Differences in the leaf functional traits of six beech (*Fagus sylvatica* L.) populations are reflected in their response to water limitation

David Sánchez-Gómez [a,*], T. Matthew Robson [b], Antonio Gascó [a,c], Eustaquio Gil-Pelegrín [d], Ismael Aranda [a,*]

[a] Centro de Investigaciones Forestales (CIFOR), Instituto Nacional de Investigaciones Agrarias (INIA), Carretera de la Coruña km 7,5, 28040 Madrid, Spain
[b] University of Helsinki, Department of Biological and Environmental Sciences, FIN-00014 Helsinki, Finland
[c] IE University, Bachelor in Biology, Segovia Campus, Cardenal Zúñiga 12, 40003 Segovia, Spain
[d] Centro de Investigación y Tecnología Agroalimentaria, Unidad de Recursos Forestales, Zaragoza 50080, Spain

ABSTRACT

Patterns of intraspecific variation in functional traits have been widely studied across plant species to find out what general suites of traits provide functional advantage under specific environmental conditions. Much less is known about this variation within tree species and, in particular, about its relationship with performance variables such as photosynthetic rates under water deficit. Nevertheless, this knowledge is fundamental to understand the adaptive potential of drought sensitive tree species to increased aridity as predicted in the context of climate change.

Intraspecific variation in photosynthetic performance and other leaf functional traits in response to water availability were examined in a glasshouse experiment using seedlings of six European beech populations. The physiological response of seedlings to a "water stress" treatment was compared to a "control" treatment along an experimental cycle of progressive soil water deficit and recovery. We found evidence of intraspecific variation in beech's photosynthetic performance and other leaf functional traits in response to water availability. We also detected intraspecific variation in leaf-level tolerance of water deficit and phenotypic plasticity to water availability suggesting a pattern shaped by both regional and local scale effects. The Swedish population was particularly sensitive to water deficit, being the only population showing impaired photochemical efficiency under the experimental water deficit. Leaf-level tolerance of water deficit was related to PNUE, but not to other functional traits, such as WUE, SLA or leaf nitrogen content, that have been described to vary across species in adaptation to drought tolerance. Our results support the idea that general trends for variation in functional traits across species do not necessarily reflect a similar pattern when observed at the intraspecific level. The observed functional variation between beech populations reaffirms the importance of local adaptation to water deficit in the context of climate change.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

The study of patterns of variation in plant functional traits along environmental and resource gradients is fundamental to understand ecological (Grubb, 1977; Silvertown, 2004; Westoby and Wright, 2006) and evolutionary processes (Ackerly et al., 2000). Most studies of variation in functional traits have been focused on interspecific rather than intraspecific differences (Fajardo and Piper, 2010). However, an increasing number of studies, since the pioneering work of Mooney and Billings (1961), have highlighted the ecological importance of intraspecific variation in functional traits of forest tree species (Arntz and Delph, 2001; Benowicz et al., 2000; Brendel et al., 2008).

Intraspecific phenotypic variation across the geographical distribution range of a species can result from phenotypic plasticity,

i.e. the property of individual genotypes to produce different phenotypes when exposed to different environmental conditions (Pigliucci et al., 2006), genotypic variation (i.e. differences in the genotype between individuals), or both. Stochastic processes, such as mutation and genetic drift, can affect genetic variation within species. However, it is generally accepted that selection is the main process driving local adaptation, reflected as genetic variation among populations occurring in divergent environments (Hereford and Winn, 2008).

Water availability is a key environmental factor limiting plant photosynthesis, growth (Flexas et al., 2002) and the regeneration of trees and shrubs (Pigott and Pigott, 1993). Thus, intraspecific variation in key functional traits is expected to occur along water availability gradients (Benowicz et al., 2000; Martin et al., 2007; Martínez-Vilalta et al., 2009). As a consequence of climate change, the frequency and severity of droughts are expected to increase in Europe, especially in summers (IPCC, 2007). In this context, the study of intraspecific variation in response to water availability is important to understand the physiological mechanisms underlying variation of drought tolerance within species and a better understanding of the potential consequences of environmental change. Overall, this knowledge is fundamental to the development of scientifically-sound forest management and conservation programmes.

European beech (*Fagus sylvatica* L.) is a widespread tree species that dominates the canopy of many forests throughout its natural distribution range in Europe. Beech is a drought sensitive species (Ciais et al., 2005; Ellenberg, 1992; Rose et al., 2009) which, in the Mediterranean basin, is confined to mountain ranges where precipitation is high (Garcia-Plazaola and Becerril, 2000; Tognetti et al., 1995). Nonetheless, in this region, beech still withstands moderate seasonal summer drought, typical of Mediterranean climates (Aranda et al., 2001; Fotelli et al., 2009). Previous studies have found that certain morphological and physiological responses to water deficit can vary among beech genotypes (Bresson et al., 2011; Meier and Leuschner, 2008; Nielsen and Jorgensen, 2003; Peuke et al., 2006; Wortemann et al., 2011). Furthermore, there is some evidence that support for a trend of decreasing drought tolerance with latitude (Fotelli et al., 2009; Robson et al., 2012). However, the way in which the variation and interplay of all these morpho-physiological traits affect photosynthetic capacity is not well understood. A reason for this stems from the difficulty of detecting potential intraspecific variation in highly dynamic gas exchange variables. In fact, despite inter-population variation in beech's stomatal conductance (Leverenz et al., 1999), only marginally-significant (Leverenz et al., 1999; Tognetti et al., 1995) or not significant differences (Bresson et al., 2011) in photosynthetic rates have been detected among populations of this species.

In this study, we investigated the variation of key leaf traits and gas exchange variables in response to water availability in one-year seedlings of six beech populations. These populations were selected so they covered the latitudinal range of the species in Europe to account for different genetic pools (Magri et al., 2006) and a range in macroclimatic conditions (Mediterranean, continental, and oceanic climates). We also considered accounting for microclimatic variation within the central continental region so we included several populations from this region (see Table 1 for climatic and location details of the studied populations).

We focused on seedlings because tree species are most vulnerable to environmental constraints at this stage (Harper, 1977; Silvertown and Charlesworth, 2001) and thus, trees experience the highest selective pressure as seedlings (Reich et al., 2003).

The objectives of this study were the following: (a) to assess differential tolerance to water shortage at the leaf level among beech populations and (b) to find the main traits underlying the observed inter-population variation in leaf-level tolerance to water shortage.

2. Materials and methods

2.1. Plant material, experimental design and microclimatic conditions

Beech seeds from the studied European populations were collected during autumn, 2009. The seeds were cold stratified at 4 °C for 10 weeks. After stratification, most of the seeds began to germinate. They were sown in pots once the radicle reached 1–2 cm length. The pots were filled with 1.2 l of a 3:1 volume mixture of peat Floragard TKS2 (Floragard Vertriebs gmbh, Oldenburg, Germany) and washed river sand. This mixture was supplemented with 2 kg m^{-3} of Osmocote Plus fertilizer (16-9-12 NPK+2 micronutrients, Scotts, Heerlen, the Netherlands). The pots were moved to a greenhouse and watered regularly. After three weeks, 50 seedlings per population were selected within a height range of 7–10 cm. A total of 300 seedlings were used in the experiment. The experimental layout was based on a factorial design with two factors: population and water availability. Two levels were established for water availability: "control" and "water deficit": half of the seedlings (25 per population) were randomly assigned to the "control" treatment and the other half was assigned to the "water deficit" treatment. The spatial distribution of the seedlings on the bench was optimized for a row-column design (15 × 20), which included the two studied factors (population and watering treatment). The software CycdesigN 3.0 (CycSoftware Ltd., Ranfurly, New Zealand) was used for this purpose. The greenhouse received natural light, temperatures and relative humidity, which varied on a daily and seasonal basis. Temperatures and relative humidity in the greenhouse were controlled within ranges close to the ambient conditions outside using cooling, heating and misting systems. Average minimum and maximum temperatures throughout the experiment were: 18.8 ± 3.1 °C and 32.5 ± 4.0 °C respectively (mean ± standard deviation provided). Average minimum relative humidity throughout the experiment was $66.6 \pm 3.8\%$. Average daily PPFD values ranged from 353 to 454 μmol m^{-2} s^{-1} throughout the experiment

2.2. Watering treatment

Seedlings assigned to the "control" treatment were watered to field capacity regularly during the whole experiment. At the beginning of the experiment (the experiment started on Julian day 112) the "control" plants were watered every 5 days. The frequency of the watering was increased as seedlings grew. At the end of the experiment, the "control" plants were watered every second day. Seedlings assigned to "water deficit" treatment were subjected to a cycle of water shortage and later recovery. This cycle had four stages with different watering protocols. During the first stage (Julian days: 112–150), these seedlings were watered to field capacity just like the "control". During the second stage (Julian days: 151–178), seedlings under "water deficit" were allowed to deplete soil water content down to a target soil volumetric water content (VWC$_s$) of 15 vol.% During the third stage (Julian days: 179–200), these seedlings were allowed to deplete soil water content down to a lower target of 13 vol.% Finally, during the fourth stage (Julian days: 201–248) seedlings were again watered to field capacity like the "control".

VWC$_s$ was individually monitored throughout the experiment with time domain reflectometry, TDR (TRIME-FM, Imko Micromodultechnik GMBH, Ettlingen, Germany). VWCs in "control" treatment for every single pot was measured once a week just before a watering event to ensure that the watering schedule kept VWC$_s$ close to 30 vol.%. VWC$_s$, of seedlings receiving the "water deficit" treatment was measured in the same way as the "control" treatment during first and fourth stages, however it was measured every other day

Table 1
Location and climatic details of the studied populations. The soil characteristics have been obtained from the ESDB v2 – 1 km × 1 km Raster Library (Panagos, 2006; Panagos et al., 2012; Van Liedekerke et al., 2006).

Population code	Sp	I	G2	G3	G1	Sw
Country	Spain	Italy	Germany	Germany	Germany	Sweden
Location	Madrid	Belluno (Province)	Kempten	Illertissen	Ingolstadt	Falkenberg
Latitude	42°01′	46°02′	47°44′	48°11′	48°56′	56°52′
Longitude	3°05′	12°23′	10°23′	10°11′	11°25′	12°51′
Altitude (m a.s.l)	1325	1130	880	560	525	150
Precipitation (mm)	1000	1800	1316	885	686	900
Average temperature (°C)	8.1	10.5	6.9	8.0	7.8	7.0
FAO classification	Humic cambisol	Rendzina	Orthic luvisol	Eutric cambisol	Orthic rendzina	Orthic podzol
Texture	Medium	Medium	Medium	Medium	Medium	Medium
Topsoil easily available water capacity	High	High	High	Medium	Very high	High
Subsoil easily available water capacity	Very low	High	High	High	Very low	High

during the second and third stages. The following protocol was carried out at these stages to keep water availability as homogeneous as possible within the "water deficit" treatment, and to compensate for individual differences in water consumption rates. First, decreasing target values for VWC_s were established beforehand: 25, 20, 17 and 15 vol.% VWC_s and pot (including the whole unit: pot, soil and seedling) weights were measured every other day. VWC_s and pot weights were simultaneously measured so we could individually relate VWC_s to pot weight in order to estimate the expected pot weight for a given VWC_s with linear regressions. Since measurements of VWC_s and pot weights were intensively taken along the experiment, we were continuously updating these regressions to account for plant growth. A weight scale DIBAL C-120 (DIBAL S.A. Derio, Bilbao, Spain) was used to measure pot weights. Water consumption rate between two consecutive measurement points was calculated for each seedling as:

$$WC_{i(n-1,n)} = S_{i(n-1)} + W_{i(n-1)} - W_{in} \tag{1}$$

where $WC_{i(n-1,n)}$ is the water consumption (units in grams, g) of seedling i between two consecutive measurement points defined by the interval $(n-1, n)$. This interval was always two days. $S_{i(n-1)}$ is the water added (g) to seedling i at the measurement point $n-1$, and W_{in} is the pot weight (g) for seedling i at measurement point n. The quantity of water to add to each seedling was calculated as:

$$S_{in} = W_{ti} + WC_{i(n-1,n)} - W_{in} \tag{2}$$

where S_{in} is the quantity of water (g) that needs to be supplemented to seedling i at measurement point n, and W_{ti} is the expected pot weight for seedling i when the target VWC_s is reached. If $S_{in} \leq 0$, the seedling is not watered. If $S_{in} > 0$, the seedling is watered with the quantity of water indicated by the value of S_{in}.

The following lower target was established when all the seedlings reached the first target of 25 vol.% Seedlings depleted soil water quickly. They all reached the final target of 15 vol.% in 12 ± 4 days.

During the third stage, the protocol was the same as previously described. The decreasing target values that were established for VWC_s at this stage were 14 and 13 vol.% All the seedlings reached the final target of 13 vol.% during the third stage in 5 ± 3 days. Measuring cylinders and syringes were used to water the seedlings assigned to "water deficit" treatment at the second and third stages.

2.3. Measurements and studied variables

At the end of the first stage, length (mm) and diameter (mm) of the main stem (at the root collar) and the base of all the branches of each seedling were measured. A ruler (±1 mm) was used to measure lengths while a digital calliper (±0.01 mm) was used to measure diameters. Initial size for each seedling was estimated using the volume of a cylinder as:

$$\sum_{i=1}^{n} \frac{\pi d_i^2 h_i}{4} \tag{3}$$

where π is the number Pi, d_i is diameter of the branch or stem i and h_i is length of the branch or stem i. The total number of branches of the seedling is n.

Gas exchange measurements were carried out at five time points throughout the experiment (T_1, T_2, T_3, T_4, and T_5) in seedlings of both "control" and "water deficit" treatments. The first one, T_1, corresponded to the end of the first stage where both "control" and "water deficit" treatments were regularly watered to field capacity. T_2 corresponded to the end of the second stage, that is, a water deficit defined by a VWC_s of 15 vol.% for seedlings assigned to "water deficit". T_3 corresponded to the end of the third stage and peak of water deficit for seedlings at "water deficit" treatment. T_4 corresponded to the first gas exchange measurement after recovery (13 days after the beginning of fourth stage). T_5 corresponded to the second gas exchange measurement after recovery (48 days after the beginning of the fourth stage). Gas exchange and chlorophyll fluorescence measurements were made on attached leaves. At each measurement time, we chose the most apical fully expanded leaf without repeating the same leaves as those used earlier. The measurements were carried out with a Li-Cor 6400 portable photosynthesis system (Li-Cor, Inc., NE, USA). The leaves were exposed to a controlled CO_2 concentration of 400 ppm using the built-in Li-Cor 6400-01 CO_2 mixer (Li-Cor, Inc.), a controlled PPFD of 800 μmol m² s^{-1}, using the Li-Cor 6400-40 fluorescence chamber (Li-Cor, Inc.), a temperature of 24 °C and a RH of 60–65%. Measurements for each time point were taken from 10 am to 1 pm throughout 4 consecutive days to complete the total 300 seedlings on each time point. Area-based maximum photosynthetic rate (A_{max}) and stomatal conductance (g_s) were obtained from gas exchange measurements while effective quantum efficiency of PSII (Φ_{PSII}) was obtained from chlorophyll fluorescence measurements at 800 μmol m^{-2} s^{-1} as:

$$\Phi_{PSII} = \frac{F_{m'} - F_s}{F_{m'}} \tag{4}$$

where F_m' is the light-adapted maximum fluorescence and F_s is "steady-state" fluorescence or fluorescence before a saturating light pulse (Genty et al., 1989).

TDR measurements for each seedling were also taken right after gas exchange and chlorophyll fluorescence. Additionally, predawn leaf water potentials were measured at the peak of water deficit (T_3) in one fully expanded leaf nearest to the one chosen for gas exchange and chlorophyll fluorescence measurements. Water potentials were measured with a pressure chamber (PMS

Instrument Co. 7000, Corvallis, OR, USA) following Scholander et al. (1965).

The Li-Cor 6400 at T_3 were harvested after measurement. The leaves were digitally scanned and leaf area (cm^2) was calculated with WINFOLIA v. 2002 (Régent, Quebec, Canada). Afterwards, the leaves were oven-dried at 65 °C for 2 days and weighed for dry mass determination. The dry leaves were grounded with a ball mill. This leaf powder was used to determine mass-based nitrogen content (N_m, g g^{-1}) by the Kjeldahl method (Vapodest 50, Gerhardt) and ^{12}C and ^{13}C abundances using a Micromass Isochrom mass spectrometer. Carbon isotope composition (δ^{13}C, $^0/_{00}$) was obtained according to the following expression:

$$\delta^{13}C = \left(\frac{Rs}{Rb} - 1 \right) \times 1000 \tag{5}$$

where Rs and Rb refer to the ^{12}C/^{13}C isotope ratio in the sample and the Pee Dee belemnite standard respectively. This method had a precision of ± 0.1 $^0/_{00}$.

In addition to the variables already mentioned (e.g. A_{max}, g_s, Φ_{PSII}, δ^{13}C, N_m), the following derived variables were also estimated: specific leaf area (SLA, m^2 kg^{-1}), area-based nitrogen content (N_a, g m^{-2}), mass-based maximum photosynthetic rate (A_{mmax}, μmol g^{-1} s^{-1}) photosynthetic nitrogen-use efficiency (PNUE, μmol g^{-1} s^{-1} as A_{mmax}/N_m) and instantaneous water-use efficiency (WUE$_i$, μmol mol^{-1} as A_{max}/g_s). The number of sampling data for each analysed trait was 300 (25 replicates × 6 populations × 2 water treatments).

2.4. Data analyses

A general linear model (GLM) was used to test for the effect of population, water treatment and "time" (repeated measures factor) on the studied photosynthetic variables (A_{max}, g_s and Φ_{PSII}). In addition to the main effects of these variables, a covariate (initial size) and the interaction term between populations and water treatment ($P \times T$) were also included in the model. Shapiro–Wilk's and Levene's tests were used to test for normality and homogeneity of variances respectively. Stomatal conductance (g_s) was log-transformed to meet the assumptions of normality and homoscedasticity. Additional GLM were fitted to test for the effects of population (P), water treatment (T), covariate (initial size) and interaction $P \times T$ on the other studied response variables (SLA, N_m, N_a, δ^{13}C, PNUE, A_{mmax}, WUE$_i$) at T_3. These variables were log-transformed when necessary to meet the assumptions of normality and homoscedasticity.

Separated log–log regression models of A_{max}, g_s and Φ_{PSII} on soil water content and initial size were fitted for each population. The aim of these models was to describe in detail the covariation of these response variables at the peak of water deficit (T_3) with respect to water availability (as a continuous variable) after removing the effect of initial seedling size. The regression coefficient from
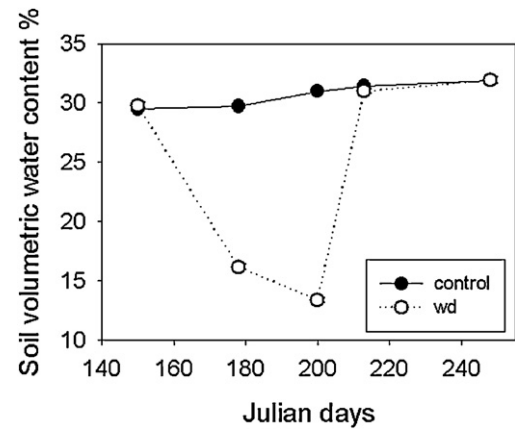

Fig. 1. The pattern of water availability through time for each treatment level: control and water deficit (wd). Populations were pooled together. Error bars denote standard error. Error bars smaller than symbols' size cannot be seen. Sample size was 150.

these models for water availability provides an estimate of the sensitivity of these photosynthetic variables to soil water depletion for each population.

Finally, a principal components analysis (PCA) was performed separately for the "control" and the "water deficit" treatments at T_3. All the studied variables were included in the analysis which aimed to identify homogeneous groups among the populations for the studied variables and find out which variables explained most of the observed variation of the data. A varimax rotation was applied to maximize the variation of factor loadings and help the interpretation of each principal component. STATISTICA v. 6.0 (Statsoft Inc., Tulsa, OK, USA) was used for the analyses.

3. Results

Soil volumetric water content VWC$_s$ varied throughout the experiment (Fig. 1). However, VWC$_s$ did not differ significantly among populations (repeated measures ANOVA, population effect: $F(2,286) = 1.83$, $p = 0.107$). The lowest VWC$_s$ (13.39 ± 2.27 vol.%, mean ± standard deviation) was reached by seedlings at "water deficit" treatment at T_3. This value corresponded to a predawn leaf water potential of -0.46 ± 0.21 MPa.

The repeated measures factor ("time") was significant for stomatal conductance (g_s) and effective quantum efficiency of PSII (Φ_{PSII}). The effect of initial size was significant for area-based maximum photosynthetic rate (A_{max}) and Φ_{PSII} (Table 2). The variable A_{max} significantly differed among populations and between water availability levels. Besides, the effect of water treatment on A_{max} differed among populations as indicated by the significant interaction term $P \times T$ (Table 2). The effect of water deficit on A_{max} was detected for most populations from T_2 onwards (Fig. 2). The

Table 2
F-Fisher values and significance levels obtained by General Linear Models, GLM analysis. This analysis was performed for the whole period of the experiment. Area-based maximum photosynthetic rate (A_{max}), stomatal conductance (g_s) and effective quantum efficiency of PSII (Φ_{PSII}) were the dependent variables analyzed. "Time" was the repeated measures factor and "Size" was the covariate effect corresponding to initial size. The interaction term between "Population" and "Treatment" ($P \times T$) was included in the model. The degrees of freedom for the F-values are provided in brackets.

Variable	Factor/covariate				
	Time	Size	Population (P)	Treatment (T)	$P \times T$
A_{max}	(4/1088) 2.11	(1/272) 4.98[*]	(5/272) 8.20[***]	(1/272) 15.22[***]	(5/272) 3.37[***]
g_s	(4/1088) 31.76[***]	(1/272) 0.39	(5/272) 3.54[**]	(1/272) 63.14[****]	(5/272) 2.01
Φ_{PSII}	(4/1088) 20.55[***]	(1/272) 10.30[**]	(5/272) 12.26[***]	(1/272) 1.58	(5/272) 1.22

[*] $0.01 \leq P < 0.05$.
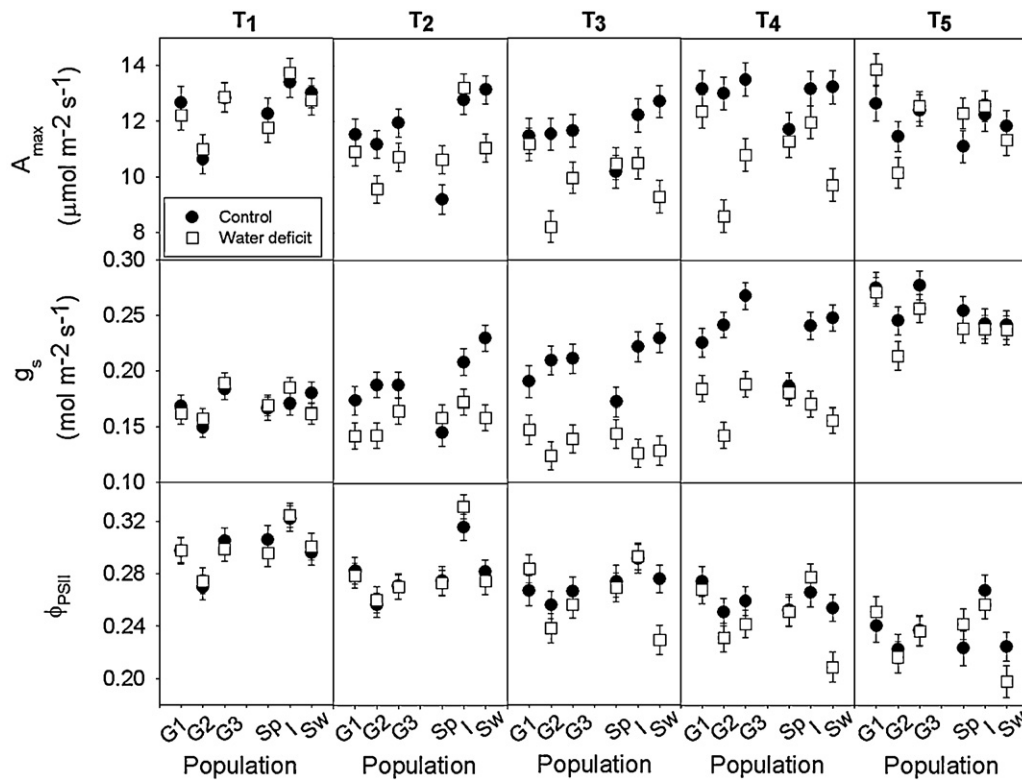[**] $0.001 \leq P < 0.01$.
[***] $P < 0.001$.

Fig. 2. Area-based maximum photosynthetic rate (A_{max}), stomatal conductance (g_s) and effective quantum efficiency of PSII (Φ_{PSII}) for each population and treatment level ("control" and "water deficit") at different measurement points during the experiment. T_1 is the initial time point where regular watering to field capacity was applied for both "control" and "water deficit" treatments. It corresponded to Julian day 150. T_2 is the first measurement under water deficit for seedlings at "water deficit" treatment. It corresponded to Julian day 178. T_3 is the second measurement under water deficit for seedlings at "water deficit" treatment. It corresponded to Julian day 200 and peak of water deficit. T_4 is the first measurement after recovery for seedlings at "water deficit" treatment. It corresponded to Julian day 213. T_5 is the second measurement after recovery for seedlings at "water deficit" treatment". It corresponded to Julian day 248. The covariate effect "initial size" was removed computing the values for initial size at its mean (GLM analysis). Error bars denote standard error. Sample size was 23–25.

variable g_s significantly differed among populations and between water availability levels, but the interaction term $P \times T$ was not significant (Table 2). As found for A_{max}, the effect of water deficit on g_s was noticeable for most populations from T_2 onwards. Φ_{PSII} significantly differed among populations but not between water availability levels (Table 2). However, Φ_{PSII} was lower in the "water deficit" treatment than in the "control" for $G2$, $G3$ and Sw (see Table 1 for population codes) at the peak of water deficit (T_3, see Fig. 2). No recovery had occurred after 13 days following the peak of water deficit for any of the three photosynthetic variables. But 48 days after the end of T_3, A_{max} recovered completely except for $G2$ (Fig. 2). The variable g_s recovered but not completely since seedlings in "water deficit" treatment consistently showed lower values than those in the "control" for every population. Again, $G2$ was the population with the worst recovery in terms of g_s (Fig. 2). Φ_{PSII} completely recovered except for Sw which maintained lower values in the "water deficit" treatment than in the "control" (Fig. 2).

The effect of "water deficit" was significant on all the rest of the physiological variables at the peak of water deficit (T_3, see Table 4). There was a population effect on SLA, N_m, N_a, $\delta^{13}C$, PNUE and WUE$_i$ but not A_{mmax}. However, there was a significant interaction effect between population and treatment ($P \times T$) on this variable as well as on $\delta^{13}C$, PNUE and WUE$_i$, (Table 4). SLA was lower under "water deficit" than under "control". I and $G1$ were the populations with the lowest SLA values under "water deficit" while Sw and $G2$ reached the highest values under this treatment (Table 5). In general, N_m was higher under "water deficit" than under "control". Sp population had the lowest N_m values under "water deficit" while Sw had the highest values (Table 5). N_a was higher under "water deficit" than under "control". Sp had the lowest N_a values under "water

deficit" while I and $G1$ reached the highest N_a values (Table 5). All the studied populations had higher $\delta^{13}C$ values under "water deficit" than under "control". Sp and I populations had the lowest $\delta^{13}C$ values under "water deficit" while $G1$ and Sw populations had the highest values (Table 5). The impact of the water treatment (the difference between mean values in "water deficit" and "control" computed with data from Table 5) on $\delta^{13}C$ was highest for Sw and $G2$ and lowest for Sp and I. PNUE was lower under "water deficit" than under "control" for all the studied populations. Sp population had the highest PNUE values under "water deficit" while $G2$, Sw and I had the lowest values (Table 5). The impact of water treatment on PNUE was highest for I and Sw and lowest for $G1$ and Sp. In general, A_{mmax} was lower under "water deficit" than under "control" for the studied populations. Sp and $G1$ had the highest A_{mmax} values under "water deficit" while $G2$ had the lowest values (Table 5). The impact of water treatment on A_{mmax} was highest for I and Sw and lowest for $G1$ and Sp. WUE$_i$ was higher under "water deficit" than under "control". I and $G1$ had the highest WUE$_i$ under "water deficit" while $G2$ had the lowest values (Table 5). The impact of water treatment on WUE$_i$ was highest for I and Sw and lowest for $G2$ and $G1$.

Principal Components Analyses (PCAs) were performed for the data at the peak of water deficit (T_3). Two separate analyses were made, one for the "control" treatment and the other for the "water deficit" treatment. Three principal components were extracted for both PCAs. Eigenvalues for PC 1, 2 and 3 were 3.7, 2.1 and 1.4 respectively for the "control" treatment and 4.1, 2.4 and 1.2 respectively for the "water deficit" treatment. The first component (PC 1) explained ca. 35% of variance in both PCAs. The second component (PC 2) explained ca. 20% of variance and the third component (PC 3) explained ca. 11% of variance (see Fig. 3 for details). For the "control"

Table 3

Coefficient values (mean ± standard error) for log–log regressions performed separately for each photosynthetic variable and population at the peak of water deficit (T_3). The independent variables included in the model were VWC_s at the measuring time, initial size and the interaction term between VWC_s and initial size. The corresponding coefficients for these independent variables were coded as "W", "S" and "$W \times S$" respectively. Dashed lines denote that the estimates were not significantly different from 0.

A_a

Population	Intercept	W	S	W × S	p-value
G1	1.04 ± 0.02	–	–	–	<0.001
G2	0.38 ± 0.14	0.44 ± 0.11	–	–	<0.001
G3	–	0.19 ± 0.08	0.19 ± 0.06	–	0.003
Sp	0.98 ± 0.02	–	–	–	<0.001
I	0.79 ± 0.11	0.19 ± 0.09	–	–	0.024
Sw	0.53 ± 0.11	0.37 ± 0.08	–	–	<0.001

g_s

Population	Intercept	W	S	W × S	p-value
G1	−1.26 ± 0.19	0.34 ± 0.15	–	–	0.026
G2	−1.60 ± 0.18	0.59 ± 0.13	–	–	<0.001
G3	−1.96 ± 0.27	0.49 ± 0.11	0.21 ± 0.09	–	<0.001
Sp	−1.17 ± 0.17	0.26 ± 0.13	–	–	0.041
I	−1.71 ± 0.13	0.69 ± 0.09	–	–	<0.001
Sw	−1.64 ± 0.13	0.66 ± 0.1	–	–	<0.001

Φ_{PSII}

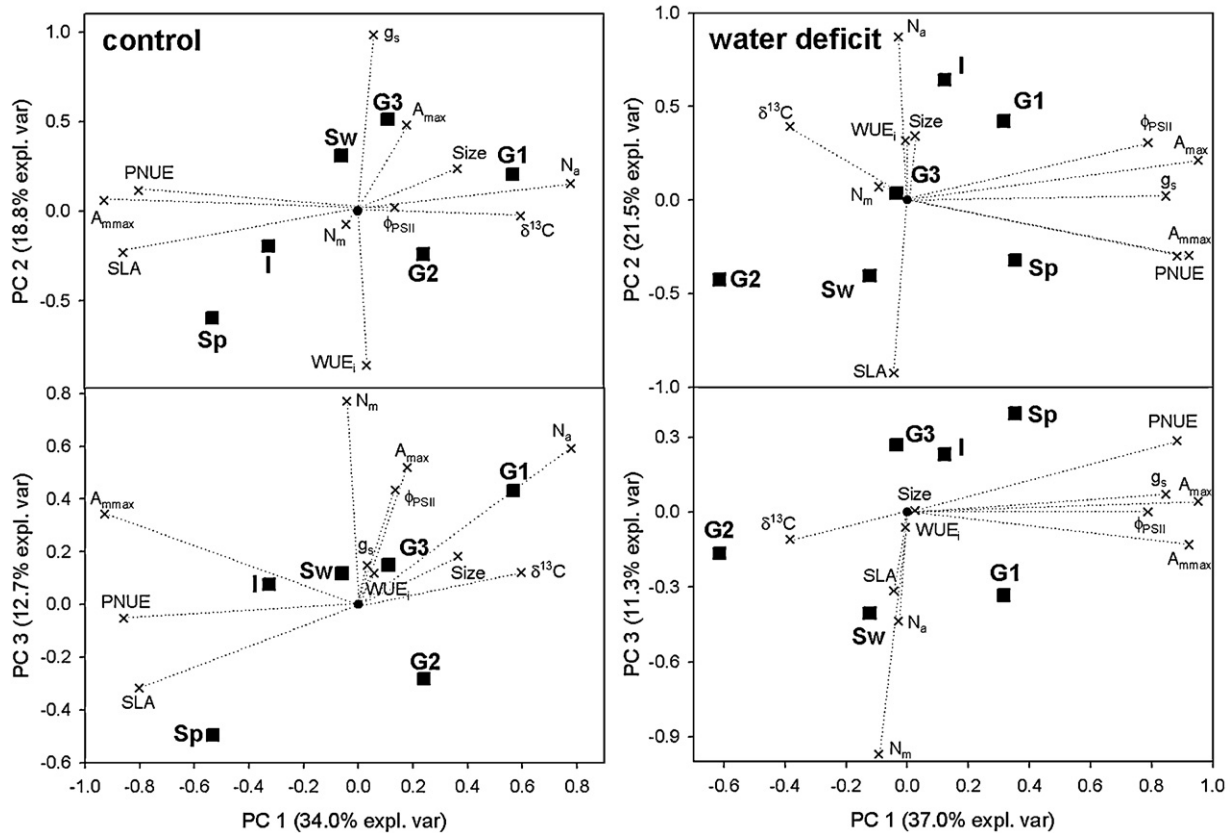| Population	Intercept	W	S	W × S	p-value
G1	−0.55 ± 0.01	–	–	–	<0.001
G2	−1.02 ± 0.16	–	0.17 ± 0.07	–	0.016
G3	−0.92 ± 0.11	–	0.14 ± 0.04	–	0.003
Sp	−0.59 ± 0.01	–	–	–	<0.001
I	−0.54 ± 0.01	–	–	–	<0.001
Sw	−0.81 ± 0.09	0.16 ± 0.07	–	–	0.03

Fig. 3. Coordinates of the variables (X) and populations (⌢) on the plane defined by the three principal components (PCs) extracted by PCA. The data correspond to time period T_3 (peak of water deficit). The variance explained by each PC is given on the axis label. The variables with the higher loadings for the control group were: A_{mmax}, SLA, N_a, $\delta^{13}C$ for PC 1, g_s and WUE_i for PC 2 and N_m for PC 3. The variables with the higher loadings for the water deficit group were: A_{max}, A_{mmax}, PNUE for PC 1, SLA and N_a for PC 2 and N_m for PC 3. The lower graphs describe PC 1 vs PC 3 while the upper graphs describe PC 1 vs PC 2.

treatment, PC 1 was positively correlated to N_a and $\delta^{13}C$ and negatively correlated to A_{mmax} and SLA. PC 2 was positively correlated to g_s and negatively correlated to WUE$_i$. PC 3 was positively correlated to N_m and negatively correlated to SLA. For the "water deficit" group, PC 1 was positively correlated to A_{max}, A_{mmax}, PNUE, g_s and Φ_{PSII} and negatively correlated to $\delta^{13}C$. PC 2 was positively correlated to N_a and negatively correlated to SLA. PC 3 was positively correlated to PNUE and negatively correlated to N_m (Fig. 3).

For the "control" group, the German populations had the highest scores on PC1. They were defined by low SLA, A_{mmax} and PNUE values but high N_a and $\delta^{13}C$ values. In contrast, Sp and I had the lowest scores on this axis with the inverse pattern for these variables. Sw had intermediate scores on this PC1. For the "water deficit" group, the ordination of populations along the PC axes changed. G1 and Sp had the highest scores on PC 1. They had high PNUE, A_{mmax}, A_{max} and g_s. G2 and Sw had the lowest scores and the inverse pattern for these variables. Although G1 and Sp had high performance under "water deficit" for the studied photosynthetic variables, they differed in SLA and N_a according to PC 2, Sp had higher SLA and lower N_a than G1 (Fig. 3).

Log-log models relating photosynthetic variables (A_n, g_s, and Φ_{PSII}) with soil volumetric water content (VWC$_s$) and initial size at T_3 were formulated and parameterized separately for each population. The fitted value of the coefficient describing the effect of soil volumetric water content (coded as W in Table 3) within each of these regression models provides an estimate of the sensitivity of each photosynthetic variable to soil water depletion since it describes the degree of variation of the variable per unit of variation of VWCs. For A_n, the populations G2 and Sw had the highest "W" values (Table 3), and consequently the highest sensitivity of A_n to water deficit. In contrast, G1 and Sp had the lowest "W" values (this coefficient was not significantly different from zero). The ordination of populations along PC 1 was inversely correlated to "W" (Pearson's $R^2 = 0.87$, $p = 0.006$). The variables with the higher weights on PC 1 (photosynthetic variables and PNUE) were consequently significantly correlated to "W" (data not shown). For g_s, I and Sw had the highest values while Sp and G1 had the lowest values. For Φ_{PSII} Sw was the only population that showed sensitivity to water depletion. The rest of populations did not change Φ_{PSII} in response to water availability (Table 3).

4. Discussion

4.1. Intraspecific variation in photosynthetic performance

In this study we found empirical evidence of inter-population variation in the area-based maximum photosynthetic rate (A_{max}), stomatal conductance (g_s) and effective quantum efficiency of PSII (Φ_{PSII}) of European beech. For A_{max} and g_s both genotypic variation and phenotypic plasticity contributed to the observed variation, while for Φ_{PSII} genotypic variation was the main source of variation. Previous recent studies accounting for a relatively high number of beech populations, have not found significant inter-population variation in either photosynthetic performance (Bresson et al., 2011) or cavitation resistance (Wortemann et al., 2011), however, inter-population variation in photosynthetic performance has been identified in other species and it has been related to general patterns of geographical variation in those species (e.g. Benowicz et al., 2000; Soolanayakanahally et al., 2009). In this study we found inter-population variation in photosynthetic performance under well watered conditions but it did not support the existence of a clinal pattern for photosynthetic performance in beech. In contrast, under water deficit we found a completely different result. At first glance, a latitudinal pattern was detected so that the southernmost populations (Spanish and Italian) were the least

sensitive populations to water availability and had the highest area-based maximum photosynthetic rates under water deficit while the northernmost population (Swedish) showed the reverse pattern. At similar latitudes, (subgroup of German populations) there was a direct relationship between sensitivity to water deficit and assimilation rates with average annual rainfall at the sites of origin. In general, the populations with higher photosynthetic performance under water deficit came also from sites with soils of lower water holding capacity. Thus, for beech seedlings, both latitudinal changes in climate at a regional scale and variation in rainfall and soil water holding capacity at a local scale appeared to be linked to inter-population variation in leaf-level tolerance to water deficit.

At the individual level, stomatal control is considered the primary short term mechanism "used" by plants under decreasing water availability to down regulate water use, with the concomitant decrease in photosynthetic rates (Chaves, 1991). Non-stomatal-limitations would not occur except under very severe droughts conditions (Flexas and Medrano, 2002). The significant effect of water availability on g_s and the non-significant effect of water availability on Φ_{PSII} found in this study agree with these expectations. However, just as the threshold for triggering some non-stomatal limitations related to alterations in photochemistry may vary depending on the species (Peguero-Pina et al., 2009), it may also vary among populations within a species, as found in this study. While the photochemistry was not affected by water availability in most of the studied populations, the effective quantum efficiency of PSII decreased in the Swedish population as water became more limited, besides it did not completely return to control values even after the whole recovery period, denoting some kind of permanent or long-lasting effect of water deficit on the photochemical machinery of this population.

4.2. Variability in other functional traits and their relationship with tolerance to water deficit

Under non-limiting water, the pattern of variation of the studied functional traits conformed to a regional trend with the southernmost populations (Spanish and Italian) forming one group clearly distinct from the German cluster and the Swedish population. However, under water deficit this pattern changed and the degree of similarity among populations did not reflect a regional cline but rather the sensitivity of these populations to water deficit, which, as discussed earlier, conformed to a more complex pattern of variation. Thus, intraspecific variation in functional traits depended strongly on the environmental conditions under which they were evaluated.

Under water deficit, SLA, PNUE, and A_{mmax} decreased as compared to the control. However, nitrogen content (both N_a and N_m), $\delta^{13}C$ and WUE$_i$ increased under water deficit. The decrease of PNUE and A_{mmax} under water limitation is mainly driven by the constraint that stomatal closure imposes on photosynthetic performance (i.e. lower internal leaf CO_2 concentrations under water deficit lead to decreased photosynthesis). Furthermore, this effect is exacerbated in PNUE due to the increased leaf nitrogen content observed under water deficit. Part of this effect could be attributed to the decrease in SLA (Soolanayakanahally et al., 2009), but the fact that higher nitrogen content was also found on a mass basis indicates that this is not only an indirect effect of variation in SLA. In fact, it has been recently demonstrated that higher leaf nitrogen content can be a functional adaptation to and not a passive consequence of water deficit (Weih et al., 2011). The observed effect of water availability on SLA has also been reported in other studies (e.g. Galmes et al., 2005) and agrees with the well-documented trend across species of lower SLA with increasing aridity (Cunningham et al., 1999; Fonseca

Table 4
F-Fisher values and significance levels obtained by General Linear Models, GLM analysis. This analysis was applied for the studied physiological variables at the peak of water deficit (T_3). "Size" was the covariate effect corresponding to initial size. The interaction term between "Population" and "Treatment" ($P \times T$) was included in the model. The degrees of freedom for the *F*-values are provided in brackets.

Variable	Factor/covariate			
	Size	Population (P)	Treatment (T)	$P \times T$
SLA	(1/287) 21.7[***]	(5/287) 2.9[*]	(1/287) 42.2[***]	(5/287) 1.9
N_m	(1/287) 0.2	(5/287) 5.1[***]	(1/287) 8.6[**]	(5/287) 0.6
N_a	(1/287) 15.0[***]	(5/287) 4.8[***]	(1/287) 66.3[***]	(5/287) 0.7
δ^{13}C	(1/287) 16.8[***]	(5/287) 2.5[*]	(1/287) 56.3[***]	(5/287) 2.3[*]
PNUE	(1/282) 1.2	(5/282) 4.1[**]	(1/282) 90.5[***]	(5/282) 2.4[*]
A_{mmax}	(1/282) 2.6	(5/282) 0.9	(1/282) 78.2[***]	(5/282) 2.9[*]
WUE$_i$	(1/282) 0.9	(5/282) 4.0[**]	(1/282) 62.9[***]	(5/282) 2.5[*]

[*] $0.01 \leq P < 0.05$.
[**] $0.001 \leq P < 0.01$.
[***] $P < 0.001$.

et al., 2000; Specht and Specht, 1989). This trend is considered to reflect leaf-level adaptations to water deficit (Reich et al., 2003).

Even though the qualitative effect of water deficit on all of the studied functional traits was consistent across all the populations (i.e. the direction of the effect on the studied traits did not differ among populations), its relative impact on particular traits did differ among populations. In general, the least tolerant populations to water deficit were also more sensitive to water deficit in terms of WUE, PNUE and A_{mmax}, while the most tolerant populations to water deficit were least sensitive to water deficit in the same traits, indicating intraspecific variation in phenotypic plasticity to water availability in this species. Bresson et al. (2011) comparing leaf gas exchange in beech and sessile oak populations across an altitudinal cline found most of the phenotypic change to result from phenotypic plasticity, with a very low relative contribution from the genotypic variation of the local population. In contrast, evidence from this study supports that both genotypic variation and phenotypic plasticity are important components of intraspecific phenotypic variability in this species.

According to the results of this study, tolerance to water deficit (based on photosynthetic performance under water shortage and sensitivity of photosynthetic performance to water shortage) was related to high PNUE. This finding suggests that an efficient use of nitrogen under water deficit might confer functional advantage, as supported by other studies (Cai et al., 2009; Sánchez-Rodríguez et al., 2011). In contrast, an increased PNUE has been linked to low leaf lifespan (Reich et al., 1992) and in general with a strategy aimed to maximize growth and production (PNUE is positively related to A_{max}, Field and Mooney, 1986) that is unlikely to provide a functional advantage under drought (Quero et al., 2006). Nevertheless the general trends observed in the leaf-economics spectrum are not universal and different patterns can be found at more local scales (Wright et al., 2005). Besides, those general trends across species might not hold when evaluated at the intraspecific level (Arntz and Delph, 2001). WUE (estimated either as δC^{13} or WUE$_i$) was not related to tolerance to water deficit in the studied populations. Despite this, WUE has been considered a central trait within the drought tolerance syndrome (Cowan, 1982; Field et al., 1983), even though this assumption is not consistently supported across the literature. There are studies that show an adaptive value of either high or low WUE. In other cases, no correlation is found between WUE and fitness components (see Nicotra and Davidson, 2010) and references therein). The patterns observed at the intraspecific level are also heterogeneous (Correia et al., 2008 and references therein).

Table 5
Least square means for the studied physiological variables at the peak of water deficit (T3). The values are computed for the covariate (initial size) at its mean. The letter codes denote homogeneous groups (Fisher-LSD post-hoc test). Upper-case letters are used to compare among provenances at the treatment level "control". Lower-case letters are used to compare among provenances at the treatment level "water deficit" (wd). The star denotes significant differences between treatment levels for a given population.

Population	Treatment	SLA (m² kg⁻¹)	N_m (g g⁻¹ × 100)	N_a (g m⁻²)	δ^{13}C (‰)	PNUE (μmol g⁻¹ s⁻¹)	A_m (μmol g⁻¹ s⁻¹)	WUE$_i$ (μmol mol⁻¹)
Sp	control	20.17	2.55	1.36	−29.01	8.39	212.72	63.27
		D	A	A	AB	BC	AB	A
	wd	16.88	2.69	1.65	−28.8	6.58	174.94	74.67
		bcd*	a	a*	a*	b*	b*	abc*
I	control	19.40	2.78	1.50	−29.72	8.73	238.35	57.27
		CD	AB	B	A	C	B	A
	wd	14.94	2.86	1.99	−28.5	5.39	153.00	87.43
		a*	abc	c*	a*	a*	ab*	d*
G2	control	19.48	2.74	1.44	−29.62	8.06	216.51	59.60
		BC	AB	B	AB	ABC	AB	A
	wd	17.81	3.01	1.77	−28.2	4.91	145.19	66.07
		cd*	bcd*	ab*	ab*	a*	a*	a
G3	control	19.02	2.78	1.50	−29.58	7.74	213.38	62.23
		AB	AB	B	B	AB	A	A
	wd	16.36	2.8	1.75	−28.2	5.72	157.96	74.87
		bc*	ab	ab*	abc*	ab*	ab*	bc*
G1	control	18.19	2.89	1.65	−28.84	7.28	203.65	72.17
		A	B	C	C	A	A	B
	wd	16.11	3.07	1.96	−27.52	5.77	175.57	81.14
		ab	cd	c*	c*	ab*	b	cd*
Sw	control	19.71	2.90	1.53	−29.62	8.60	245.84	57.15
		BCD	B	B	AB	BC	B	A
	wd	17.64	3.20	1.81	−27.47	5.35	159.06	71.56
		d*	d*	ab*	bc*	a*	ab*	ab*

All these conflicting results reflect: (1) different patterns of water availability and consequently different plant strategies to cope with water deficit (2) trade-offs between water conservation and gain of other resources or tolerance of other conditions and (3) differential selection pressure on WUE along different developmental stages that could be especially relevant for those particular forest tree species with a long lasting juvenile phase (see Nicotra and Davidson, 2010) for further details). Evidence from this study indicates that WUE is not a key trait involved in beech's response to water deficit, at least, during early developmental stages. Regarding SLA and leaf nitrogen, the expected pattern for the most tolerant populations to water deficit would be high leaf nitrogen content and low SLA (see discussion above). We did find this pattern, but we also found that the reverse provided high levels of tolerance to water deficit among the studied populations. This result suggests that SLA and leaf nitrogen are not traits directly linked to variation in beech's tolerance to water deficit and again supports the idea that general patterns observed across species are not necessarily reflected at the intraspecific level. Alternatively, it might also reflect different adaptive strategies to different patterns of water scarcity. In fact the range of studied populations covered variation at several levels (total amount of rainfall, seasonality in water availability, and soils with slightly different water holding capacities). Comparison of populations from very different genetic pools could also have contributed to the observed differences. In fact, the studied populations are thought to come from very different glacial refuges (Magri et al., 2006).

The estimation of tolerance to water deficit in this study refers to photosynthetic rates at the leaf level so care should be taken when linking this to performance at the whole-plant level or fitness (e.g. plant growth or survival) in a tree species with a long life-span. Nevertheless, the link between photosynthetic rates and fitness when explicitly tested has been demonstrated within genotypes of the same species (Arntz et al., 2000). Besides, the intuitive causal sequence: higher photosynthetic rates, higher carbon gain, higher biomass accumulation and in turn higher fitness is probably stronger at early life stages when maximizing carbon gain is critical given the small size and high sensitivity to environmental stresses of a seedling. Assuming this is the case, the rate of decrease of photosynthetic rate with water depletion should reflect the seedling's tolerance to water deficit. However, further long-term studies should address to what extend the findings of this study hold true at different ontogenetic stages.

Acknowledgements

References

Ackerly, D.D., Dudley, S.A., Sultan, S.E., Schmitt, J., Coleman, J.S., Linder, C.R., Sandquist, D.R., Geber, M.A., Evans, A.S., Dawson, T.E., Lachowicz, M.J., 2000. The evolution of plant ecophysiological traits: recent advances and future directions. Bioscience 50, 979–995.

Aranda, I., Gil, L., Pardos, J.A., 2001. Effects of thinning in a Pinus sylvestris L. stand on foliar water relations of Fagus sylvatica L. seedlings planted within the pinewood. Trees-Structure and Function 15, 358–364.

Arntz, A.M., Delph, L.F., 2001. Pattern and process: evidence for the evolution of photosynthetic traits in natural populations. Oecologia 127, 455–467.

Arntz, A.M., DeLucia, E.H., Jordan, N., 2000. From fluorescence to fitness: variation in photosynthetic rate affects fecundity and survivorship. Ecology 81, 2567–2576.

Benowicz, A., Guy, R.D., El-Kassaby, Y.A., 2000. Geographic pattern of genetic variation in photosynthetic capacity and growth in two hardwood species from British Columbia. Oecologia 123, 168–174.

Brendel, O., Le Thiec, D., Scotti-Saintagne, C., Bodenes, C., Kremer, A., Guehl, J.M., 2008. Quantitative trait loci controlling water use efficiency and related traits in Quercus robur L. Tree Genetics and Genomes 4, 263–278.

Bresson, C.C., Vitasse, Y., Kremer, A., Delzon, S., 2011. To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech? Tree Physiology 31, 1164–1174.

Cai, Z.Q., Schnitzer, S.A., Bongers, F., 2009. Seasonal differences in leaf-level physiology give lianas a competitive advantage over trees in a tropical seasonal forest. Oecologia 161, 25–33.

Chaves, M.M., 1991. Effects of water deficits on carbon assimilation. Journal of Experimental Botany 42, 1–16.

Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogee, J., Allard, V., Aubinet, M., Buchmann, N., Bernhofer, C., Carrara, A., Chevallier, F., De Noblet, N., Friend, A.D., Friedlingstein, P., Grunwald, T., Heinesch, B., Keronen, P., Knohl, A., Krinner, G., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourcival, J.M., Papale, D., Pilegaard, K., Rambal, S., Seufert, G., Soussana, J.F., Sanz, M.J., Schulze, E.D., Vesala, T., Valentini, R., 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437, 529–533.

Correia, I., Almeida, M.H., Aguiar, A., Alía, R., David, T.S., Pereira, J.S., 2008. Variations in growth survival and carbon isotope composition (d^{13}C) among P. pinaster populations for different geographic origins. Tree Physiology 28, 1545–1552.

Cowan, I.R., 1982. Regulation of water use in relation to carbon gain in higher plants. In: Lange, O.L., Nobel, P.S., Osmond, C.B., Zeiger, H. (Eds.), Water Relations and Carbon Assimilation. Encyclopedia Plant Physiology. Springer Verlag, Berlin Heidelberg, New York, pp. 589–613.

Cunningham, S.A., Summerhayes, B., Westoby, M., 1999. Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. Ecological Monographs 69, 569–588.

Ellenberg, H., 1992. Vegetation Mitteleuropas mit den Alpen. Eugen Ulmer Verlag, Stuttgart, Germany.

Fajardo, A., Piper, F.I., 2010. Intraspecific trait variation and covariation in a widespread tree species (Nothofagus pumilio) in southern Chile. New Phytologist 189, 259–271.

Field, C., Merino, J., Mooney, H.A., 1983. Compromises between water-use efficiency and nitrogen-use efficiency in five species of California evergreens. Oecologia 60, 384–389.

Field, C., Mooney, H.A., 1986. The photosynthesis-nitrogen relationship in wild plants. In: Givnisch, T.J. (Ed.), On the Economy of Plant Form and Function. Cambridge University Press, Cambridge, pp 25–55.

Flexas, F., Medrano, H., 2002. Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. Annals of Botany 89, 183–189.

Flexas, J., Bota, J., Escalona, J.M., Sampol, B., Medrano, H., 2002. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Funtional Plant Biology 29, 461–471.

Fonseca, C.R., Overton, J.M., Collins, B., Westoby, M., 2000. Shifts in trait-combinations along rainfall and phosphorus gradients. Journal of Ecology 88, 964–977.

Fotelli, M.N., Nahm, M., Radoglou, K., Rennenberg, H., Halyvopoulos, G., Matzarakis, A., 2009. Seasonal and interannual ecophysiological responses of beech (Fagus sylvatica) at its south-eastern distribution limit in Europe. Forest Ecology and Management 257, 1157–1164.

Galmes, J., Cifre, J., Medrano, H., Flexas, J., 2005. Modulation of relative growth rate and its components by water stress in Mediterranean species with different growth forms. Oecologia 145, 21–31.

Garcia-Plazaola, J.I., Becerril, J.M., 2000. Photoprotection mechanisms in European beech (Fagus sylvatica L.) seedlings from diverse climatic origins. Trees-Structure and Function 14, 339–343.

Genty, B., Briantais, J.M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta 990, 87–92.

Grubb, P.J., 1977. The maintenance of species-richness in plant communities: the importance of the regeneration niche. Biological Reviews 52, 107–145.

Harper, J.L., 1977. Population Biology of Plants. Academic Press, London.

Hereford, J., Winn, A.A., 2008. Limits to local adaptation in six populations of the annual plant Diodia teres. New Phytologist 178, 888–896.

IPCC, 2007. Climate change 2007. The physical science basis: working group I contribution to the fourth assessment report of the IPCC. Cambridge University Press, Cambridge, UK.

Leverenz, J.W., Bruhn, D., Saxe, H., 1999. Responses of two provenances of Fagus sylvatica seedlings to a combination of four temperature and two CO_2 treatments during their first growing season: gas exchange of leaves and roots. New Phytologist 144, 437–454.

Magri, D., Vendramin, G.G., Comps, B., Dupanloup, I., Geburek, T., Gomory, D., Latalowa, M., Litt, T., Paule, L., Roure, J.M., Tantau, I., van der Knaap, W.O., Petit, R.J., de Beaulieu, J.L., 2006. A new scenario for the quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. New Phytologist 171, 199–221.

Martin, R.E., Asner, G.P., Sack, L., 2007. Genetic variation in leaf pigment, optical and photosynthetic function among diverse phenotypes of Metrosideros polymorpha grown in a common garden. Oecologia 151, 387–400.

Martínez-Vilalta, J., Cochard, H., Mencuccini, M., Sterck, F., Herrero, A., Korhonen, J.F.J., Llorens, P., Nikinmaa, E., Nole, A., Poyatos, R., Ripullone, F., Sass-Klaassen, U., Zweifel, R., 2009. Hydraulic adjustment of scots pine across Europe. New Phytologist 184, 353–364.

Meier, I.C., Leuschner, C., 2008. Genotypic variation and phenotypic plasticity in the drought response of fine roots of European beech. Tree Physiology 28, 297–309.

Mooney, H.A., Billings, W.D., 1961. Comparative physiological ecology of artic and alpine populations of *Oxyria digyna*. Ecological Monographs 31, 1–29.

Nicotra, A.B., Davidson, A., 2010. Adaptive phenotypic plasticity and plant water use. Funtional Plant Biology 37, 117–127.

Nielsen, C.N., Jorgensen, F.V., 2003. Phenology and diameter increment in seedlings of European beech (*Fagus sylvatica* L.) as affected by different soil water contents: variation between and within provenances. Forest Ecology and Management 174, 233–249.

Panagos, P., 2006. The European soil database. GEO: conexion 5, 32–33.

Panagos, P., Van Liedekerke, M., Jones, A., Montanarella, L., 2012. European Soil Data Centre: response to European policy support and public data requirements. Land Use Policy 29, 329–338.

Peguero-Pina, J.J., Sancho-Knapik, D., Morales, F., Flexas, J., Gil-Pelegrin, E., 2009. Differential photosynthetic performance and photoprotection mechanisms of three Mediterranean evergreen oaks under severe drought stress. Functional Plant Biology 36, 453–462.

Peuke, A.D., Gessler, A., Rennenberg, H., 2006. The effect of drought on C and N stable isotopes in different fractions of leaves, stems and roots of sensitive and tolerant beech ecotypes. Plant, Cell & Environment 29, 823–835.

Pigliucci, M., Murren, C.J., Schlichting, C.D., 2006. Phenotypic plasticity and evolution by genetic assimilation. Journal of Experimental Biology 209, 2362–2367.

Pigott, C.D., Pigott, S., 1993. Water as a determinant of the distribution of trees at the boundary of the Mediterranean zone. Journal of Ecology 81, 557–566.

Quero, J.L., Villar, R., Maranon, T., Zamora, R., 2006. Interactions of drought and shade effects on seedlings of four quercus species: physiological and structural leaf responses. New Phytologist 170, 819–833.

Reich, P.B., Walters, M.B., Ellsworth, D.S., 1992. Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. Ecological Monographs 62, 365–392.

Reich, P.B., Wright, I.J., Cavender-Bares, J., Craine, M., Oleksyn, J., Westoby, M., Walters, M.B., 2003. The evolution of plant functional variation: traits spectra and strategies. International Journal of Plant Sciences 164, S143–S164.

Robson, T.M., Sánchez-Gómez, D., Cano, F.J., Aranda, I., 2012. Variation in functional leaf traits among beech provenances during a Spanish summer reflects the differences in their origin. Tree Genetics and Genomes, http://dx.doi.org/10.1007/s11295-11012-10496-11295.

Rose, L., Leuschner, C., Kockemann, B., Buschmann, H., 2009. Are marginal beech (*Fagus sylvatica* L.) provenances a source for drought tolerant ecotypes? European Journal of Forest Research 128, 335–343.

Sánchez-Rodríguez, E., Rubio-Wilhelmi, M.M., Blasco, B., Constán-Aguilar, C., Romero, L., Ruiz, J.M., 2011. Variation in the use efficiency of N under moderate water deficit in tomato plants (*Solanum lycopersicum*) differing in their tolerance to drought. Acta Physiologiae Plantarum 33, 1861–1865.

Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemmingsen, E.A., 1965. Sap pressure in vascular plants. Science 148, 339–346.

Silvertown, J., 2004. Plant coexistence and the niche. Trends in Ecology & Evolution 19, 605–611.

Silvertown, J., Charlesworth, D., 2001. Introduction to Plant Population Biology. Blackwell Science, Carlton, Australia.

Soolanayakanahally, R.Y., Guy, R.D., Silim, S.N., Drewes, E.C., Schroeder, W.R., 2009. Enhanced assimilation rate and water use efficiency with latitude through increased photosynthetic capacity and internal conductance in balsam poplar (*Populus balsamifera* L.). Plant Cell and Environment 32, 1821–1832.

Specht, R.L., Specht, A., 1989. Canopy structure in Eucalyptus-dominated communities in Australia along climatic gradients. Acta Oecologica 10, 191–213.

Tognetti, R., Johnson, J.D., Michelozzi, M., 1995. The response of European beech (*Fagus sylvatica* L) seedlings from 2 Italian populations to drought and recovery. Trees-Structure and Function 9, 348–354.

Van Liedekerke, M., Jones, A., Panagos, P., 2006. SDBv2 Raster Library – a set of raster derived from the European Soil Database distribution v2.0. The European Commission and the European Soil Bureau Network (CD-ROM, EUR 19945 EN).

Weih, M., Bonosi, L., Ghelardini, L., Rönnberg-Wästljung, A.C., 2011. Optimizing nitrogen economy under drought: increased leaf nitrogen is an acclimation to water stress in willow (Salix spp.). Annals of Botany 108, 1347–1353.

Westoby, M., Wright, I.J., 2006. Land-plant ecology on the basis of functional traits. Trends Ecology and Evolution 21, 261–268.

Wortemann, R., Herbette, S., Barigah, T.S., Fumanal, B., Alía, R., Ducousso, A., Gomory, D., Roeckel-Drevet, P., Cochard, H., 2011. Genotypic variability and phenotypic plasticity of cavitation resistance in *Fagus sylvatica* L. across Europe. Tree Physiology 31, 1175–1182.

Wright, I.J., Reich, P.B., Cornelissen, J.H.C., Falster, D.S., Garnier, E., Hikosaka, K., Lamont, B.B., Lee, W., Oleksyn, J., Osada, N., Poorter, H., Villar, R., Warton, D.I., Westoby, M., 2005. Assessing the generality of global leaf trait relationships. New Phytologist 166, 485–496.