



Seasonal and interannual ecophysiological responses of beech (*Fagus sylvatica*) at its south-eastern distribution limit in Europe

Mariangela N. Fotelli^{a,1}, Michael Nahm^b, Kalliopi Radoglou^{a,*},
Heinz Rennenberg^b, George Halyvopoulos^a, Andreas Matzarakis^c

^a Forest Research Institute, National Agricultural Research Foundation, Vassilika 57006, Thessaloniki, Greece

^b Institute of Forest Botany and Tree Physiology, Albert-Ludwigs-University Freiburg, Georges Köhler Allee Geb.053/054, 79110 Freiburg i. Br., Germany

^c Meteorological Institute, Albert-Ludwigs-University Freiburg, Werderring 10, 79085 Freiburg i. Br., Germany

ARTICLE INFO

Article history:

Received 25 July 2008

Received in revised form 21 November 2008

Accepted 24 November 2008

Keywords:

Carbon isotopes

Effective quantum yield

Fagus sylvatica

Leaf water potential

South-eastern Europe

ABSTRACT

Due to its wide European distribution and its drought-susceptibility, beech (*Fagus sylvatica* L.) received intensive attention recently in the light of global warming. Contrary to central European beech ecosystems, little is known about the ecophysiology of beech at its south-eastern European distribution limit. Here we tested whether climatic fluctuations during a three-year period affected the ecophysiology of a beech site in Greece. Attention was paid at comparing our findings to the intense effects the 2003 extreme drought had on beech forests in central Europe.

We assessed the interannual and seasonal variation of certain physiological parameters in a beech stand of north-western Greece during three consecutive growing seasons of the period 2003–2005. Leaf water potential and effective quantum yield of PSII were measured as well-known indicators of plant's responses to environmental stresses. Furthermore, plant carbon isotopic composition ($\delta^{13}\text{C}$) of tissues and extracts with different turnover times was determined, since it can reveal short- or long-term environmental effects on the water and carbon balance of the plant. Moreover, a number of micrometeorological parameters were measured and their effect on ecophysiological responses was tested.

Precipitation of 2003 at the study site was comparable to that in central Europe, but it did not differ from the local range of precipitation in NW-Greece. Still, 2003 was more xeric, compared to 2004 and 2005. Despite this, leaf water potential, effective quantum yield and $\delta^{18}\text{O}$ showed no significant variation between years and their values were not indicative of plants suffering from drought stress. Foliar $\delta^{13}\text{C}$, on the other hand, appeared to be more sensitive to the climatic differences between the years and it was higher during the more xeric 2003 compared to later on. Regression analysis revealed that its response was largely controlled by current soil water content and vapour pressure deficit of the preceding month. Regarding $\delta^{13}\text{C}$ of phloem from both twigs and trunk, their patterns were influenced only by short-term changes in air vapour pressure deficit.

Within the climatic range recorded in this study, which is typical for beech ecosystems in Greece, no substantial drought-driven limitations were observed on beech ecophysiology. Our observations contradict those from central European beech sites, rarely subjected to drought, where similarly low water availability had a great impact on the ecophysiology of beech.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Owing to climate change (Schär et al., 2004; IPCC, 2007) the future survival and sustainability of European beech (*Fagus sylvatica*) ecosystems in Europe has become of great concern

(e.g. Cescatti and Piutti, 1998; Peñuelas and Boada, 2003; Geßler et al., 2004a, 2007; Bréda et al., 2006), due to the species' high sensitivity to drought (Fotelli et al., 2001; Leuschner et al., 2001; Granier et al., 2007). Since European beech is one of the widely spread forest species in Europe (Ellenberg, 1996), any possible adverse effects on its sustainability and regeneration may have great ecological and economical impacts.

In the Mediterranean region, beech is limited to mountainous areas where it could not be reached by ice during the Quaternary period allowing, thus, its survival. Although beech populations found close to the southern limit of the species' distribution are

* Corresponding author. Tel.: +30 2310 461172; fax: +30 210 929806.

E-mail address: radoglou@fri.gr (K. Radoglou).

¹ Present address: Laboratory of Plant Physiology and Morphology, Department of Agricultural Biotechnology, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece.

characterized by high genetic diversity compared to northern populations (Demesure et al., 1996; Scarascia-Mugnozza et al., 2000), they might be quite sensitive to extreme environmental conditions since they grow at the limit of their ecological requirements (e.g. Jump et al., 2006a). Therefore, beech ecosystems of the Mediterranean might be especially prone to climate change due to the already xeric conditions of this area (e.g. Scarascia-Mugnozza et al., 2000). A recent study of Jump et al. (2006a) reports a rapid growth decline of beech forests in north-eastern Spain, as a direct consequence of intense drought and warming during the last decades. As a result, beech may be forced to shift to higher altitudes (Peñuelas and Boada, 2003) and restrict its distribution in southern Europe. On the other hand, beech of southern origins might respond better to intensified drought and heat events due to a potential acclimation to such environmental conditions (Tognetti et al., 1995; García-Plazaola et al., 2008). Recently, evidence is provided that current climatic changes are resulting in genetic alterations and, thus, adaptive responses to climate change in certain beech populations of the Mediterranean (Jump et al., 2006b). The observed increase in water use efficiency of lower elevation beech sites in Spain, subjected to intense warming during the last decades (Peñuelas et al., 2008) further emphasizes that some beech population have the potential to respond to climate change.

Studies on the effects of drought on water relations and photosynthetic performance of beech (e.g. Tognetti et al., 1995; Backes and Leuscher, 2000; Fotelli et al., 2001; Leuschner et al., 2001; Bréda et al., 2006) generally showed that although beech possesses mechanisms for responding to water deficits, it is not a drought-tolerant species. To date there is limited information on the ecophysiology of beech in the typically xeric Mediterranean ecosystems (e.g. Tognetti et al., 1995; Aranda et al., 2000, 2005; Sabate et al., 2002; Skomarkova et al., 2006). Particularly in Greece, the south-eastern distribution limit of beech in Europe, only few studies focused on this species till lately (Raftoyannis and Radoglou, 2002; Nahm et al., 2006b; Zerva et al., 2008), since beech forests in Greece were of low economical importance, but may reveal to be of great ecological importance. Studying the ecophysiology of these beech ecosystems, generally characterized by xeric conditions, may offer valuable information on the species' potential to acclimate and adapt to climatic changes. For example, the summer of 2003 was extremely dry and the hottest of the last 180 years in central and western Europe (Schär et al., 2004; Ciais et al., 2005; Löw et al., 2006; Rebetez et al., 2006; Granier et al., 2007) and led to intensified research on the physiological responses of beech to drought (e.g. Löw et al., 2006; Geßler et al., 2007; Granier et al., 2007; Nahm et al., 2007). However, little is known about the ecophysiological performance of beech in Greece during this well-studied dry year.

In this study we have measured certain physiological parameters, indicative of the plant's water and carbon balance, in order to assess the performance of beech in NW-Greece under the influence of the typical Mediterranean climate of this region. Carbon isotopic compositions of plant tissues and extracts offer useful insights on plants' responses to environmental stresses, such as limited water availability (e.g. Damesin et al., 1998; Adams and Grierson, 2001; Fotelli et al., 2003). In C_3 plants, the slower diffusion of the heavier ^{13}C isotope, compared to ^{12}C , from the atmosphere to the site of carboxylation, and the strong discrimination of Rubisco against ^{13}C , are largely responsible for the depletion of plant material in ^{13}C relative to the atmosphere. The $\delta^{13}C$ composition of a plant tissue is described by Farquhar et al. (1989) as:

$$\delta^{13}C_{\text{plant}} = \delta^{13}C_{\text{atm}} - \alpha - (b - \alpha) \frac{C_i}{C_a}$$

where $\delta^{13}C$ is expressed in units of parts per thousand (‰), α is the discrimination during diffusion ($\sim 4.4\%$), b is the discrimination

during carboxylation by Rubisco ($\sim 29\%$), C_i is the CO_2 concentration inside the stomatal cavities, and C_a is the atmospheric CO_2 concentration.

Water deficits lead, thus, in reductions in the C_i/C_a factor and in increases in $\delta^{13}C$ of organic matter (^{13}C -enriched tissues). However, structural carbon of bulk material like leaves, may carry an isotopic signature affected by storage and remobilisation processes (Helle and Schleser, 2004; Skomarkova et al., 2006). On the other hand, $\delta^{13}C$ of recently fixed carbon, allocated among others in phloem sap, is indicative of short-term environmental fluctuations (Keitel et al., 2003; Scartazza et al., 2004). Moreover, variations in water availability may affect the downstream processes of carbon metabolism and ^{13}C -isotopic signature during allocation from leaf to stem (Damesin and Lelarge, 2003) or to root (Keitel et al., 2003). Therefore, $\delta^{13}C$ analysis of leaves, as well as of phloem sap, which are characterized by different turnover times may reveal differences in the effect of environmental factors, such as water availability (e.g. Peuke et al., 2006).

Fluorescence parameters are also well-established measures of plants' responses to environmental stresses. Effective quantum yield of PSII in light-adapted leaves is a reliable indicator of photoinhibition in plants in response to stresses (Colom and Vazzana, 2003). Furthermore, leaf water potential is traditionally measured for characterizing plant's responses to drought and other stresses, and is among the very few ones already studied in beech native to Greece (Raftoyannis and Radoglou, 2002; Nahm et al., 2006b), providing thus comparability to existing data.

To assess the seasonal and interannual variation of critical physiological traits of adult beech trees in a beech forest in Greece, carbon isotopic analysis of various plant compounds were combined to chlorophyll fluorescence and water relations measurements. We aimed at comparing the seasonal ecophysiological performance of beech during three consecutive growing periods from 2003 to 2005 and at studying how this performance is affected by climatic conditions. In the light of the great impact the 2003 drought had on beech forests of central Europe, we also focused on comparing our findings with those of studies on beech ecosystems in central Europe, generally characterised by less xeric summers than in Greece.

2. Materials and methods

2.1. Site description

The experimental site of this study is located in north-western Greece, at the area of Kastaneri on Paiko mountain (longitude: $22^\circ 20' E$; latitude $40^\circ 58' N$), about 100 km north-northwest from Thessaloniki. Paiko is characterized as a Site of Community Interest, according to the 92/43 EU Regulation, due to the particular importance of its natural resources. European beech forms natural stands occupying the greatest part of mountain Paiko, followed by oaks (*Quercus frainetto*, *Quercus petraea* sub. *medwediewii*, *Quercus pubescens*), chestnut (*Castanea sativa*) and eastern hornbeam (*Carpinus orientalis*).

The study area is established in a pure beech stand with average tree height 17 ± 3 m and diameter at breast height 0.2 ± 0.09 m. The beech stand is 30–60 years old and is regenerated mainly with sprouts and partly with seeds. Silviculturally it is regarded a low quality, degraded stand and the applied thinning aims at converting it from a sprout-origin to a productive seed-origin forest. The forest site is located at 1140 m.a.s.l., within the submontane level of beech distribution, which in Greece lies between 800 and 1700 m.a.s.l. (Bergmeier and Dimopoulos, 2001).

The experimental site is characterized by moderate to steep slope (30–80%) and is S-SW-exposed. The soil depth at field site is 40–50 cm. More than 50% of the live fine root biomass of the

studied beech trees is developed at the upper 20 cm of the soil (Zerva et al., 2008). The geologic substrate consists of marbles, crystalline limestones, and clay schist; formations characterized by great depth and high consolidation. The soil pH (H₂O) is slightly acidic (5.0 at 0.1 m depth and 4.8 at 0.3 m depth).

2.2. Environmental conditions

During the three years of study, climatic conditions of the site were monitored by a weather station located close to the forest edge, at ca. 10 m distance from the first (closest) studied beech tree. Air temperature and air relative humidity (RHT2nl, Delta-T Devices Ltd., Cambridge, UK), and photosynthetically active radiation (SKP215; Skye Instruments Ltd., Llandrindod Wells, UK) were measured at 1.5 m height. Moreover, precipitation (Rain Collector II, Davis Instruments, Hayward, USA) and soil water content at 30 cm depth (Theta Probe ML1, Delta-T Devices Ltd., Cambridge, UK), were recorded. A recent study showed that more than 50% of live fine root biomass of the studied beech trees was developed in the upper 20 cm of soil and this biomass decreased with depth (Zerva et al., 2008). Thus, the soil water content of the upper 30 cm of soil should be decisive for the water uptake by the trees. All parameters were data-logged hourly (DL2e Delta-T Logger, Delta-T Devices Ltd., Cambridge, UK).

In order to characterize the climatic conditions of the three years of study, air temperature and precipitation data have additionally been obtained by the following meteorological stations established at forest sites of N-NW Greece: Loutra Thermis, Chrisopigi and Paggaiio. The climate diagrams of these stations are presented in Fig. 1.

Stand evaporation, transpiration and evapotranspiration of the studied site were calculated with the water balance model WBS3, a forest-hydrological model that requires daily mean value of air temperature and daily total precipitation as meteorological inputs (Schmidt, 1990; Matzarakis et al., 2000). Time-independent input variables of the WBS3-simulations are: basal area of the stand, mixing ratio of deciduous trees, mixing ratio of coniferous trees, type of soil, plant available soil water, slope angle, slope direction

and geographical latitude. In comparison to other water balance models, WBS3 uses a simple approach but requires less meteorological parameters. For evapotranspiration, evaporation, transpiration and interception of forests, validations of WBS3 showed a good agreement between results from model calculations and measurements for different areas and slopes (Fritsch, 1998; Matzarakis et al., 2000; Nahm et al., 2006a) and has been applied for such purposes in ecophysiological studies (Keitel et al., 2003, 2006; Nahm et al., 2006a).

2.3. Measurement campaigns

Eight non-neighboring adult beech trees of the experimental area, distanced at least 10 m from each other, were selected for measurements and sampling, according to their height (Vertex III Hypsometer; Haglof Inca., Madison, USA) and diameter at breast height (DBH). The studied trees were chosen as representative of the forest stand, covering the entire range of height (10–22 m) and DBH (9–51 cm).

Field measurements of effective quantum yield of PSII and leaf water potential were conducted once per month during the growing season. Collection of plant material was performed three times per growing season, between 09:00 h and 11:00 h: in May (at the beginning of the growing season), in July (mid-summer) and in September (at the end of the growing season). The plant material sampled consisted of leaves and phloem exudates derived both from twigs and the tree trunk.

2.4. Collection of phloem sap from twigs and the trunk

For phloem exudation from the twigs, a bark piece of approximately 300 mg fresh weight was removed from the cut end of one twig per studied tree (Nahm et al., 2006a,b). Small pieces of bark of the same fresh weight were removed from the trunk of each tree at breast height with a corer and used for phloem exudation (Geßler et al., 2004b; Keitel et al., 2006).

All bark samples were washed with double-distilled water in order to exclude contamination of phloem exudates with xylem

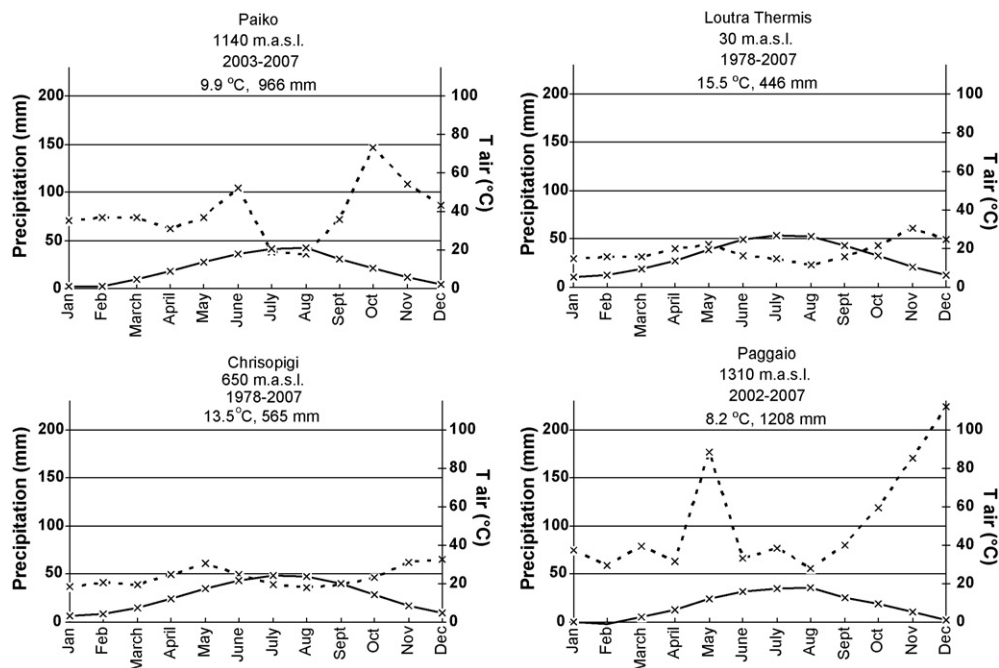


Fig. 1. Climate diagrams of the studied beech site on Paiko Mtn., as well as of additional three forest sites of N-NW Greece (Loutra Thermis, Chrisopigi and Paggaiio), characterised by different elevation.

sap. Subsequently, in 2003 phloem exudation was conducted with the EDTA-technique described by [Rennenberg et al. \(1996\)](#) and [Schneider et al. \(1996\)](#). The bark pieces were incubated in 6-ml vials with 2 ml exudation solution consisting of 10 mM EDTA and 0.015 mM chloramphenicol at pH 7.0 for 5 h. In 2004 and 2005, EDTA was replaced by Polyphosphate puffer (Sodium hexameta-phosphate—“Phosphate Glass”) (15 mM), as it was observed that EDTA affected the $\delta^{13}\text{C}$ signature of the exudates. To overcome this problem, $\delta^{13}\text{C}$ of EDTA was measured and the $\delta^{13}\text{C}$ signatures of phloem exudates from 2003 were corrected accordingly.

Phloem exudates were finally frozen in liquid N_2 and stored at -80°C until analysis. Previous studies ([Schneider et al., 1996](#)) showed that contamination of phloem exudates with cellular constituents is negligible under the experimental conditions applied.

In *Pinus sylvestris* [Brandes et al. \(2006\)](#) observed no intracanal gradient of $\delta^{13}\text{C}$ in twigs. It can, therefore, be assumed that twig sampling in the present study was representative of the tree canopy.

2.5. Determination of tissues C isotopic composition

Leaf material was ground with mortar and pestle into a homogenous fine powder and then oven-dried (3 days, 65°C). Samples of 0.5–1.0 mg were transferred into tin capsules (IVA Analysentechnik, Meerbusch, Germany) and then injected into an elemental analyser (NA 2500; CE Instruments, Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT GmbH, Bremen, Germany) by a ConFlo II interface (Finnigan MAT GmbH, Bremen, Germany).

For $\delta^{13}\text{C}$ analysis in the phloem exudates, 100 μl were injected into tin capsules and left to dry for 24 h at 65°C . This procedure was followed twice before the tin capsules were closed and injected into the elemental analyzer–isotope ratio mass spectrometer system. The $\delta^{13}\text{C}$ values were expressed as: $\delta^{13}\text{C}$ (‰) = $[(R_{\text{sample}}/R_{\text{VPDB}}) - 1] \times 1000$, where R_{sample} and R_{VPDB} are the $^{13}\text{C}/^{12}\text{C}$ ratios of sample and VPDB (Vienna Pee Dee Belemnite) standard, respectively.

2.6. Determination of effective quantum yield of PSII and leaf water potential

Effective quantum yield was recorded on a monthly basis on seven of the selected beech trees between 10:00 h and 12:00 h. Measurements of the effective quantum yield of PSII were performed on six leaves per tree. The saturation pulse method associated with the pulse-amplitude-modulation technique was applied for fluorescence measurements using a MINI-PAM fluorometer (Heinz Walz, Elteltrich, Germany). The tip of the fiberoptics was located 0.01 m from and 60° to the leaf surface. The effective quantum yield of PSII was calculated as $\Delta F/F'_m = (F'_m - F)/F'_m$, where F and F'_m are the fluorescence yields before and after the saturation pulse is applied on the leaf, respectively. Effective quantum yield was measured on fully expanded southeast-exposed leaves at the same layer in the lower sunlit crown. Measurements were conducted on leaves of peripheral twigs, being under steady-state at a light intensity range of 150–300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. According to [Kreuzwieser et al. \(1997\)](#) this light intensity enables photosynthesis in the shade-tolerant beech to proceed to ca. 70–100% of the rate at light saturation, which occurs at PAR values around 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Leaf (xylem) water potential (Ψ) was determined on three fully expanded leaves of the twig harvested for phloem sampling, from eight adult beech trees, according to [Scholander et al. \(1965\)](#) using a pressure chamber (Wescor Inc., Logan, UH, USA). Leaf water potential measurements were conducted between 09:00 h and

10:00 h, directly after leaf harvest, given that technical difficulties did not allow pre-dawn measurements.

2.7. Data analysis

Statistical analysis was performed using SPSS 12.0 (SPSS, Inc., Chicago, IL, USA). For all parameters, statistically significant differences between and within the three measuring years were assessed by applying Univariate Analysis of Variance, after data has been tested for normal distribution. Homogenous groups were detected with a Duncan post hoc test at a 95% level of significance. Regression models between physiological parameters, as well as between physiological and environmental parameters were built with the stepwise procedure which removes from the model any entered parameter with $p > 0.05$. Water potential, effective quantum yield of PSII and carbon and oxygen isotopic compositions were tested as dependent variables. The respective environmental parameters were rainfall, air relative humidity, air vapour pressure deficit, mean, maximum and minimum air temperature, and soil water content averaged over (a) the respective months the dependent variable was measured, (b) the preceding month and (c) five days prior to measurement.

3. Results

3.1. Climatic characterization of the studied years

The studied beech site is characterized by a relatively dry season in July and August, as indicated by the climate diagram of the area ([Fig. 1](#)). Cumulative climatic parameters during the growing season, which are of particular biological importance for plants' growth and survival, were calculated for the three years of study ([Fig. 2](#)). Cumulative air temperature, stand evapotranspiration and maximum evaporation were the highest in 2003 compared to the other years, while cumulative precipitation of 2003 was comparable to that of 2005 but lower than in 2004 ([Fig. 2](#)). When averaged over the growing season, 2003 was characterized by the highest air temperature, stand transpiration, evapotranspiration and maximum evaporation, compared to next years ([Table 1](#)). Although precipitation of the 2003 growing season was not lower than that of 2005, the soil water content of 2003 was the lowest of all years, probably because of the high temperature and evapotranspiration during this year ([Table 1](#)). Similar air temperature and precipitation patterns during the three study years were also observed in three sites of N-NW Greece ([Table 1](#)). In combination the above-mentioned meteorological recordings indicate that the growing season of 2003 was more xeric compared to that of 2004 and 2005.

Particularly regarding the growing season of 2003, [Table 2](#) shows that air temperature of the studied beech site in Greece was higher than that of beech sites from central Europe. Furthermore, despite the higher elevation of our beech site, compared to central European sites, precipitation on Paiko Mtn. fell within the range recorded along this European transect. Therefore, the summer of 2003 was climatically similar in NW-Greece and in central Europe ([Table 2](#)).

3.2. Leaf water potential and effective quantum yield of PSII

In general, leaf water potential followed a similar seasonal trend during all years and no significant differences were detected between the years ([Fig. 3a](#)). The highest values were measured at the beginning of the growing season, followed by a sharp decrease in June. Thereafter, leaf water potential increased again and presented some smaller fluctuations and gradually declined towards the end of the growing season. Regression analysis

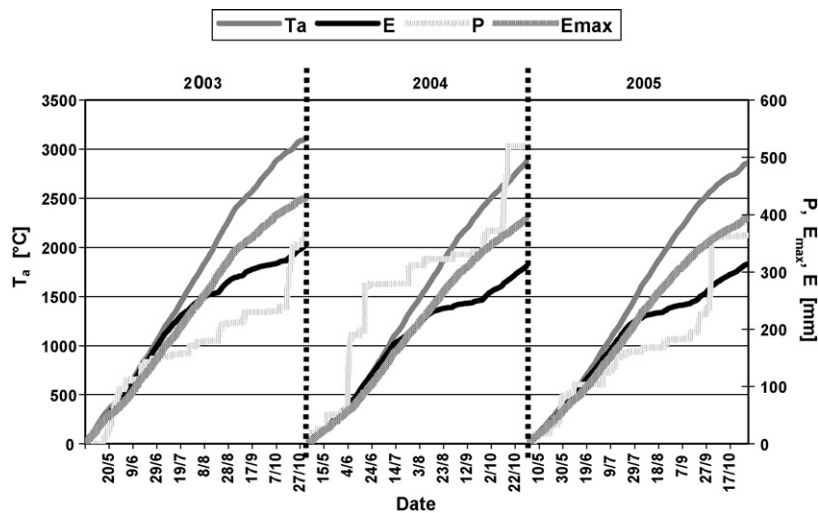


Fig. 2. Seasonal accumulation of mean daily air temperature (T_a), evaporation (E), precipitation (P) and maximum evaporation (E_{max}) during the growing season (May to October) of 2003, 2004 and 2005 in the study site on Paiko Mtn.

Table 1

Comparison of climatic parameters between the growing seasons (May to October) of the years 2003, 2004 and 2005. Data were obtained from meteorological stations of the study site (Paiko Mtn.) and of other three sites of N-NW Greece.

	Elevation [m.a.s.l.]	Latitude	Longitude	Year	Precipitation [mm]	Air temperature [°C]	Accumulated air temperature [°C]	Evapotranspiration [mm]	Maximum evaporation [mm]	Soil water content [%]
Paiko	1140	40°58'	22°20'	2003	406	16.9	3107	347	430	21.6
				2004	520	15.6	2872	310	393	24.2
				2005	365	15.7	2858	314	394	22.4
Loutra Thermis	30	40°30'	23°04'	2003	291	22.5				
				2004	269	21.6				
				2005	296	21.8				
Chrisopigi	650	41°10'	23°34'	2003	314	19.2				
				2004	378	18.5				
				2005	310	18.9				
Paggaio	1310	40°50'	23°12'	2003	403	15.1				
				2004	403	13.9				
				2005	565	13.1				

All values shown are averages of mean daily values, except for accumulation of air temperature which represents the sum of mean daily air temperature and precipitation that represents the sum of daily totals. Stand evaporation and evapotranspiration of the study area (Paiko Mtn.) were calculated by means of the WBS3 water balance model (Matzarakis et al., 2000; for details see Section 2).

revealed that seasonal variation in leaf water potential was solely controlled by the respective variation in soil water content ($R^2 = 0.92$, $p < 0.001$; Fig. 4a), since no significant regression model could be built with any of the other environmental or physiological parameters, or with combinations between them, tested as determinants.

The seasonal variation and gradual decline of leaf water potential was not mirrored in effective quantum yield of PSII, which remained quite more stable and was only slightly lowered in September and October (Fig. 3b).

3.2.1. Carbon isotopic composition

In beech leaves, $\delta^{13}C$ composition presented the same seasonal pattern during all years of measurements (Table 3); $\delta^{13}C$ values were the highest (less negative) at the beginning of the growing season and decreased significantly thereafter. However, 2003 was characterized by more ^{13}C -enriched leaves throughout the growing season, compared to 2004 and 2005.

In 2004 and 2005, the $\delta^{13}C$ composition of phloem from twigs and the trunk presented no significant variation during the growing season (Table 3). In 2003, $\delta^{13}C$ was the lowest in May and

Table 2

Comparison of air temperature and precipitation during the growing season (May to October) 2003 between the study area and different beech forests of Central Europe.

Parameter	Paiko Greece	Freising Germany	Freiburg Germany	Sisteron France	Tuttlingen Germany		Mühlhausen Germany
					NE	SW	
Altitude [m]	1140	485	270	1200	750		400
Precipitation [mm]	406	359	374	266	391		260
Air temperature [°C]	16.9	16.2	-	14.9	14.8		14.9
Source or citation	Present study	Löw et al. (2006)	Rebetez et al. (2006)	Météo France (St. Auban)	Deutscher Wetter Dienst		

Characteristics of the climate stations in Sisteron/France, Tuttlingen/Germany, and Mühlhausen/Germany are given by Nahm et al. (2007).

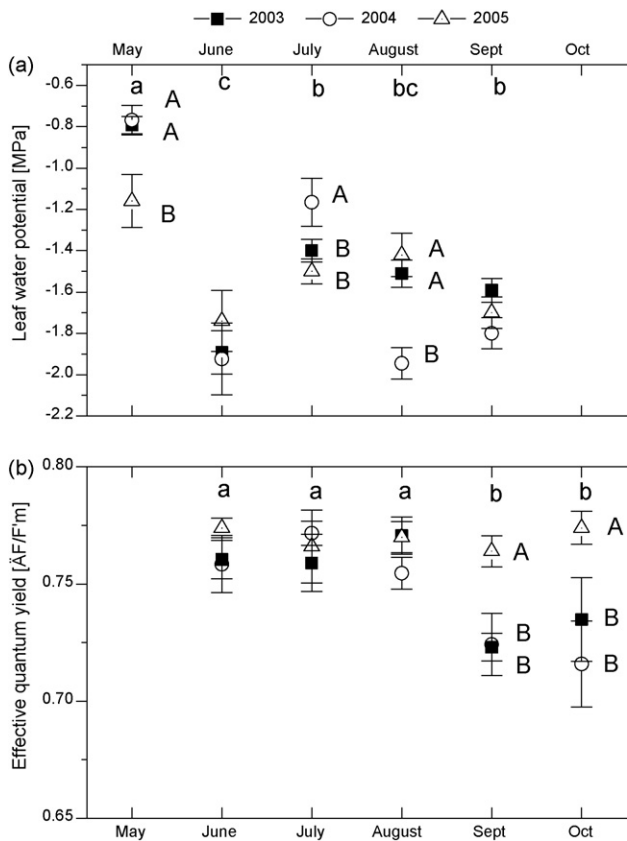


Fig. 3. Seasonal variation of (a) leaf water potential and (b) effective quantum yield of PSII during the growing season of 2003, 2004 and 2005. Both parameters were measured once per month; leaf water potential from May till September and effective quantum yield from June till October. All values shown are means (\pm SE) of eight beech trees. Statistically significant differences between the three years and within each year between the different months are indicated with upper and lower case letters, respectively. In the case of absence of significant differences no symbol is used. Means that are different at a 95% level of significance share no common letter.

increased during the growing season. Consistent to the patterns found for leaves, phloem from twigs and the trunk was significantly more ^{13}C -enriched in July and September 2003, compared to 2004 and 2005.

Regression analysis showed that the seasonal variation of foliar $\delta^{13}\text{C}$ was largely explained by the combined effect of soil water content of the current month and air vapour pressure deficit of the preceding month ($R^2 = 0.85$, $p < 0.001$; Fig. 4b). Among all climatic parameters, both twig and trunk phloem $\delta^{13}\text{C}$ were solely controlled by air vapor pressure deficit of the five days prior to plant sampling ($R^2 = 0.58$, $p = 0.011$; Fig. 4c and $R^2 = 0.50$, $p = 0.02$; Fig. 4d, respectively).

4. Discussion

The 2003 growing season was more xeric compared to the following two years, but it fell within the climatic range of the typical relatively warm and dry summers in Greece. Moreover, this growing period was warmer and with similar water availability in our site on Paiko Mtn. as in some studied beech sites of central Europe.

Contrary to effective quantum yield of PSII which presented a rather stable seasonal course, leaf water potential exhibited some seasonal variation, which was uniform in all studied years. Effective quantum yield might be less responsive to the environmental fluctuations of Mediterranean sites, thanks to biochemical mechanisms assigned to avoid any actual photoinhibition effect (Mulkey and Percy, 1992). Consistent to this, even during the

more xeric 2003 effective quantum yield remained high (ca. 0.75), at a range comparable to that measured on young beech seedlings at the absence of photoinhibition (Tognetti et al., 1998).

Furthermore, the warmer and drier 2003 did not result in differences in the leaf water potential of beech, relative to the other years. However, the fact that water potential was not measured at pre-dawn may have dampened some effects. During the exceptionally dry summer of 1998, Raftoyannis and Radoglou (2002) reported substantially lower values of beech leaf water potential, also measured between 10:00 a.m. and 12:00 p.m., which fluctuated between -2MPa and -4MPa . It can, thus, be supported that the studied beech trees maintained a favorable water status through the entire study period, despite the differences in climatic traits between the years. Contrary to our findings, studies in central European beech sites reported decreases of gas exchange and electron transport rate (Löw et al., 2006) and lower pre-dawn water potentials (Leuzinger et al., 2005) during the summer of 2003, when compared to the wetter 2004.

The seasonal fluctuation of foliar $\delta^{13}\text{C}$ was similar during all three years of measurements: foliar $\delta^{13}\text{C}$ was high at the beginning of the growing season and decreased thereafter, possibly due to the effect of stored, isotopically heavy carbohydrates remobilisation that are deposited to new tissues, as similarly reported by Skomarkova et al. (2006) for $\delta^{13}\text{C}$ in tree-rings of beech. Moreover, Holtum and Winter (2005) found that $\delta^{13}\text{C}$ of juvenile leaves are on average 1.5‰ less negative than that of mature leaves. The same seasonal, not-climatically driven, pattern of foliar $\delta^{13}\text{C}$ was reported in adult beech trees along a European gradient by Nahm et al. (2007).

The climatic difference between the years seems to be imprinted in foliar $\delta^{13}\text{C}$, which was higher during the more xeric 2003, compared to the following growing seasons. ^{13}C -enriched whole leaf dry matter is indicative of higher water use efficiency (e.g. Farquhar et al., 1989; Adams and Grierson, 2001) along the vegetation period 2003. Peñuelas et al. (2008) reported increased water use efficiency only in certain low elevation beech populations of Spain, under the pressure of warming. They suggest that some of the south European beech populations have the potential to genetically adapt to climate changes that threaten their distribution. In our study, the lower soil water content and higher air temperature during 2003, compared to next years, explain the need for higher water use efficiency and the effect of mean soil water content and vapour pressure deficit over foliar $\delta^{13}\text{C}$. Fotelli et al. (2003) and Keitel et al. (2006) also reported foliar $\delta^{13}\text{C}$ being correlated to a number of climatic parameters in beech studied along a range of environmental regimes.

Consistent to foliar $\delta^{13}\text{C}$, phloem exudates from both twigs and the trunk were ^{13}C -enriched in July and September of the drier and warmer 2003, compared to next years. Pate and Arthur (1998) and Peuke et al. (2006) similarly reported ^{13}C -enrichment of phloem extracts from eucalypt and beech, respectively, due to drought. Overall the response of phloem $\delta^{13}\text{C}$ was affected only by short-term changes in air vapor pressure deficit during the preceding five days. Keitel et al. (2003, 2006) also observed that phloem $\delta^{13}\text{C}$ was indicative only of short-term variations in micrometeorological parameters. Although short-term regulated, phloem $\delta^{13}\text{C}$ was generally affected by the drier and warmer conditions of 2003, apparently also characterized by higher air vapor pressure deficits.

In conclusion, during the summer of the relatively xeric 2003 the climatic conditions of the beech forest on Paiko Mtn., NW-Greece, were comparable to those of central European beech sites. However, comparison with long-term climatic records, and current measurements of physiological parameters revealed that beech of NW-Greece experienced only a mild drought stress in 2003, but no such stress in the next two years. In contrast, severe drought effects have been reported for beech of central Europe during the heat of 2003 (Leuzinger et al., 2005; Löw et al., 2006). Thus, similar

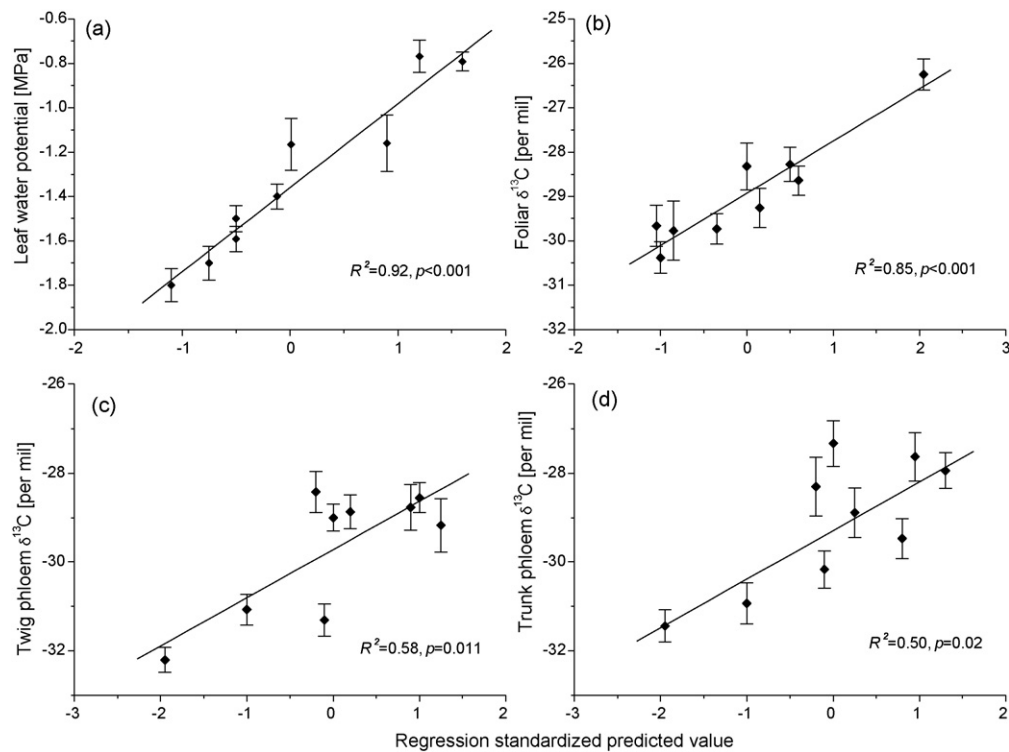


Fig. 4. Regression models describing the relationships between (a) foliar $\delta^{13}\text{C}$ and the combined effect of soil water content of the current month and air vapour pressure deficit of the preceding month of measurements, (b) leaf water potential and soil water content of the current month of measurements, (c) twig phloem $\delta^{13}\text{C}$ and air vapor pressure deficit of the preceding five days, and (d) trunk phloem $\delta^{13}\text{C}$ and air vapor pressure deficit of the preceding five days.

Table 3

Seasonal and interannual variation in $\delta^{13}\text{C}$ abundance of leaves and phloem exudates from twigs and from the trunk in adult trees of the studied beech forest on Paiko Mtn., NW-Greece.

	2003	2004	2005
Leaves			
May	$-26.25 \pm 0.4^A a$	$-28.64 \pm 0.3^B a$	$-28.28 \pm 0.4^B a$
July	$-28.32 \pm 0.5^A b$	$-29.73 \pm 0.3^B b$	$-29.77 \pm 0.7^B b$
September	$-29.25 \pm 0.4^A b$	$-29.66 \pm 0.5^{AB} b$	$-30.38 \pm 0.4^B b$
Phloem twigs			
May	$-28.86 \pm 0.3^A b$	$-28.55 \pm 0.3^A a$	$-28.42 \pm 0.5^A a$
July	$-26.57 \pm 0.3^A a$	$-29.00 \pm 0.3^B a$	$-29.17 \pm 0.6^B a$
September	$-27.05 \pm 0.4^A a$	$-28.87 \pm 0.4^B a$	$-28.77 \pm 0.5^B a$
Phloem trunk			
May	$-27.32 \pm 0.4^A c$	$-27.63 \pm 0.5^A a$	$-28.30 \pm 0.7^A a$
July	$-26.29 \pm 0.4^A b$	$-27.94 \pm 0.5^B a$	$-28.89 \pm 0.6^B a$
September	$-25.15 \pm 0.4^A a$	$-27.33 \pm 0.4^B a$	$-29.47 \pm 0.5^C a$

Upper and lower case letter refer to the comparison between years and between months of the same year, respectively. Two means are significantly different at a 95% level of significance when they share no common letter.

climatic conditions in central and Mediterranean Europe during 2003 determined different ecophysiological responses in beech populations adapted to contrasting climatic regimes. This is in agreement with the view that beech of south European origin, which is most-threatened by shifts of its geographical range, is capable of genetic adaptive changes in response to climate change (Jump et al., 2006b). Our findings may reveal to be particularly important for the ecology and management of the widely distributed European beech, in the light of the intensified drought events expected in Europe in the near future.

Acknowledgements

We thank Bettina Amtage for the assistance in field measurements, and Martin Cérnak for the biometrical characterisation of

the site. This study was supported by the IKYDA project, funded by the Greek national scholarships foundation [IKY] and the German service for academic exchanges [DAAD].

References

- Adams, M.A., Grierson, P.F., 2001. Stable isotopes at natural abundance in terrestrial plant ecology and ecophysiology: an update. *Plant Biology* 3, 299–310.
- Aranda, I., Gil, L., Pardos, J.A., 2000. Water relations and gas exchange in *Fagus sylvatica* L. and *Quercus petraea* (Mattuschka) Liebl. in a mixed stand at their southern limit of distribution in Europe. *Trees* 14, 344–352.
- Aranda, I., Gil, L., Pardos, J.A., 2005. Seasonal changes in apparent hydraulic conductance and their implications for water use of European beech (*Fagus sylvatica* L.) and sessile oak [*Quercus petraea* (Matt.) Liebl.] in South Europe. *Plant Ecology* 179, 155–167.
- Backes, K., Leuschner, C., 2000. Leaf water relations of competitive *Fagus sylvatica* and *Quercus petraea* trees during 4 years differing in soil drought. *Canadian Journal of Forest Research* 30, 335–356.
- Bergmeier, E., Dimopoulos, P., 2001. *Fagus sylvatica* forest vegetation in Greece: syntaxonomy and gradient analysis. *Journal of Vegetation Science* 12, 109–126.
- Brandes, E., Kodama, N., Whittaker, K., Weston, C., Rennenberg, H., Keitel, C., Adams, M.A., Geßler, A., 2006. Short-term variations in the isotopes signatures of organic matter allocated from the leaves to the axis of *Pinus sylvestris*—effects of photosynthetic and post-photosynthetic carbon isotope fractionation. *Global Change Biology* 12, 1922–1939.
- Bréda, N., Huc, R., Granier, A., Dreyer, E., 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science* 63, 625–644.
- Cescatti, A., Piutti, E., 1998. Silvicultural alternatives, competition regime and sensitivity to climate in a European beech forest. *Forest Ecology and Management* 102, 213–223.
- 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.
- Colom, M.R., Vazzana, C., 2003. Photosynthesis and PSII functionality of drought-resistant and drought-sensitive weeping love grass plants. *Environmental and Experimental Botany* 49, 135–144.
- Damesin, C., Lelarge, C., 2003. Carbon isotope composition of current-year shoots from *Fagus sylvatica* in relation to growth, respiration and use of reserves. *Plant Cell & Environment* 26, 207–219.

- Damesin, C., Rambal, S., Joffre, R., 1998. Seasonal and annual changes in leaf $\delta^{13}\text{C}$ in two co-occurring Mediterranean oaks: relations to leaf growth and drought progression. *Functional Ecology* 12, 778–785.
- Demesure, B., Comps, B., Petit, R.J., 1996. Chloroplast DNA phylogeography of the common beech (*Fagus sylvatica* L.) in Europe. *Evolution* 50, 2515–2520.
- Ellenberg, H., 1996. Vegetation Mitteleuropas mit den Alpen in ökologischer, dynamischer und historischer Sicht. Ulmer, Stuttgart.
- Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. In: Briggs, W.R. (Ed.), *Annual Review of Plant Physiology and Plant Molecular Biology*. Annual Reviews Inc., Palo Alto, CA, pp. 503–538.
- Fotelli, M.N., Geßler, A., Peuke, A.D., Rennenberg, H., 2001. Drought affects the competition between *Fagus sylvatica* L. seedlings and an early successional species (*Rubus fruticosus*): growth, water status and $\delta^{13}\text{C}$ composition. *New Phytologist* 151, 427–435.
- Fotelli, M.N., Rennenberg, H., Holst, T., Mayer, H., Geßler, A., 2003. Carbon isotope composition of various tissues of beech (*Fagus sylvatica*) regeneration is indicative of recent environmental conditions within the forest understorey. *New Phytologist* 159, 229–244.
- Fritsch, J., 1998. Energiebilanz und Verdunstung eines bewaldeten Hanges im Hochschwarzwald. Bericht des Meteorologischen Instituts der Universität Freiburg 1, 168.
- García-Plazaola, J.I., Esteban, R., Hormaetxe, K., Fernandez-Marin, B., Becerril, J.M., 2008. Photoprotective responses of Mediterranean and Atlantic trees to the extreme heat-wave of summer 2003 in Southwestern Europe. *Trees* 22, 385–392.
- Geßler, A., Keitel, C., Nahm, M., Rennenberg, H., 2004a. Water shortage affects the water and nitrogen balance in central European beech forests. *Plant Biology* 6, 289–298.
- Geßler, A., Rennenberg, H., Keitel, C., 2004b. Stable isotope composition of organic compounds transported in the phloem of European beech—evaluation of different methods of phloem sap collection and assessment of gradients in carbon isotope composition during leaf-to-stem transport. *Plant Biology* 6, 721–729.
- Geßler, A., Keitel, C., Kreuzwieser, J., Matyssek, R., Seiler, W., Rennenberg, H., 2007. Potential risks for European beech (*Fagus sylvatica* L.) in a changing climate. *Trees* 21, 1–11.
- Granier, A., Reichstein, M., Bréda, N., Janssens, I.A., Falge, E., Ciais, P., Grunwald, T., Aubinet, M., Berbigier, P., Bernhofer, C., Buchmann, N., Facini, O., Grassi, G., Heinesch, B., Ilvesniemi, H., Kerönen, P., Knohl, A., Köstner, B., Lagergren, F., Lindroth, A., Longdoz, B., Loustau, D., Mateus, J., Montagnani, L., Nys, C., Moors, E., Papale, D., Peiffer, M., Pilegaard, K., Pita, G., Pumpanen, J., Rambal, S., Rebmann, C., Rodrigues, A., Seufert, G., Tenhunen, J., Vesala, I., Wang, Q., 2007. Evidence for soil water control on carbon and water dynamics in European forests during the extremely dry year: 2003. *Agricultural and Forest Meteorology* 143, 123–145.
- Helle, G., Schleser, G.H., 2004. Beyond CO_2 -fixation by Rubisco—an interpretation of $^{13}\text{C}/^{12}\text{C}$ variations in tree rings from novel intra-seasonal studies on broad-leaf trees. *Plant Cell & Environment* 27, 367–380.
- Holtum, J.A.M., Winter, K., 2005. Carbon isotope composition of canopy leaves in a tropical forest in Panama throughout a seasonal cycle. *Trees* 19, 545–551.
- IPCC, 2007. *Climate Change 2007: The Physical Science Basis*. Summary for Policymakers, Paris.
- Jump, A.S., Hunt, J.M., Peñuelas, J., 2006a. Rapid climate change-related growth decline at the southern range edge of *Fagus sylvatica*. *Global Change Biology* 12, 2163–2174.
- Jump, A.S., Hunt, J.M., Martínez-Izquierdo, J.A., Peñuelas, J., 2006b. Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. *Molecular Ecology* 15, 3469–3480.
- Keitel, C., Adams, M.A., Holst, T., Matzarakis, A., Mayer, H., Rennenberg, H., Geßler, A., 2003. Carbon and oxygen isotope composition of organic compounds in the phloem sap provides a short-term measure for stomatal conductance of European beech (*Fagus sylvatica* L.). *Plant Cell & Environment* 26, 1157–1168.
- Keitel, C., Matzarakis, A., Rennenberg, H., Geßler, A., 2006. Carbon isotopic composition and oxygen isotopic enrichment in phloem and total leaf organic matter of European beech (*Fagus sylvatica* L.) along a climate gradient. *Plant Cell & Environment* 29, 1492–1507.
- Kreuzwieser, J., Herschbach, C., Stulen, I., Wiersema, P., Vaalburg, W., Rennenberg, H., 1997. Interactions of NH_4^+ and L-glutamate with NO_3^- transport processes of non-mycorrhizal *Fagus sylvatica* roots. *Journal of Experimental Botany* 48, 1431–1438.
- Leuschner, Ch., Backes, K., Hertel, D., Schipka, F., Schmitt, U., Terborg, O., Runge, M., 2001. Drought responses at leaf, stem and fine root levels of competitive *Fagus sylvatica* L. and *Quercus petraea* (Matt.) Liebl. trees in dry and wet years. *Forest Ecology and Management* 149, 33–46.
- Leuzinger, S., Zotz, G., Asshoff, R., Körner, C., 2005. Responses of deciduous forest trees to severe drought in Central Europe. *Tree Physiology* 25, 641–650.
- Löw, M., Herbinger, K., Nunn, A.J., Häberle, K.-H., Leuchner, M., Heerd, C., Werner, H., Wipfler, P., Pretzsch, H., Tausz, M., Matyssek, R., 2006. Extraordinary drought of 2003 overrules ozone impact on adult beech trees (*Fagus sylvatica*). *Trees* 20, 539–548.
- Matzarakis, A., Mayer, H., Schindler, D., Fritsch, J., 2000. Simulation des Wasserhaushaltes eines Buchenwaldes mit dem forstlichen Wasserhaushaltsmodell WBS3. Bericht des Meteorologischen Instituts der Universität Freiburg 5, 137–146.
- Mulkey, S.S., Pearcy, R.W., 1992. Interactions between acclimation and photoinhibition of photosynthesis of a tropical forest understory herb, *Alocasia macrorrhiza*, during simulated canopy formation. *Functional Ecology* 6, 719–729.
- Nahm, M., Holst, T., Matzarakis, A., Mayer, H., Rennenberg, H., Geßler, A., 2006a. Soluble N compound profiles and concentrations in European beech (*Fagus sylvatica* L.) are influenced by local climate and thinning. *European Journal of Forest Research* 125, 1–14.
- Nahm, M., Radoglou, K., Rennenberg, H., Chalyvopoulos, G., Fotelli, M.N., 2006b. Physiological performance of beech (*Fagus sylvatica* L.) at its south-eastern distribution limit in Europe: seasonal changes in nitrogen, carbon and water balance. *Plant Biology* 8, 52–63.
- Nahm, M., Matzarakis, A., Rennenberg, H., Geßler, A., 2007. Seasonal courses of key parameters of nitrogen, carbon and water balance in European beech (*Fagus sylvatica* L.) grown on four different study sites along a European North–South climate gradient during the 2003 drought. *Trees* 21, 79–92.
- Pate, G., Arthur, D., 1998. $\delta^{13}\text{C}$ analysis of phloem sap carbon: novel means of evaluating seasonal water stress and interpreting carbon isotope signatures of foliage and trunk wood of *Eucalyptus globulus*. *Oecologia* 117, 301–311.
- Peñuelas, J., Boada, M., 2003. A global change-induced biome shift in the Montseny mountains (NE Spain). *Global Change Biology* 9, 131–140.
- Peñuelas, J., Ogaya, R., Hunt, J.M., Jump, A.S., 2008. 20th century changes of tree-ring $\delta^{13}\text{C}$ at the southern range-edge of *Fagus sylvatica*. Increasing water-use efficiency does not avoid the growth decline induced by warming at low altitudes. *Global Change Biology* 14, 1076–1088.
- 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.
- Raftoyannis, Y., Radoglou, K., 2002. Physiological responses of beech and sessile oak in a natural mixed stand during a dry summer. *Annals of Botany* 89, 723–730.
- Rebetez, M., Mayer, H., Dupont, O., Schindler, D., Gartner, K., Kropp, J., Menzel, A., 2006. Heat and drought 2003: a climate synthesis. *Annals of Forest Science* 63, 569–577.
- Rennenberg, H., Schneider, S., Weber, P., 1996. Analysis of uptake and allocation of nitrogen and sulphur compounds by trees in the field. *Journal of Experimental Botany* 47, 1491–1498.
- Sabate, S., Gracia, C.A., Sanchez, A., 2002. Likely effects of climate change on growth of *Quercus ilex*, *Pinus halepensis*, *Pinus pinaster*, *Pinus sylvestris* and *Fagus sylvatica* forests in the Mediterranean region. *Forest Ecology and Management* 162, 23–37.
- Scarascia-Mugnozza, G., Oswald, H., Piussi, P., Radoglou, K., 2000. Forests of the Mediterranean region: gaps in knowledge and research needs. *Forest Ecology and Management* 132, 97–109.
- Scartazza, A., Mata, C., Matteucci, G., Yakir, D., Moscatello, S., Brugnoli, E., 2004. Comparisons of $\delta^{13}\text{C}$ of photosynthetic products and ecosystem respiratory CO_2 and their response to seasonal climatic variability. *Oecologia* 140, 340–351.
- Schär, C., Vidale, P.L., Lüthi, D., Frei, C., Häberli, C., Liniger, M.A., Appenzeller, C., 2004. The role of increasing temperature variability in European summer heatwaves. *Nature* 427, 332–336.
- Schmidt, J., 1990. Überlegungen zur Erfassung und Beschreibung von Wachstumsgängen am Beispiel der Durchmesserzuwachsentwicklung der letzten Jahrzehnte von Fichtenbeständen in Bayern unter besonderer Berücksichtigung witterungsbedingter Zuwachsreaktionen. Forstliches Forschungsbericht 104, 164.
- Schneider, S., Geßler, A., Weber, P., von Sengbusch, D., Hanemann, U., Rennenberg, H., 1996. Soluble N compounds in trees exposed to high loads of N: a comparison of spruce (*Picea abies*) and beech (*Fagus sylvatica*) grown under field conditions. *New Phytologist* 134, 103–114.
- Scholander, P.F., Hammel, T., Branstreet, E.D., Hemmingsen, E.A., 1965. Sap pressure in vascular plants. *Science* 148, 339–345.
- Skomarkova, M.V., Vaganov, E.A., Mund, M., Knohl, A., Linke, P., Boerner, A., Schulze, E.-D., 2006. Inter-annual and seasonal variability of radial growth, wood density and carbon isotope ratios in tree rings of beech (*Fagus sylvatica*) growing in Germany and Italy. *Trees* 20, 571–586.
- Tognetti, R., Johnson, J.D., Michelozzi, M., 1995. The response of European beech (*Fagus sylvatica* L.) seedlings from two Italian populations to drought and recovery. *Trees* 9, 348–354.
- Tognetti, R., Minotta, G., Pinzauti, S., Michelozzi, M., Borghetti, M., 1998. Acclimation to changing light conditions of long-term shade-grown beech (*Fagus sylvatica* L.) seedlings at two different geographic origins. *Trees* 12, 326–333.
- Zerva, A., Halyvopoulos, G., Radoglou, K., 2008. Fine root biomass in a beech (*Fagus sylvatica* L.) stand on Paiko Mountain, NW Greece. *Plant Biosystems* 142, 381–385.