil water content by up to 5% in gap and understory plots of beech seedlings in a mixed beech forest. Root-collar diameter was increased by our watering treatment in understory seedlings. Neither drought-avoidance through stomatal closure nor physiological drought-tolerance mechanisms were able to mitigate the effects of water stress in the understory seedlings, whereas osmotic adjustment enhanced the ability of the gap seedlings to tolerate water stress. Overall, high photosynthetic rates in the gaps, despite the photoinhibitory effects of high radiation, allowed gap seedlings to survive and grow better than the understory seedlings irrespective of water availability. Our results indicate that further intensification of summer drought, predicted for the Iberian Peninsula, will hinder the establishment of a beech seedling bank in the understory because of the conflicting seedling trait responses to simultaneously withstand water stress and to tolerate shade.

Keywords: chlorophyll fluorescence, climate change, *Fagus sylvatica*, functional traits, *P–V* curves, water-use efficiency, ¹³C.

Introduction

Beech is one of the most emblematic and widespread broad-leaved tree species in Europe, and it has great

cultural and environmental importance (Huntley et al. 1989, Ellenberg 1996), but climate change scenarios predict that suitable forest areas for beech across Europe will decrease over the next century (Sabaté et al. 2002, Geßler et al. 2007). As beech is sensitive to extremes of water logging and drought stress (Dreyer 1994, Bréda et al. 2006), the predicted changes in precipitation patterns across Europe may critically affect its potential to regenerate (Peuke et al. 2002). In common with other shade-tolerant trees, beech may be particularly susceptible to the effects of climate change because of its relatively low capacity for physiological acclimation (Grime and Mackey 2002, Valladares et al. 2002), and so it may lack the plasticity to respond to unpredictable extreme weather (Valladares et al. 2007).

A strategy that maximizes light capture allows beech to establish a persistent seedling bank in the forest understory; however, this shade-tolerant strategy may hinder seedling regeneration under drought conditions, because conflicting responses at both the leaf and whole-seedling scale tend to prevent plants from being both drought and shade tolerant (Sánchez-Gómez et al. 2006, Valladares et al. 2007). In understory shade, seedlings must maximize light capture to maintain a positive carbon balance through photosynthesis, but this precludes stomatal closure to conserve water under drought stress (Smith and Huston 1989). Furthermore, hydraulic architecture acclimated to shaded conditions may restrict water uptake because of the large gradients of resistance along the transpiration stream (Cochard et al. 1999). Likewise, greater aboveground biomass allocation by trees under shaded conditions runs counter to the shift in allocation expected when they experience water stress (Veneklaas and Poorter 1998). In forest gaps, high radiation and large vapor pressure deficit (VPD) hinder water retention. However, the non-limiting light environment facilitates the maintenance of a positive carbon balance in gap seedlings allowing greater physiological activity and flexibility in mechanisms to

tolerate higher tissue dehydration through an enhanced capacity for osmotic adjustment compared with understory seedlings (Abrams and Mostoller 1995, Aranda et al. 2001).

In the central mountain range (*Sistema Central*) of the Iberian Peninsula scattered beech-oak forests survive at the range limit of beech in Europe (Jump et al. 2006, Magri 2008). The recent increase in severe summer droughts has detrimentally affected the health and physiological responses of both adults and seedlings in this population (Aranda et al. 2004). Light and drought are the main environmental factors influencing morphological and physiological traits during the establishment of beech seedlings (Madsen and Bo Larsen 1997, Aranda et al. 2002), and their promotion to the canopy (Aranda et al. 2004, Balandier et al. 2007).

We compared the effects of a current summer drought with those of a watering treatment that increased the ambient soil water content by up to 5% on regenerating seedlings in experimental forest plots. We tested two hypotheses: (1) Despite a relatively shade-tolerant strategy, beech seedlings grow less and exhibit symptoms of greater stress under moderate understory shade than in small gaps, because acclimation to shade constrains the seedling response to drought for selected physiological variables (Tognetti et al. 1994, Aranda et al. 2004). (2) Increased water availability benefits understory seedlings more than gap seedlings, because the physiological plasticity of gap seedlings allows them greater flexibility in their response to drought. To test our hypotheses, we measured seasonal changes in leaf traits that govern gas exchange, photoinhibition and water relations in response to an intensifying summer drought, and examined whether the responses are expressed in reduced seedling growth and survival. We also monitored soil water content to determine whether the watering treatment affected understory and gap conditions similarly.

Materials and methods

Site characteristics

The experiment was conducted in Hayedo de Montejo (41°1' N and 3°5' W, 1400 m a.s.l.), a site of conservation and biodiversity value within the UNESCO World Biosphere Reserve Cuenca alta del Rio Manzanares. This ancient semi-natural sub-Mediterranean forest is at the south western distribution limit of European beech (*Fagus sylvatica* L.), where it occurs in mixed stands with *Quercus petraea* (Matt.) Liebl., *Quercus pyrenaica* Willd., and *Ilex aquifolium* L. Soils are deep, fertile sandy loams. Climate is continental sub-Mediterranean, characterized by cold winters (−6 °C minimum) and hot summers (mean of 18 °C for July–August, maximum of 42 °C; Figure 1B). Much of the annual precipitation of about 1100 mm falls in early spring followed by a prolonged summer drought (about 40 mm of rain during July–August).

Experimental design

Beech nuts were sown in plots (3 × 2 m) about 15 m apart within a 50 × 50-m area of forest. All plots were of similar topography and inclination on an east-facing slope. Three plots were in natural gaps created by fallen canopy trees, and these were paired with three understory plots in moderate shade under mature beech trees (Table 1). Each plot was split into two 1.3 × 2 m² subplots for the dry and watered treatments, separated by a 0.4 × 2 m² buffer zone. Pre-stratified germinating beech nuts were sown (100 beech

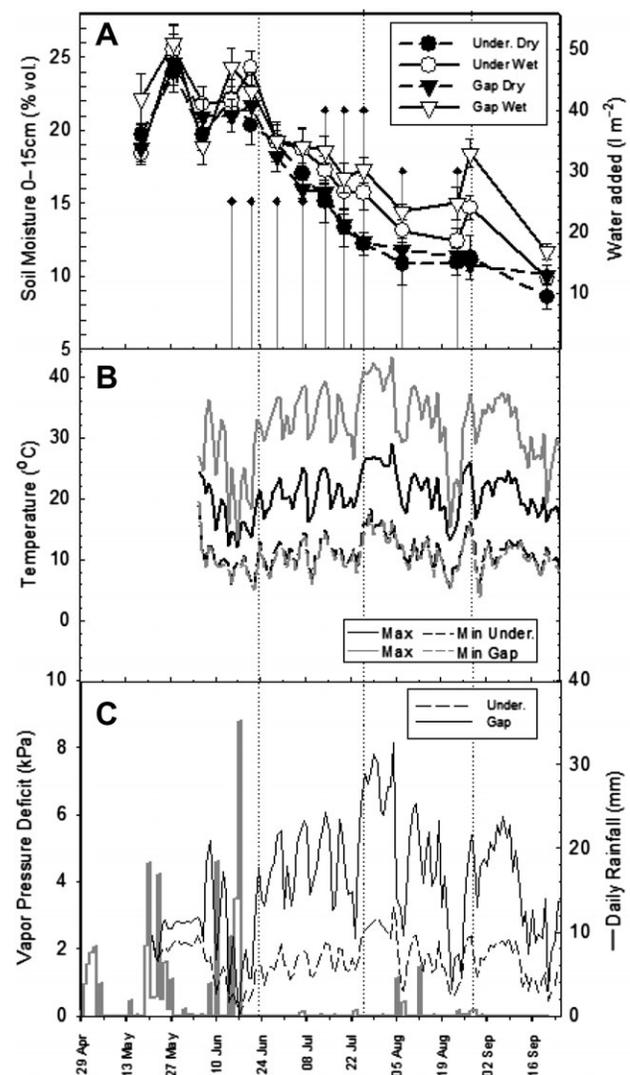


Figure 1. (A) Soil water content at 0–15 cm depth measured by time domain reflectometry (TDR). Values are mean \pm 1 SE of three plots (two tubes per plot). Dashed vertical lines mark the sampling dates for photosynthetic measurements. The quantity of water added per m² of the watered subplots is indicated on the date of each addition. (B) Maximum and minimum daily temperature, and (C) relative humidity, recorded every 3 min by micro-meteorologic stations. Values are means from three gap and three understory plots. (C) Site-level precipitation was recorded daily by a rain gauge in a tower at canopy level. Abbreviation: Under. = understory.

Table 1. Punctual values of environmental conditions. Values are means of three plots \pm 1 SE. Light (photosynthetic photon flux density (PPFD)) and vapor pressure deficit (VPD) were recorded during the gas exchange and chlorophyll fluorescence measurements of each seedling.

Plot treatment	Understory + dry	Understory + watered	Gap + dry	Gap + watered
<i>June 21</i>				
Air temperature ($^{\circ}\text{C}$)	18 \pm 2	17 \pm 1	18 \pm 1	19 \pm 1
Light ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	20 \pm 6	19 \pm 6	298 \pm 36	380 \pm 77
Soil water (% vol)	18.1 \pm 0.8	20.2 \pm 0.7	17.7 \pm 0.5	22.8 \pm 0.9
VPD (kPa)	2.06 \pm 0.18	1.95 \pm 0.29	1.97 \pm 0.08	2.14 \pm 0.08
<i>July 26</i>				
Air temperature ($^{\circ}\text{C}$)	32 \pm 2	32 \pm 3	39 \pm 3	38 \pm 3
Light ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	25 \pm 3	29 \pm 8	1798 \pm 62	1821 \pm 50
Soil water (% vol)	11.8 \pm 0.6	15.3 \pm 0.5	11.1 \pm 0.8	14.5 \pm 0.9
VPD (kPa)	4.70 \pm 0.61	4.92 \pm 0.88	6.33 \pm 0.76	5.66 \pm 0.57
<i>August 27</i>				
Air temperature ($^{\circ}\text{C}$)	29 \pm 3	29 \pm 2	33 \pm 1	31 \pm 2
Light ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	22 \pm 3	22 \pm 5	1271 \pm 48	1373 \pm 89
Soil water (% vol)	9.9 \pm 0.6	14.6 \pm 0.7	9.7 \pm 0.9	12.1 \pm 0.8
VPD (kPa)	4.07 \pm 0.66	3.98 \pm 0.48	4.70 \pm 0.17	4.04 \pm 0.38

nuts per subplot spaced 15 cm apart and 2 cm deep) during the second week of April 2007 and the existing litter layer (about 2 cm) was evenly replaced. Beech masts were collected the previous autumn from five nearby mother trees, and the nuts were distributed evenly among the treatments. At the time of sowing, the area in and around all plots was free of vegetation, and weeds that germinated in the plots or surrounding area during the experiment were removed. The final height and root-collar diameter attained by the seedlings were measured with calipers at the end of the growing season. Seedling leaf number was monitored every 7–10 days during the spring and summer.

In each plot, natural drought conditions were retained in one subplot (dry treatment), and water stress was alleviated in the other subplot by irrigation (watered treatment). Irrigation commenced on June 15, using water from a nearby canal, and was repeated every 7–10 days until August 24. Initially 25 l m⁻² and later 40 l m⁻² of water was added to achieve a mean soil water content of about 5% above the dry subplots during July–August accounting for fluctuations between each watering date (Figure 1). Measurements of soil water content and seedling performance were made at least 1 week after watering.

Air temperature and relative humidity were continuously monitored at 15-cm height above the ground in each plot with mini weather stations (Hobo H8, pro onset). Volumetric soil water content was quantified by sonic time domain reflectometry, TDR (Trace System I, Soil Moisture Equipment, Santa Barbara, CA) every week in two 0–50-cm profiles per subplot (Figure 1).

Hemispherical photographs were taken in the evening during late summer to characterize the light environment at two locations in each subplot. A global site factor (GSF, %) was calculated for each plot based on a solar transmissivity of 0.8 and 0.1 diffuse:direct radiation (Canopy Analysis Software

Hemiview Version 2.1, Delta-T Devices Ltd., Houston, TX). Gap GSF was 0.43 \pm 0.06 (10.3 \pm 1.5 mol m⁻² day⁻¹) and understory GSF was 0.11 \pm 0.01 (2.5 \pm 0.2 mol m⁻² day⁻¹) ($F_{1,6} = 55.3$, $P < 0.001$). There was no difference in radiation received between dry and watered subplots ($F_{1,6} = 0.02$, $P = 0.890$).

Gas exchange and chlorophyll fluorescence

Net photosynthetic rate (A_{net}), transpiration rate, and stomatal conductance of water vapor were measured (IRGA LcPro Analytical Development Corporation, UK) on the same attached fully expanded leaves of three seedlings per subplot on three occasions during the growing season – in late spring (June 21), midsummer (July 26) and late summer (August 26) – paralleling the progressive increase in drought intensity. Measurements were made at mid-morning on cloud-free days in July–August, and about 1 h earlier in June because of the prospect of approaching cloud. The leaves were later harvested for morphological and chemical analyses (data not shown).

Chlorophyll fluorescence parameters were recorded (FMS 2, Hansatech Instruments Ltd., UK) on the same leaves and on the same day as the gas exchange measurements. Three sets of measurements were recorded on each sampling date, dark-adapted fluorescence, mid-morning light fluorescence and midday light fluorescence. Maximum fluorescence was measured by applying a 0.8-s saturating light pulse (6600 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and minimum light fluorescence was measured after applying a 5-s far-red light pulse. Fluorescence signals were used to calculate the potential quantum yield of photosystem II (PSII) (F_v/F_m), the effective quantum yield (Φ_{PSII}), the efficiency of open reaction centers (F_v'/F_m'), photochemical quenching (qP), and non-photochemical quenching (NPQ), according to the equations given by Maxwell and Johnson (2000).

Water relations

Predawn and midday leaf water potentials (Ψ_{pd} and Ψ_{md}) were measured with a pressure chamber (PMS Instrument Co. 7000, Corvallis, OR) on August 26–27 at the peak of the summer drought on leaves previously used for gas exchange measurements. The leaves measured for Ψ_{pd} were kept hydrated and used to construct pressure–volume (P – V) curves by the free transpiration method (Robichaux 1984), to derive the following parameters: leaf water potential at full turgor ($\Psi\pi_{100}$) and turgor loss ($\Psi\pi_0$), elastic modulus at maximum turgor (ϵ_{max}) and relative water content at turgor loss (RWC^0).

Efficiency of water flow through the transpiration stream was estimated by calculating the apparent soil–leaf hydraulic conductance (K_L) as the quotient between transpiration and the difference between soil water potential (using Ψ_{pd} as surrogate) and minimum daily leaf water potential (Ψ_{md}).

The leaves excised for the water potential measurements were subsequently analyzed by isotope-ratio mass spectrometry to determine their stable carbon isotope composition ($\delta^{13}C$, ‰), given by $\delta^{13}C = (R_{sample}/R_{standard} - 1)1000$. This assessment of $\delta^{13}C$ was used as a proxy for water-use efficiency (WUE) of the leaf integrated over its life span.

Statistical analysis

The effects of shade and drought on beech seedlings were tested with a mixed-model two-way analysis of variance (ANOVA), based on a completely randomized split-plot design, with repeated measures. All computations were performed in SAS Version 8.2 software (SAS Institute, Cary, NC). As summer drought intensified, successive monthly values were expected to be most closely correlated with those from the previous month, so a first-order autoregressive variance/covariance structure was used, with the Kenward–Roger method for degrees of freedom calculations. The pairwise interactions between drought treatments were tested by single-factor ANOVA. Additional analyses showed that the variation explained by a seedlings' mother tree was much less than that explained by the treatments (data not shown), therefore, this co-variant was not included in the model.

Results

Environmental data

The summer drought reduced volumetric soil water content from 25% during May to < 10% by the end of September (Figure 1) at 0–15 cm depth. The soil water profile revealed a gradient of increasing dryness with depth (measured to 50 cm) both in the gap and understory plots: the gradient was slightly steeper in the understory plots than in the gap plots and the difference increased as summer progressed (data not shown), presumably reflecting water uptake by

the roots of mature trees. The watering treatment successfully raised the soil water content to about 5% above ambient values during July–August, and this difference did not vary significantly between the gap and understory plots (Figure 1A).

Maximum daily temperature in the gap plots was about 10 °C higher than that in the understory plots throughout the summer, but minimum nighttime temperature was similar in both environments (Figure 1B). Consistent with our expectations, relative humidity was up to 20% higher in the understory plots than in the gap plots, which contributed to a 2–3 times higher daytime VPD in the gap plots than in the understory plots (Figure 1C).

Seedling growth and survival

As summer progressed, the population of seedlings in the gaps became higher than that in the understory because of high understory seedling mortality during July–August (Figure 2; Table 2). The watering treatment had no significant overall effect on understory seedling population size, although when the data for August and September only were considered there was a tendency for the seedling population size to be less in the dry understory subplots (26 ± 5) than in the watered understory subplots (36 ± 7) (Figure 2; $F_{1,6} = 5.2$, $P = 0.063$). Gap seedlings produced more leaves than understory seedlings, which mostly remained at the two-leaf stage.

Height, diameter and leaf number, measured at the end of the summer, were all significantly higher in the gap seedlings than in the understory seedlings (Table 3). Root-collar diameter, but not height, was lower in the drought-grown seedlings than in the watered seedlings. The understory \times drought interaction was marginally significant for root-collar diameter (Table 3; $P = 0.084$), because the drought-induced reduction in root-collar diameter occurred only in the understory seedlings and not in the gap seedlings. For both gap and understory seedlings, leaf number

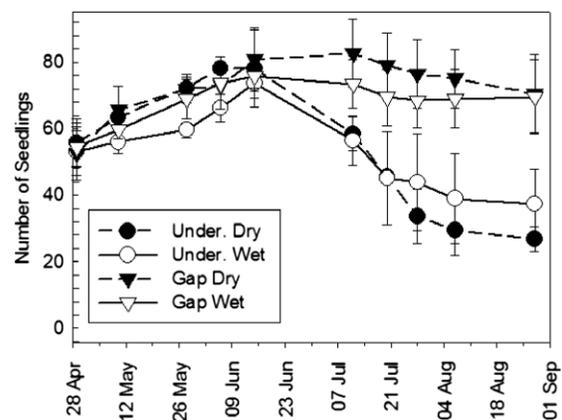


Figure 2. Survival of individual seedlings in the population during the field season. Values are mean number of seedlings \pm 1 SE from three plots for each treatment combination. Abbreviation: Under. = understory.

Table 2. The *P* values from ANOVAs corresponding to each table and figure. The *F* values with degrees of freedom are given.

Effect	Figures/Tables	Understory (U)	Dry (D)	S × D	Month (M)	U × M	D × M	U × D × M
Repeated measures		$F_{1,2}$	$F_{1,2}$	$F_{1,2}$	$F_{2,86}$	$F_{2,86}$	$F_{2,86}$	$F_{2,86}$
Temperature	Table 1	0.050	0.733	0.839	< 0.0001	< 0.0001	0.215	0.132
Light	Table 1	0.0009	0.269	0.275	< 0.0001	< 0.0001	0.760	0.738
VPD	Table 1	0.057	0.492	0.334	< 0.0001	0.002	0.760	0.738
Soil water	Table 1	$F_{1,2}$ 0.788	$F_{1,2}$ 0.016	$F_{1,2}$ 0.904	$F_{2,86}$ < 0.0001	$F_{2,86}$ 0.194	$F_{2,86}$ 0.989	$F_{2,86}$ 0.186
Seedling population	Figure 2	$F_{1,2}$ 0.091	$F_{1,2}$ 0.543	$F_{1,2}$ 0.457	$F_{9,72}$ 0.008	$F_{9,72}$ 0.031	$F_{9,72}$ 0.532	$F_{9,72}$ 0.099
A_{net}	Figure 3	$F_{1,2}$ 0.022	$F_{1,2}$ 0.036	$F_{1,2}$ 0.030	$F_{2,87}$ < 0.0001	$F_{2,87}$ 0.003	$F_{2,87}$ 0.005	$F_{2,87}$ 0.035
g_{wv}	Figure 3	0.048	0.018	0.138	< 0.0001	0.004	0.097	0.719
IWUE	Figure 3	0.004	0.326	0.340	0.001	0.0003	0.096	0.065
F_v/F_m am	Not shown	$F_{1,2}$ 0.027	$F_{1,2}$ 0.537	$F_{1,2}$ 0.408	$F_{2,86}$ < 0.0001	$F_{2,86}$ < 0.0001	$F_{2,86}$ 0.143	$F_{2,86}$ 0.063
F_v'/F_m' am	Figure 4	0.039	0.368	0.456	< 0.0001	< 0.0001	0.476	0.203
F_v'/F_m' pm	Figure 4	0.001	0.131	0.133	< 0.0001	< 0.0001	0.289	0.277
ΦPSII am	Figure 4	0.028	0.532	0.650	< 0.0001	< 0.0001	0.529	0.362
ΦPSII pm	Figure 4	0.003	0.307	0.248	< 0.0001	< 0.0001	0.901	0.611
<i>q</i> P am	Figure 4	0.056	0.780	0.757	< 0.0001	0.001	0.472	0.418
<i>q</i> P pm	Figure 4	0.029	0.361	0.296	0.007	0.018	0.037	0.055
NPQ am	Figure 4	0.027	0.451	0.221	< 0.0001	< 0.0001	0.783	0.191
NPQ pm	Figure 4	0.004	0.406	0.318	< 0.0001	< 0.0001	0.339	0.258

Table 3. Seedling vertical height (mm) and root-collar diameter (mm), and the proportion of living seedlings with < 2, 2–3, or 4+ leaves in each treatment at the end of the growing season. Values are mean ± 1 SE of the population from three plots in each treatment type.

Treatment	Height (mm)	Diameter (mm)	< 2 leaves	2–3 leaves	4+ leaves
Under (U) + dry (D)	86.8 ± 5.7	1.23 ± 0.09	0.91 ± 0.01	0.08 ± 0.01	0
Under + watered	89.8 ± 7.6	1.47 ± 0.10	0.76 ± 0.07	0.24 ± 0.07	0
Gap + dry	99.4 ± 11.6	1.88 ± 0.18	0.28 ± 0.07	0.41 ± 0.15	0.31 ± 0.13
Gap + watered	98.8 ± 11.4	1.91 ± 0.16	0.27 ± 0.06	0.09 ± 0.04	0.64 ± 0.05
<i>P</i> values					
(U) $F_{1,2}$	0.018	< 0.0001	< 0.0001		
(D) $F_{1,4}$	0.762	0.008	0.585		
(U × D) $F_{1,24}$	0.959	0.084	0.354		

was higher in the watered subplots than in the dry subplots (Table 3).

Gas exchange and chlorophyll fluorescence

Gas exchange, transpiration and chlorophyll fluorescence measurements reflected monthly differences in environmental conditions (Table 1) and the effects of shade and the watering treatments (Figures 3 and 4; Tables 1 and 2). Net photosynthetic rate (A_{net}) was the most responsive parameter to drought: in gap seedlings, it was about 50% lower in the dry subplots than in the watered subplots, and in understory seedlings A_{net} was similarly reduced by drought, although the very low photosynthetic rates of these seedlings made the effect hard to discern (Figure 3A; Table 2). The effects of drought on stomatal conductance (g_{wv}) were similar to those on photosynthesis:

g_{wv} was lower in understory seedlings by about 67% and in gap seedlings by about 43%, in the dry subplots compared with the watered subplots (Figure 3B). Instantaneous water use efficiency (IWUE), calculated from the gas exchange measurements, was much higher in gap seedlings than in understory seedlings, but did not differ between the wet and dry treatments. By August, IWUE had increased slightly in the gap seedlings as they became larger and better established, but it did not increase in the understory seedlings (Figure 3C).

Dark fluorescence F_v/F_m was always above 0.83 in the understory seedlings and was unaffected by watering. In the gap seedlings, there was little evidence of irreversible photodamage caused by excess light in either the dry or watered subplots. Values of F_v/F_m in gap seedlings were lowest in June (gap + dry: 0.780 ± 0.009 , gap +

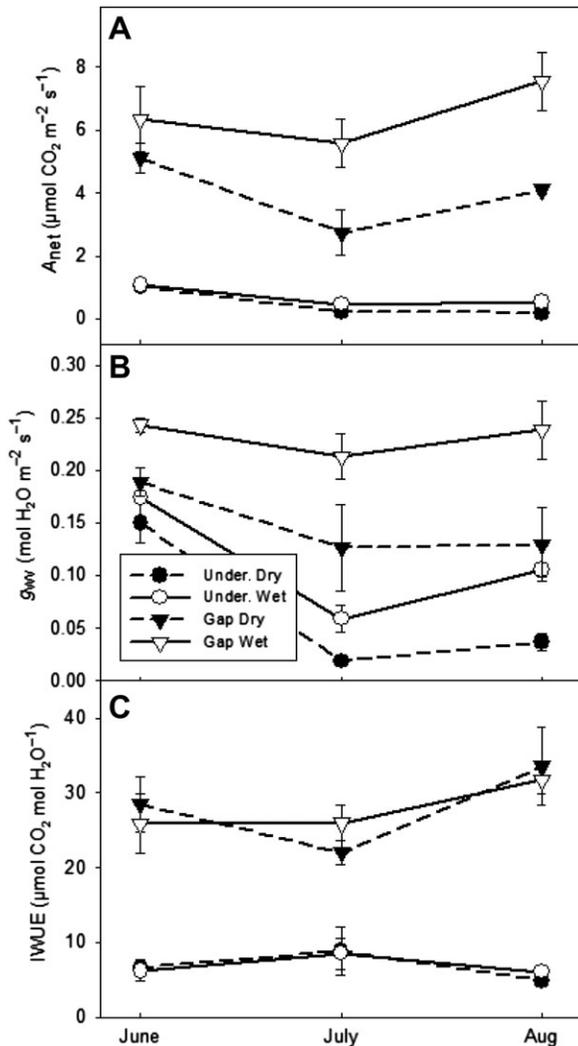


Figure 3. (A) Net photosynthetic rate (A_{net}), (B) stomatal conductance (g_{wv}), and (C) intrinsic water-use efficiency (IWUE) calculated from these data. Gas exchange was measured under ambient conditions (Table 1) at mid-morning on June 21, July 26 and August 27, in three plants from each plot. Values are mean \pm 1 SE of three plots of each treatment combination. Abbreviation: Under. = understory.

watered: 0.773 ± 0.008), and gradually increased in both subplots during the growing season (August, gap + dry: 0.828 ± 0.005 , gap + watered: 0.838 ± 0.004 , Table 2).

Differences in quantum efficiency (F_v'/F_m'), yield (Φ_{PSII}), and quenching between gap and understory seedlings were consistent with the different irradiances (Table 1) and seasonal changes between sampling dates (Figure 4). However, increased values of F_v'/F_m' and Φ_{PSII} in the morning as the growing season progressed suggest that as leaves matured they were better able to utilize high irradiances, dissipate excess radiation, or both. There was a small but statistically significant effect of drought on both F_v'/F_m' and Φ_{PSII} in July, and this was more apparent in the gap seedlings than in the shade seedlings at midday (Figure 4A–D: July midday, Dry $P = 0.035$, U \times D,

$P = 0.039$). The only effect of watering on quenching was a trend toward higher NPQ ($F_{1,45} = 6.2$, $P = 0.017$) in gap seedlings in the dry subplots compared with the watered subplots (Figure 4G).

Water relations

At the end of summer, predawn leaf water potential (Ψ_{pd}) was higher (closer to zero), whereas midday leaf water potential (Ψ_{md}) was lower (more negative), in the gap seedlings than in the understory seedlings, in accordance with the lower daytime transpirational losses in the shade. Seedlings in the dry subplots had lower Ψ_{pd} and Ψ_{md} than seedlings in the watered subplots (Figure 5A).

Calculations from P – V curves revealed that leaf water potentials at both full turgor ($\Psi_{\pi 100}$) and at turgor loss ($\Psi_{\pi 0}$) were lower in gap seedlings than in understory seedlings. However, the difference $\Psi_{\pi 0} - \Psi_{\pi 100}$ was greater in gap seedlings than in understory seedlings. Unlike the understory seedlings, gap seedlings had lower $\Psi_{\pi 100}$ and $\Psi_{\pi 0}$ in the dry subplots than in the watered subplots (Figure 5B).

The modulus of elasticity at maximum turgor (ϵ_{max}) was dependent on the combination of shade and drought treatments. Comparing the four treatment combinations, there was no difference in ϵ_{max} between understory and gap seedlings in the watered subplots, but ϵ_{max} was highest in gap seedlings in the dry subplots and lowest in understory seedlings in the dry subplots (Table 4). The RWC^0 also depended on the interaction of shade and drought. Gap and understory seedlings in the dry subplots had similar values of RWC^0 , whereas gap seedlings in the watered subplots had a higher RWC^0 (Table 4). Apparent soil-leaf hydraulic conductance (K_L) was lower in understory seedlings than in gap seedlings, and lower in the dry subplots than in the watered subplots. This effect was significantly greater in the understory seedlings than in the gap seedlings, as is evident from the significant interaction term (Table 4).

Leaf $\delta^{13}C$ in August was significantly more depleted in the understory seedlings than in the gap seedlings (-31.0 versus -29.0‰); assuming that atmospheric $\delta^{13}C$ is equivalent in the nearby gap and understory plots, this should reflect higher WUE integrated over the lifespan of the leaf in the gap seedlings. In both light environments, leaf $\delta^{13}C$ was further depleted by the watering treatment (Table 4).

Discussion

Implications of shade and drought for survival and growth

The results generally support our hypothesis that beech seedlings grow better and exhibit fewer symptoms of stress in small gaps than in the understory. Low water potentials in seedlings from all plots suggest that plants endured some drought stress even in the watered treatments. Soil water content in the understory plots was lower than that in the gap plots, presumably because of greater uptake by the roots of adult trees beneath the understory plots, and

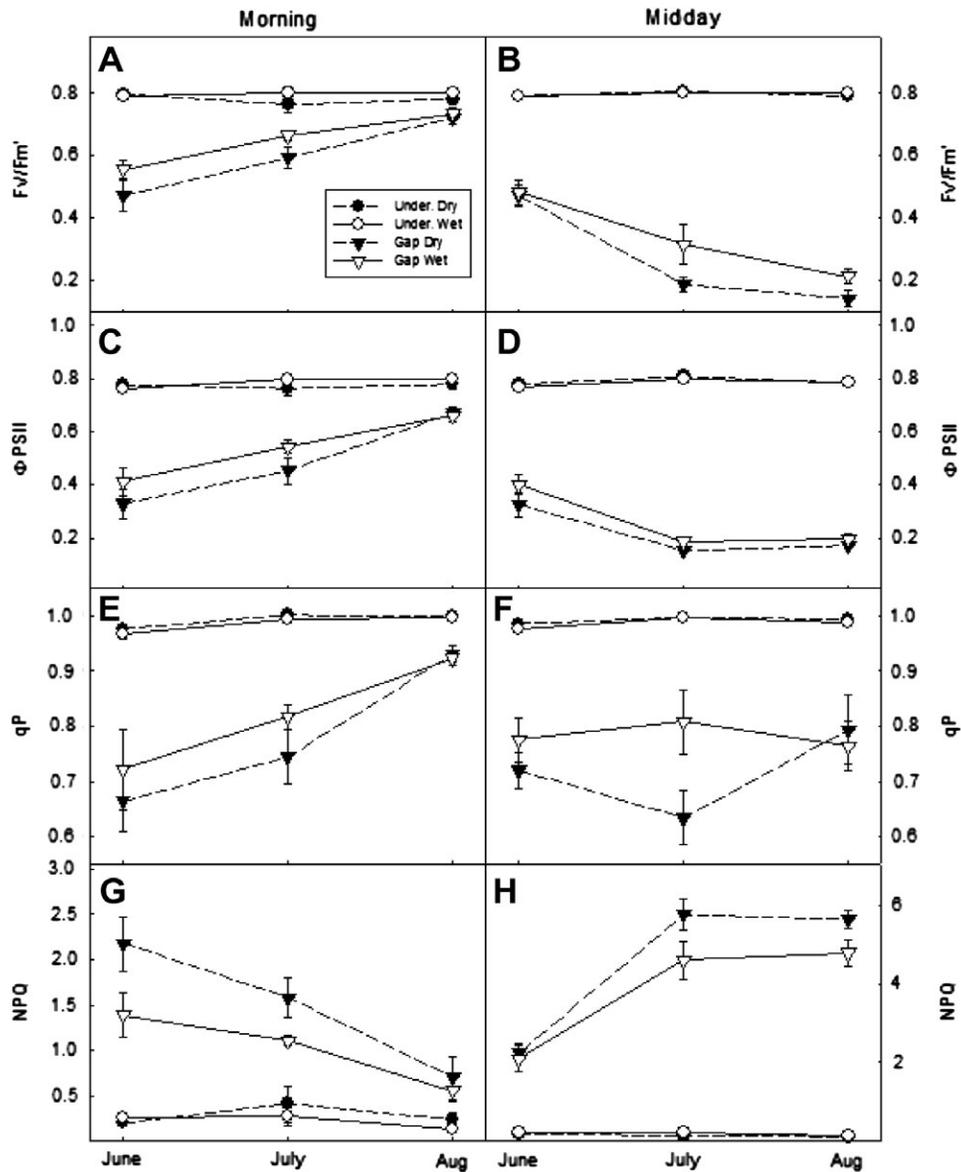


Figure 4. Chlorophyll fluorescence parameters and quenching coefficients measured in the morning and at midday on the same leaves and on the same days as the gas exchange measurements. The efficiency of open reaction centers, F_v'/F_m' (A and B); the effective quantum yield, Φ_{PSII} (C and D); photochemical quenching, qP (E and F); and non-photochemical quenching, NPQ (G and H). Abbreviation: Under. = understory.

possibly also because rainfall was partially intercepted by the canopy above the understory plots. These two factors affecting soil water content in the understory outweighed the drying effect of direct radiation on the soil in the gap plots. We speculate that because the gap seedlings received higher radiation than the understory seedlings, they were better able to establish during the spring before the onset of the summer drought, and as a result seedling mortality was higher in the understory plots than in the gap plots later in the summer.

Although the effects of shade were clear-cut, seedling responses to the partial alleviation of drought differed in the gap and understory plots. In the gaps, the effects of

the watering treatment on seedling physiology and water relations did not translate into a difference in growth or mortality. But in understory seedlings, diameter growth increased in response to the watering treatment, which is consistent with more severe drought stress in the understory than in the gap plots, as implied by the lower leaf water potentials and soil water content in the understory.

Effects of shade and drought on gas exchange

The maintenance of relatively constant mid-morning photosynthetic rates and high IWUE by the gap seedlings through July–August as summer drought intensified suggests that

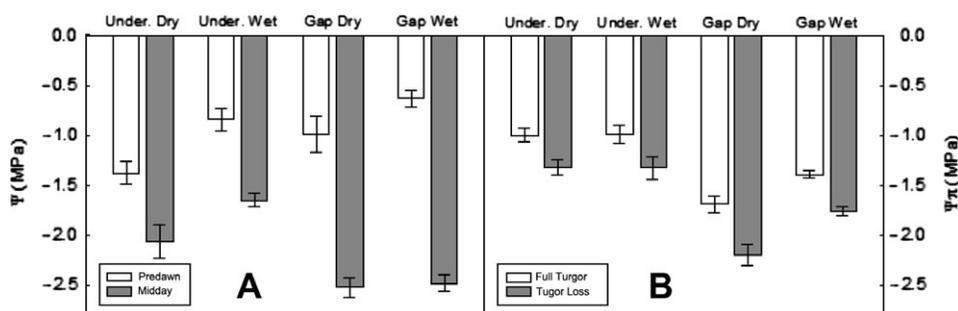


Figure 5. Plant water relations measured on August 28 on the same leaves as the gas exchange and chlorophyll fluorescence measurements. (A) Predawn water potential (Ψ_{pd}) (understory (U), $P < 0.0001$; dry (D), $P < 0.0001$; and $U \times D$, $P = 0.235$) and midday water potential (Ψ_{md}) (U, $P < 0.0001$; D, $P = 0.030$; and $U \times D$, $P = 0.069$). (B) Leaf water potential at full turgor ($\Psi\pi_{100}$) (U, $P < 0.001$; D, $P = 0.014$; and $U \times D$, $P = 0.050$) and leaf water potential at turgor loss ($\Psi\pi_0$) (U, $P < 0.0001$; D, $P = 0.014$; and $U \times D$, $P = 0.011$).

Table 4. Plant water relations measured on August 28 on the same leaves as gas exchange and chlorophyll fluorescence measurement. Relative water content at turgor loss (RWC⁰). Dry mass:full-turgor mass ratio (DM:TM). Apparent soil-leaf hydraulic conductance (K_L). Modulus of elasticity at maximum turgor (ϵ_{max}). Leaf $\delta^{13}C$ content measured as a proxy for WEU.

Treatment	RWC ⁰ (%)	DM:TM	K_L	ϵ_{max}	$\delta^{13}C$
Understory (U) + dry (D)	86 ± 2	0.357 ± 0.019	0.51 ± 0.08	3.04 ± 0.70	-30.6 ± 0.5
Understory + watered	84 ± 1	0.364 ± 0.016	1.44 ± 0.12	5.20 ± 0.61	-31.5 ± 0.3
Gap + dry	86 ± 1	0.479 ± 0.016	1.32 ± 0.15	7.77 ± 1.56	-28.8 ± 0.1
Gap + watered	89 ± 1	0.460 ± 0.009	1.78 ± 0.11	5.40 ± 0.72	-29.2 ± 0.1
<i>P</i> values					
(U) $F_{1,2}$	0.067	0.647	< 0.0001	0.007	< 0.0001
(D) $F_{1,4}$	0.568	< 0.0001	< 0.0001	0.980	0.023
(U × D) $F_{1,24}$	0.035	0.303	0.029	0.049	0.412

drought-tolerance mechanisms were relatively robust in these seedlings compared with those of the understory seedlings. Photoinhibition and activation of NPQ support our assertion that photosynthetic photon flux density (PPFD) exceeded seedling capacity for carbon assimilation rate in the gap plots. This was more evident in the dry subplots, where low water availability exacerbated stomatal closure in response to high VPD (Herbst 1995), and increased the susceptibility of plants to high radiation load. The effects of drought on fluorescence parameters in the gap seedlings were transient and only apparent in July, when the seedlings were still relatively small and both radiation and drought stress were high. Most photoinhibitory differences between the watered and dry subplots in the gaps were more apparent in July–August than in June, a similar response to that previously reported in beech saplings subjected to an imposed drought (Gallé and Feller 2007), and in desiccated seedlings (García-Plazaola and Becerril 2000). However, recovery of PSII by the morning in gap seedlings suggests that the water stress endured by gap seedlings was within the range where midday photoinhibition remained largely reversible (Tognetti et al. 1998).

Punctual IWUE was greater in the gap seedlings than in the understory seedlings across sampling dates, suggesting a greater potential for carbon assimilation despite high VPD and saturating PPFD in the gap seedlings than in the

understory seedlings, which struggled to maintain a positive carbon balance even though the VPD was lower in the understory plots and the seedlings showed no signs of photoinhibition. Both photosynthesis and g_{wv} were lower in unwatered seedlings, and although these effects were of similar magnitudes in the gap and understory seedlings, the consequences for overall seedling carbon assimilation were greater for the understory seedlings because of their lower baseline photosynthetic rates (Balandier et al. 2007), and their non-conservative water use even under water-limited conditions. As the summer drought intensified, a positive carbon balance allowed unwatered gap seedlings to maintain greater flexibility in osmoregulation and stomatal control of water loss than unwatered understory seedlings. These results support previous reports of reduced potential for photosynthesis in beech seedlings growing under combined shade and drought-stressed conditions (Tognetti et al. 1994, Aranda et al. 2004). The data also corroborate the declines in photosynthesis of adult trees attributed to drought based on inter annual comparisons (Aranda et al. 2001, Leuzinger et al. 2005, Löw et al. 2006).

Effects of shade and drought on leaf water relations

The threshold of -0.5 MPa Ψ_{pd} for optimal performance of beech in this forest (Aranda et al. 2004) was exceeded by seedlings in all treatments by the end of summer. The most

severe drought stress was suffered by unwatered understory seedlings, the Ψ_{pd} of watered understory seedlings was similar to that of unwatered gap seedlings but both these groups of seedlings were considerably more stressed than watered gap seedlings.

Predawn water potentials (Ψ_{pd}) were more negative in understory seedlings than in gap seedlings, reflecting slightly higher soil water content in the gaps because of lower canopy transpiration and less intense competition for belowground resources, but in both cases drought further lowered Ψ_{pd} . Leaf water potential values of less than -2.0 MPa can induce embolisms in beech seedlings (Hacke and Sauter 1995), and in our experiment seedling Ψ_{md} in all but the watered understory seedlings exceeded this threshold. Such low water potentials may put plants at risk of desiccation, especially the understory seedlings where August Ψ_{pd} reached similar values to the osmotic potential at turgor loss calculated from the $P-V$ curves. Although evaporative demand and transpiration were low in the understory, the very low soil water content may have caused higher tension in the xylem leading to embolisms in understory seedlings with a narrower safety margin for cavitation than gap seedlings (Cochard et al. 1999). In gaps, recovery of Ψ_{pd} values together with a higher apparent K_L may allow beech to compensate for high evaporative demand for water at midday, maintain photosynthesis, and withstand high pressure gradients in the xylem (Sack et al. 2003). Together with higher osmotic adjustment capacity, this more efficient hydraulic architecture would also contribute to the maintenance of turgor at more negative water potentials in gap seedlings than is possible in understory seedlings.

Adjustment of the modulus of elasticity at ϵ_{max} has been suggested as a potential drought-tolerance mechanism (Saito and Terashima 2004, Lenz et al. 2006). We found that ϵ responded to drought but in contrasting ways in the gap and understory seedlings. High ϵ in the unwatered gap seedlings indicates that relatively rigid cell walls, consistent with higher dry mass:full-turgor mass ratio (DM:TM), allowed cell water potential (Ψ) to drop quickly, consistent with a greater osmotic adjustment to maintain turgor. This would cause strong water potential gradients to establish within the xylem, as suggested by the Ψ differences in gap seedlings. In contrast, unwatered understory seedlings had lower ϵ than their watered counterparts. Lower maximum temperature and VPD in the shade reduce evaporative demand, and the imperative to maximize light capture by these understory seedlings is consistent with a strategy of high cell wall elasticity (low ϵ) which maintains cell function despite water loss (Lenz et al. 2006). This feature would contribute to a postponement of turgor loss despite impaired osmoregulation capacity under dry shade conditions (Aranda et al. 2001).

The greater depletion of leaf $\delta^{13}C$ in the understory seedlings compared with the gap seedlings reflects lower intercellular partial pressures of CO_2 in shade leaves operating

at a low photosynthetic rate, consistent with reported sun-shade differences in beech (Grams et al. 2007). The further depletion of leaf $\delta^{13}C$ in the watered seedlings compared with the unwatered seedlings agrees with other studies of drought-stress responses in beech (Peuke et al. 2006 and references therein). This effect of the watering treatment on the understory seedlings as well as the gap seedlings suggests that, even at low PPFD, seedlings must compromise their C assimilation through stomatal closure in response to drought. In the gap seedlings assimilation rate is sufficiently high to compensate for this closure, whereas in the understory seedlings this compromise could be critical.

Consequences for beech regeneration in sub-Mediterranean regions

Understory beech seedlings were unable to cope with severe drought. The consequences of a relatively moderate change in soil water content for beech seedling physiology have repercussions for the potential persistence of beech in sub-Mediterranean forests if the recurrence and intensity of seasonal drought increases in the future (IPCC 2007). The weather during the summer of 2007 caused a relatively mild drought compared with other recent years in this forest, and yet physiological responses of beech were translated into a reduction in growth in the understory seedlings. Although survival was not reduced, we would expect that if repeated summer droughts of a similar or greater magnitude were to continue or escalate the resultant impairment of physiology and growth would reduce the viability of regeneration. Furthermore, the niche available for beech at its south-western limit would become very narrow because of: (1) functional incompatibilities between shade and drought-tolerance strategies that may preclude beech establishment or development of a seedling bank in moderate shade (10–15% sunlight); and (2) increased competition from coexisting species, such as *Quercus petraea* (Matt.) Liebl and *Quercus pyrenaica* Willd., which are better able to exploit canopy gaps under drought conditions (Rodríguez-Calcerrada et al. 2007).

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