Seasonal variations in photosynthesis, intrinsic water-use efficiency and stable isotope composition of poplar leaves in a short-rotation plantation

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Received April 2, 2014; accepted June 3, 2014; published online July 28, 2014; handling Editor Ülo Niinemets

Photosynthetic carbon assimilation and transpirational water loss play an important role in the yield and the carbon sequestration potential of bioenergy-devoted cultures of fast-growing trees. For six poplar (Populus) genotypes in a short-rotation plantation, we observed significant seasonal and genotypic variation in photosynthetic parameters, intrinsic water-use efficiency (WUEi) and leaf stable isotope composition (δ13C and δ18O). The poplars maintained high photosynthetic rates (between 17.8 and 26.9 µmol m⁻² s⁻¹ depending on genotypes) until late in the season, in line with their fast-growth habit. Seasonal fluctuations were mainly explained by variations in soil water availability and by stomatal limitation upon photosynthesis. Stomatal rather than biochemical limitation was confirmed by the constant intrinsic photosynthetic capacity (Vcmax) during the growing season, closely related to leaf nitrogen (N) content. Intrinsic water-use efficiency scaled negatively with carbon isotope discrimination (Δ13Cbl) and positively with the ratio between mesophyll diffusion conductance (gm) and stomatal conductance. The WUEi–Δ13Cbl relationship was partly influenced by gm. There was a trade-off between WUEi and photosynthetic N-use efficiency, but only when soil water availability was limiting. Our results suggest that seasonal fluctuations in relation to soil water availability should be accounted for in future modelling studies assessing the carbon sequestration potential and the water-use efficiency of woody energy crops.

Keywords: leaf nitrogen, maximum rate of carboxylation, mesophyll conductance, photosynthetic nitrogen-use efficiency, short-rotation-coppice, soil water deficit, stomatal conductance.

Introduction

Fast-growing tree species, such as poplar and willow, implemented in short-rotation bioenergy cultures (SRC), represent a promising renewable energy source (AEBIOM 2012). The success of this renewable bioenergy largely depends on the yields that can be achieved. The large genetic variability found within the Populus genus (Dunlap and Stettler 1998, Al Afas et al. 2005, Paris et al. 2011, Broeckx et al. 2012a, 2012b) offers the possibility to select highly productive genotypes. The high productivity of poplar has been associated with its high water use (water consumption) (Zsuffa et al. 1996, Allen et al. 1999, Meiresonne et al. 1999) and with its sensitivity to drought (Lindroth et al. 1994, Liang et al. 2006, Monclus et al. 2009). The increasing probability of seasonal droughts (Easterling et al. 2000, Seneviratne et al. 2010) and the prospects of freshwater scarcity (Berndes 2002) emphasize the
importance of traits such as water-use efficiency (WUE) and drought tolerance as the selection criteria for biomass production under future climate conditions (King et al. 2013).

At the whole-plant level, WUE is defined as plant dry matter production per unit of water loss via transpiration. Substantial species and genotypic variation in whole-plant WUE have been reported (Cernusak et al. 2007, Linderson et al. 2007, Rasheed et al. 2013). At the leaf level, intrinsic water-use efficiency (WUEi) is defined as the instantaneous ratio between net CO2 assimilation rate (A) and stomatal conductance to water vapour (gs). The carbon isotope discrimination (Δ13C) is expected to scale negatively with WUEi (Farquhar and Richards 1984), and has been commonly used as an indicator of WUEi in poplar (Ripullone et al. 2004, Monclus et al. 2006, Bonhomme et al. 2008, Dillen et al. 2008, Fichot et al. 2011, Rasheed et al. 2013). However, the relationship between Δ13C and WUEi can be disturbed because of differences in the respective time of integration (Ponton et al. 2002, Ripullone et al. 2004) or of variable mesophyll diffusion conductances (gm) (Warren and Adams 2006, Soolanayakanahally et al. 2009). Selecting genotypes for low Δ13C—and assuming high WUEi—may not necessarily result in a selection towards higher productivity, as this depends on the main source of variation driving WUEi (Gilbert et al. 2011). As such, Δ13C does not allow distinguishing between the effects of A and gs. On the contrary, the oxygen composition of organic matter (δ18O) may be used to independently estimate variations in WUEi originating from variations in gs (Scheidegger et al. 2000, Barbour 2007). As water in the leaf is the most important source of oxygen, bulk leaf oxygen isotope composition (δ18O) integrates gs over the leaf life span. It thus combines source water oxygen isotope composition and leaf water enrichment, partly affected by evaporative processes. When combined with Δ13C data, δ18O is a means to distinguish between the different sources of variation in WUEi.

Seasonal variations in photosynthetic parameters and resource-use efficiency largely affect the modelling of ecosystem carbon uptake (Wilson et al. 2001, Wang et al. 2004, Kosugi and Matsuo 2006, Zhu et al. 2011), determining the efficiency of bioenergy cultures. Strong seasonal variations in photosynthetic parameters have been reported for deciduous species, but mostly under Mediterranean climate conditions in relation to water availability (Wilson et al. 2000b, 2001, Xu and Baldocchi 2003, Limousin et al. 2010, Misson et al. 2010). Stomatal closure is generally the primary diffusive limitation to carbon assimilation rate and one of the earliest responses to drought during the growing season (Wilson et al. 2000b, Chaves et al. 2002, Flexas and Medrano 2002). Mesophyll conductance to CO2 (gm) also decreases in response to decreasing soil water availability, adding an additional resistance to CO2 diffusion to the chloroplasts (Rouspard et al. 1996, Gras and Magnani 2005, Limousin et al. 2010, Misson et al. 2010). Photosynthetic limitations because of biochemical impairments are generally observed under severe water stress (Bota et al. 2004, Flexas et al. 2004). Besides increasing WUE, stomatal closure decreases photosynthetic nitrogen-use efficiency (PNUE), defined as the ratio between A and leaf nitrogen (N) concentration (Warren and Adams 2006). The trade-off between WUE and PNUE arises from the generally observed relationship between light-saturated photosynthesis (A sat) and leaf N (Xu and Baldocchi 2003). Stomatal closure has a smaller effect on photosynthesis when compared with the direct impact on transpiration, and has no effect on leaf N. However, gm influences the variation in PNUE, and hence its relationship with WUE (Warren and Adams 2006). The role of gm and its relationship to gs is important for a better understanding of the economics of photosynthetic and N use in a changing climate (Buckley and Warren 2014).

For SRC plantations under temperate climate conditions, the seasonal evolution of photosynthesis, transpirational water loss and WUE are of utmost importance for their productivity and biomass yield. This is especially true when one considers that (i) seasonal variability in photosynthesis is a strong determinant of carbon balance and therefore of the environmental benefit of bioenergy-devoted plantations (Zona et al. 2012); (ii) SRC plantations devoted to biomass production rely on high-yielding species which are generally very sensitive to fluctuations in water availability, such as poplars (Lindroth et al. 1994, Liang et al. 2006, Monclus et al. 2009); (iii) high planting densities (6000–20,000) are likely to exacerbate competition for water acquisition and lead to faster water depletion (Toillon et al. 2013); and (iv) climate change might result in increased frequency and duration of abnormal drought episodes (IPCC 2007, Seneviratne et al. 2010). In view of the above, the seasonal evolution in Δ13C and δ18O as a potential indicator of WUEi, in combination with seasonal changes in photosynthesis provides a more detailed study, in particular under field conditions of changing soil water availability. The rationale of the present study is also to quantify genotypic variation in the aforementioned seasonal evolution and in the WUE, relationships for poplar.

The objectives of this study were (i) to investigate and characterize seasonal and genotypic variation in photosynthesis, WUE, and leaf stable isotope composition (13C and 18O); and (ii) to examine how genotypes and timing throughout the growing season affect the relationships between the aforementioned leaf traits. Measurements were performed in a young SRC plantation on six genotypes throughout the growing season (from early May to the end of September 2011). An atypical dry spring to summer period allowed studying the effect of soil water availability. We hypothesized that decreased soil water availability would lead to an increased WUEi, and to a decreased PNUE, mainly due to diffusional (stomatal and mesophyll) limitation of assimilation. We investigated the relationship between WUEi, Δ13C and δ18O, as well as between PNUE and WUEi, including the potential effect of gm. Based on the theory, we expected an
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Experimental site and plant material

The experimental site was located in Lochristi, East-Flanders, Belgium (51°06′44″N, 3°51′02″E; 6.25 m above sea level). The poplar bioenergy plantation (http://uahost.uantwerpen.be/popfull) was established in April 2010 on 18.4 ha of former agricultural land. The long-term average annual temperature at the site is 9.5 °C and the average annual precipitation is 726 mm, equally distributed over the year. A detailed soil analysis of the experimental site was performed repeatedly on the same trees during the 2011 growing season in seven measurement campaigns (MCs): 4–6 May (MC1), 18–20 May (MC2), 4–8 July (MC3), 27–29 July (MC4), 16–19 August (MC5), 5–9 September (MC6) and 26–30 September 2011 (MC7). For the six genotypes, we sampled leaves from the upper canopy, on the first fully mature sunlit leaf of the current-year main axis. To minimize differences in leaf age across MCs, we sampled leaves of the same leaf rank. Leaves were first acclimated for 10 min in the chamber at a CO2 concentration of 400 ppm and under a photosynthetic photon flux density (PPFD) of 1500 µmol m−2 s−1. Preliminary test experiments had shown that this PPFD was enough to ensure saturating light conditions for all genotypes. Afterwards, light-saturated assimilation rate at atmospheric CO2 concentration (A_sat, µmol m−2 s−1) and stomatal conductance (gs_sat, mol m−2 s−1) were recorded before establishing the response of the net assimilation rate (A) to varying intercellular CO2 concentrations (Ci), i.e., the A–Ci curve. Each curve consisted of 10 steps of external CO2 concentrations set in succession to 400, 300, 250, 150, 100, 50, 500, 750, 1000 and 1250 ppm (Monclus et al. 2006). Leaves were allowed to equilibrate at least 3 min at each step before data were collected. Net assimilation rates were corrected for the effect of CO2 diffusion, according to the instrument manual (LI-6400XT Version 6), using a diffusion correction term of 0.46 µmol s−1. Before logging at each step of the A–Ci curves, steady state (F_s) and maximum fluorescence (F_m′) were measured during a light-saturating pulse (7 mmol m−2 s−1) and the efficiency of Photosystem II (ΦPSII) was determined as:

ΦPSII = F_m′ − F_s
F_m′
Then, the CO₂ concentration in the chamber was set back to 400 ppm. Once the net assimilation rate had stabilized, the response to varying light intensities was recorded (A-light curve). Leaf photosynthesis was measured at eight PPFD intensities in the following order: 1500, 1000, 800, 600, 400, 200, 100, 0 μmol m⁻² s⁻¹. A minimum of 2 min of leaf equilibration was set at each step before data were logged. Dark respiration was defined as the absolute CO₂ exchange rate measured during the last step of the A-light curve. All measurements were done at a constant block temperature (25 °C) and a controlled VPD close to 1 kPa (1.2 ± 0.04, mean ± SE). Intrinsic water-use efficiency under saturating conditions (WUE, mmol CO₂ mol H₂O⁻¹) was calculated as the ratio between the values of A_sat and gₛ_sat obtained from the A–C and A-light curves under reference conditions (PPFD of 1500 μmol m⁻² s⁻¹ and CO₂ concentration of 400 ppm).

Once gas exchange measurements were completed, a minimum of six chlorophyll readings was taken on the same leaf with a portable chlorophyll content meter (CCM-200, Opti-Sciences, Inc., Hudson, NH, USA). Total chlorophyll content (Chl) was estimated from the CCM values according to the equations reported in Richardson et al. (2002). The leaf sample was then harvested and the individual leaf area (LA) was measured using a LI-3000 leaf area meter (Li-Cor). A subsample was punched out of the leaf lamina to determine leaf mass per area (LMA, g m⁻²) after drying at 70 °C; LMA was only available from MC3 onwards. The dried leaf material was then ground and used for the assessment of the leaf N content per unit mass (N₁₀⁻¹, mg g⁻¹) with an elemental analyser (Carlo Erba, NA 1500-NC, Milan, Italy). The values of N₁₀ were converted to N content per unit area (Nₛ, mg cm⁻²) using LMA values. Photosynthetic nitrogen-use efficiency (μmol mg⁻¹ s⁻¹) was calculated as the ratio of A_sat (μmol m⁻² s⁻¹) to Nₛ.

**Estimation of mesophyll conductance and photosynthetic parameters**

The mesophyll diffusion conductance to CO₂ from the substomatal cavities to the chloroplast (gₘ) was estimated by combining gas exchange and chlorophyll fluorescence measurements (Pons et al. 2009). The rate of photosynthetic electron transport (IₑTR) was calculated as:

\[ IₑTR = \alpha \times 0.5 \times PPFD \times \phi_{PSH}, \]

where \( \alpha \) is the leaf absorptance and 0.5 is the fraction of photons absorbed by Photosystem II. Absorptance was derived from the CCM readings according to Bauerle et al. (2004) after conversion of the CCM readings to soil plant analysis development (SPAD) values (Richardson et al. 2002). Mesophyll conductance was then estimated following the equation of Harley et al. (1992):

\[ gₘ = \frac{A}{C_i - \left(42.7\left[IₑTR + 8(A + R_d)\right]/[IₑTR - 4(A + R_d)]\right)^{1/2}}, \]

where 42.7 is the CO₂ compensation point in the absence of dark respiration, as taken from Bernacchi et al. (2001), and \( R_d \) is the mitochondrial respiration in the light, taken as half of the dark respiration obtained from the A-light curves (Piel et al. 2002, Niinemets et al. 2005). The values of \( gₘ \) were then used to convert A–C curves to A–Cₑ curves, with Cₑ being the CO₂ concentration in the chloroplast stroma calculated as (Limousin et al. 2010, Misson et al. 2010):

\[ Cₑ = C_i - \frac{A}{gₘ}. \]

The maximum carboxylation rate (\( V_{cmax} \)) and the maximum rate of electron transport (\( Iₐₘₐₓ \)) were estimated by fitting the A–Cₑ curves to the biochemical photosynthesis model of Farquhar et al. (1980) using the routine developed by Sharkey et al. (2007). The Michaelis constant of Rubisco for carbon dioxide (\( K_c \)), the inhibition constant of Rubisco for oxygen (\( K_o \)) and the photocompensation point (\( i^* \)) used for fitting were taken from Sharkey et al. (2007).

**Carbon and oxygen stable isotope analyses**

Isotopic analyses were performed at the Stable Isotope Laboratory of the James Hutton Institute (Invergowrie, Dundee, UK). Bulk leaf carbon isotope composition (\( \delta^{13}\text{C}_{bl} \)) was determined on the leaves used for gas exchange measurements. Subsamples of ground leaf material were enclosed and weighed in tin capsules and combusted in a continuous flow isotope ratio mass spectrometer (IRMS) (Delta V, Thermo Fisher Scientific, Bremen, Germany). The CO₂ produced by combustion was purified and its \( ^{13}\text{CO}_₂/^{12}\text{CO}_₂ \) ratio was analysed by the IRMS. The \( \delta^{13}\text{C}_{bl} \) (%) was expressed relative to the Pee Dee Belemnite standard (Craig 1957). The accuracy of measurements was assessed by repeated measures of laboratory standards and was ±0.08‰ (standard deviation). Carbon isotope discrimination between the atmosphere and the bulk leaf organic matter (\( \Delta^{13}\text{C}_{bi} \) %) was then calculated as in Farquhar et al. (1989):

\[ \Delta^{13}\text{C}_{bi} = \frac{\delta^{13}\text{C}_{air} - \delta^{13}\text{C}_{bl}}{[1+(\delta^{13}\text{C}_{bi}/1000)]}, \]

with \( \delta^{13}\text{C}_{air} \) assumed to equal −8‰.

The same leaf powder used for \( ^{13}\text{C} \) analyses was used to measure the \( ^{18}\text{O} \) composition of bulk leaf matter (\( \delta^{18}\text{O}_{bi} \)). Leaf material was enclosed and weighed in silver capsules. Analyses were conducted with a continuous flow IRMS (Delta Plus XP, Thermo Fisher Scientific) interfaced with a high temperature elemental analyser. Bulk leaf oxygen isotope composition was expressed relative to the sea mean ocean water standard.
and the analytical precision for repeated measurements was ±0.09‰ (standard deviation).

**Statistical analysis**

Some data were missing at random (MAR) and could be ignored for parameters and genotypes that were measured from the third MC onwards (Verbeke and Molenberghs 2000). The mixed procedure for repeated measurements was used to analyse the effects of genotype and seasonality on the above-mentioned parameters. Measurements were performed seven times during the growing season (repeated variable ‘MC’) on the same four replicate trees of each genotype (subject variable ‘Tree’). A linear mixed model with fixed effects genotype, MC and their interaction—indicating the genotype-specific behaviour in time—was used. The unstructured repeated covariance structure was chosen and the variance component was estimated using restricted maximum likelihood (REML). When significant genotype or MC effects were found, pairwise comparisons of the means were performed using the Bonferroni adjustment (see Table S2A available as Supplementary Data at Tree Physiology Online). Relationships between photosynthetic, WUE and isotopic parameters were examined using linear and non-linear regression analyses and coefficients of determination ($R^2$) as well as genotype-specific Spearman’s rank correlation coefficients. All statistical tests were considered significant when $P<0.05$. All statistical analyses were performed in SPSS 20.0 (IBM Corp., SPSS Statistics for Windows, Armonk, NY, USA).

**Results**

The seasonal course of precipitation, air temperature, daytime (SWR $>20$ W m$^{-2}$) maximum VPD ($V_{PD_{max}}$) and soil water availability at the site during the 2011 growing season (May–September) is presented in Figure 1. Although the 2011
The growing season showed a normal pattern of temperature and rainfall throughout the season, there were a few periods with dry conditions. The first drop in soil water potential ($\Psi_s$) was observed at the end of May in the upper soil layer (20 cm depth). Close to MC2 $\Psi_s$ peaked at approximately $-1.8$ MPa, while there was no apparent response in the 40-cm depth layer. The precipitation and lower VPD that occurred afterwards led to a progressive recovery. The second drop in $\Psi_s$ to $-1.5$ MPa was observed in mid-July (close to MC3) for both soil layers, concomitantly with high air temperatures and high VPD. The high amount of precipitation after mid-July combined with a progressive decrease in VPD resulted in the recovery of $\Psi_s$ close to zero for the rest of the growing season (MC4–MC7).

Overall, nearly all of the photosynthetic leaf traits differed significantly among genotypes and fluctuated during the growing season, i.e., along the MCs (Figure 2, Table 1; see Table S2A available as Supplementary Data at Tree Physiology Online). Considering the average pattern across the six genotypes, $A_{sat}$, $g_{s\text{-}sat}$ and $\Delta^{13}C_{bl}$ exhibited a similar time course with a pronounced decrease in mid-July (MC3), when the soil water potential was low at both 20 and 40 cm depths, and a progressive increase towards the end of the growing season (Figure 2a, b and d). The values of WUE, followed an opposite trend (Figure 2c). The time course observed for $V_{cmax}$, $J_{max}$ and $g_m$ was slightly different. $V_{cmax}$ and $J_{max}$ increased progressively during the growing season and $g_m$ decreased, especially in July (MC3; Figure 2f and g; $J_{max}$ data not shown). Mesophyll conductance showed overall a similar seasonal evolution to that of $g_{s\text{-}sat}$ for the dry period May–July but remained higher than $g_{s\text{-}sat}$ (Figure 2b and g). From August onwards, however, $g_{s\text{-}sat}$ and $g_m$ followed an opposite pattern; $g_m$ decreased progressively (Figure 2b and g). The overall means of $V_{cmax}$ and $J_{max}$ were $125.2$ and $172.1 \mu$mol m$^{-2}$ s$^{-1}$, respectively (Figure 2f), both parameters being strongly and linearly correlated across genotypes and MCs ($R^2 = 0.79; P < 0.0001$). A decrease throughout the growing season was also observed in $\delta^{18}O_{bl}$ (Figure 2e). The seasonal trends in $N_A$ and in LMA were less obvious (despite a significant time effect; Table 1), potentially due to some missing data points in the beginning of the growing season (Figure 2h and i). Photosynthetic nitrogen-use efficiency was lower in July at low soil water availability (MC3; Figure 2j), but—as for $N_A$ and LMA—the response to water availability was less clear. Genotypes were not significantly different in their seasonal evolution of $N_A$ and PNU (Table 1).

A closer look at the observed results showed that the six genotypes did not respond in the same way or with the same amplitude with time in the growing season, as indicated by the significant genotype × MC interactions observed for most traits (Table 1; see also Figure 2). Differences among genotypes were particularly reinforced during the dry period around MC3 (Figure 2). Genotypes Wolterson and Oudenberg were clearly less responsive to the dry period than the other genotypes in terms of $A_{sat}$, $g_{s\text{-}sat}$, WUE, and PNUE (Figure 2a–c and j). Overall, genotype Wolterson showed the highest values of $A_{sat}$, $g_{s\text{-}sat}$, $V_{cmax}$ and $N_A$ throughout most of the growing season (Figure 2a, b, f and h), while WUE, was at the lower end of the genotypic range (Figure 2c). The ranking of the other genotypes changed substantially during the growing season, although genotypes Bakan and Skado remained consistently at the lower end of the range for $A_{sat}$ and $g_{s\text{-}sat}$ (Figure 2a and b). Genotype Skado had the lowest $\Delta^{13}C_{bl}$ and the highest WUE, throughout the entire growing season (Figure 2d and e). On the other end, the highest $\Delta^{13}C_{bl}$ values were observed for genotype Grimminge, which also showed the lowest $V_{cmax}$ and $J_{max}$ values with an early decrease from August onwards (MC5–7; Figure 2d and f). In contrast to other leaf traits, the genotypic ranking for $N_A$ did not significantly change throughout the growing season (no significant genotype × MC interaction, Table 1; Figure 2h). Genotypes Wolterson and Grimminge generally showed the highest and lowest $N_A$ values, respectively (Figure 2h).

The values of $A_{sat}$ and $g_{s\text{-}sat}$ were significantly, but non-linearly, related ($A_{sat} = 36.25 \times g_{s\text{-}sat} / (0.22 + g_{s\text{-}sat})$, with $A_{sat}$ reaching saturation at high $g_{s\text{-}sat}$ (Figure 3). A similar but less significant pattern was found between $A_{sat}$ and $g_m$ ($A_{sat} = 22.67 \times (1 - e^{-7.27 \times g_{s\text{-}sat}})$), with $A_{sat}$ saturating at high $g_m$. The relationship between $g_{s\text{-}sat}$ and $g_m$ was linear at low values, with a decoupling among both parameters at higher values ($g_m = 0.33 \times (1 - e^{-15.55 \times g_{s\text{-}sat}})$). The linear part of the relationship was mainly determined by values recorded during MC3, when soil water availability was reduced.

Net assimilation rate was linearly and negatively related to WUE (Figure 4a). A stronger negative, but non-linear (WUE = $(0.15 \times 0.31) / (0.31 + g_{s\text{-}sat})$) relationship was found between $g_{s\text{-}sat}$ and WUE (Figure 4b). The ratio of $g_m$ to $g_{s\text{-}sat}$ was significantly and positively related to $\Delta^{13}C_{bl}$ and $\Delta^{13}C_{ci}$ (Figure 5a). However, a significant and positive relationship was found between $\delta^{18}O_{bl}$ and WUE, (Figure 5a), while a significant and negative (non-linear; $\delta^{18}O_{bl} = (28.24 \times 2.51) / (2.51 + g_{s\text{-}sat})$; $R^2 = 0.42; P < 0.0001$) relationship was observed between $\delta^{18}O_{bl}$ and $g_{s\text{-}sat}$.

The maximum rate of carboxylation scaled positively with leaf N content, especially when expressed on an area basis (Figure 6). In addition, $V_{cmax}$ and $N_A$ scaled positively with Chl (data not shown). Leaf N content on an area basis was significantly and negatively correlated to WUE ($R^2 = 0.18; P = 0.0123$) but no relationship could be observed with $\Delta^{13}C_{bl}$. No correlation was observed between $N_A$ and LMA while $N_{bl}$ was significantly and
positively correlated to LMA ($R^2 = 0.42, P < 0.0001$). Neither $g_m$ nor $V_{cmax}$ was correlated to LMA. A significant and negative relationship was found between WUE$_i$ and PNUE, mainly due to the observations during MC3 (Figure 7a). Similarly, the significant and positive relationship between $g_m$ and PNUE was mainly driven by MC3 readings.


Discussion

The results reported above illustrate that photosynthesis-related leaf traits, including WUE$_i$, significantly varied during the growing season in an SRC bioenergy plantation, and that this variation was genotype dependent in poplar. Furthermore, our results indicate that the relation between $\Delta^{13}C_{bl}$ and WUE$_i$ did not always hold throughout the growing season, and that water availability played a significant role in this relationship. Since we have shown that seasonal variations and genotypic differences in photosynthetic parameters are substantial in SRC poplar plantations, they need to be accounted for in future modelling studies. Seasonality of leaf gas exchange can result from leaf ontogeny and dynamic changes in environmental conditions such as light, nutrients, temperature or water availability. In our study, potential effects of leaf ontogeny were minimized by always measuring recently matured leaves emerging on the current-year axis, taking advantage of the indeterminate growth of poplars. As all measurements were performed under saturating irradiance and constant VPD, seasonal variations could be mostly attributed to variations in soil water availability. Temporal variation in photosynthetic parameters is important in determining the seasonality and magnitude of ecosystem carbon fluxes and is therefore an important factor to consider for modelling studies (Wilson et al. 2001).

Table 1. Output of the mixed model analysis (REML) showing the effects of genotype and time in the season (MC) on photosynthetic and related parameters. The different parameters have been identified and described in the text. *, 0.01 < $P$ ≤ 0.05; **, 0.001 < $P$ ≤ 0.01; ***, $P$ ≤ 0.001; MC, measurement campaign.

| Parameters | df | Genotype | Df | MC | df | Genotype × MC |
|------------|----|----------|----|----|----|---------------|
| $A_{sat}$  | 5  | ***      | 6  | *** | 25 | ***           |
| $g_{s-sat}$| 5  | ***      | 6  | *** | 25 | ***           |
| WUE$_i$    | 5  | **       | 6  | *** | 25 | *             |
| $\Delta^{13}C_{bl}$ | 5  | ***      | 6  | *** | 26 | *             |
| $\delta^{18}O_{bl}$ | 5  | **       | 6  | *** | 26 | ***           |
| $V_{cmax}$ | 5  | **       | 6  | *** | 25 | ***           |
| $J_{max}$  | 5  | **       | 6  | *** | 25 | ***           |
| $g_{m}$    | 5  | ***      | 6  | *** | 24 | **            |
| $N_{A}$    | 5  | ***      | 4  | **  | 20 |               |
| LMA        | 5  | **       | 4  | *** | 20 | ***           |
| PNUΕ       | 5  | **       | 4  | *** | 19 |               |

*Heterogeneous Toeplitz covariance structure was used.

Figure 3. Curvilinear relationships between (a) stomatal conductance ($g_{s-sat}$) and net assimilation rate ($A_{sat}$); (b) mesophyll conductance ($g_{m}$) and net assimilation rate ($A_{sat}$); and (c) $g_{s-sat}$ and $g_{m}$. Data points indicate the first letter of each genotype (B, Bakan; S, Skado; K, Koster; O, Oudenberg; G, Grimminge; W, Woltersen) followed by the number of MCs (1–7) and represent the mean of four individuals. For (a) the genotype-specific Spearman's correlation coefficients are presented (**, 0.001 < $P$ ≤ 0.01; ***, $P$ ≤ 0.001).
Figure 4. Relationships between (a) net assimilation rate ($A_{\text{sat}}$) and intrinsic water-use efficiency (WUE$_i$); (b) stomatal conductance ($g_{\text{s-sat}}$) and WUE$_i$; (c) the ratio of mesophyll conductance ($g_m$) to stomatal conductance ($g_{\text{s-sat}}$) and WUE$_i$. Data points indicate the first letter of each genotype (B, Bakan; S, Skado; K, Koster; O, Oudenberg; G, Grimminge; W, Wolterson) followed by the number of MCs (1–7) and represent the mean of four individuals. For (b) the genotype-specific Spearman’s correlation coefficients are presented (*, 0.01 < $P$ ≤ 0.05; **, 0.001 < $P$ ≤ 0.01; ***, $P$ ≤ 0.001).

Figure 5. Relationships between (a) intrinsic water-use efficiency (WUE$_i$) and bulk leaf oxygen isotope composition ($\delta^{18}O_{\text{bl}}$); (b) WUE$_i$ and bulk leaf carbon isotope discrimination ($\Delta^{13}C_{\text{bl}}$); (c) bulk leaf oxygen isotope composition ($\delta^{18}O_{\text{bl}}$) and bulk leaf carbon isotope discrimination ($\Delta^{13}C_{\text{bl}}$). Data points indicate the first letter of each genotype (B, Bakan; S, Skado; K, Koster; O, Oudenberg; G, Grimminge; W, Wolterson) followed by the number of MCs (1–7) and represent the mean of four individuals. For (b) the genotype-specific Spearman’s correlation coefficients are presented (*, 0.01 < $P$ ≤ 0.05; **, 0.001 < $P$ ≤ 0.01; ***, $P$ ≤ 0.001).
Leaf photosynthetic parameters recorded in this study were consistent with data previously reported for several poplar species and hybrids (Roupsard et al. 1996, Pons and Westbeek 2004, Ripullone et al. 2004, Monclus et al. 2006, Gornall and Guy 2007, Soolanayakanahally et al. 2009, Fichot et al. 2010, 2011). Eddy covariance measurements of net ecosystem CO$_2$ fluxes performed during the same period confirmed a net ecosystem carbon uptake until the end of September (Broeckx et al. 2013, Zona et al. 2013). This indicated a good agreement between the timing of leaf-level and canopy-level photosynthetic processes in the plantation. High photosynthetic activity until the end of September has already been reported for three different genotypes of *P. alba* L., *P. nigra* L. and *P. deltoides* × *P. nigra* (Bernacchi et al. 2003). However, this pattern contrasts with data reported for other temperate deciduous species such as ash, maple and oak, for which photosynthetic uptake and
photosynthetic capacity already showed a substantial decline by early or mid-September (Wilson et al. 2000a, Grassi et al. 2005, Dillen et al. 2012). Delayed senescence with sustained carbon uptake is most likely associated with the pioneering and fast-growth habit of poplar species. Drought-induced variations in leaf photosynthesis can be mediated by stomatal closure, by changes in mesophyll conductance to CO₂ and by alterations of photosynthetic capacities. Reduced \( g_\text{m} \) was observed concomitantly with reduced \( g_{\text{s-sat}} \) during the period of low water availability, as already documented for different species (Roupsard et al. 1996, Warren et al. 2004, Galmes et al. 2007, Flexas et al. 2008). However, in the present study not all poplar genotypes responded in the same way to drier soil conditions, indicating substantial genotypic variation in the degree of this response. Local measurements of soil water potential around the mast could not exclude the possibility of genotypic differences in soil water potential related to genotypic differences in total LA. Bigger trees encounter a more rapid and more severe water shortage due to their high transpiratory water loss. In contrast, the values of \( V_{\text{cmax}} \) and \( J_{\text{max}} \) did not show any clear pattern during the dry period, suggesting that the decrease in photosynthesis was mostly caused by diffusional limitations (stomatal and non-stomatal) rather than by biochemical limitations. This is consistent with the idea that biochemical limitations become quantitatively important only during severe droughts (Grassi and Magnani 2005, Galmes et al. 2007). The values of \( V_{\text{cmax}} \) were tightly correlated with leaf N contents expressed either on a unit mass or on an area basis, suggesting that leaf N contents were a reliable estimator of photosynthetic capacities across all poplar genotypes along the growing season (Zhu et al. 2011). The constant \( N_\text{a} \) observed during the period of low water availability is in line with the absence of a reduction in \( V_{\text{cmax}} \) and suggests that there was no marked seasonal change in N allocation to the photosynthetic apparatus (i.e., Rubisco and chlorophyll) (Demarez et al. 1999, Montpied et al. 2009).

Stomatal conductance remained higher than \( g_\text{m} \) during the drier period, suggesting that stomatal conductance was actually the most limiting process to photosynthesis at this time. This is in line with other studies (Wilson et al. 2000b, Grassi and Magnani 2005, Niinemets et al. 2005, Limousin et al. 2010, Flexas et al. 2012). The relative contribution of stomatal vs mesophyll conductance to total limitations may however vary with species, with drought intensity and also with canopy position (Diaz-Espejo et al. 2007, Galmes et al. 2007, Cano et al. 2013, Flexas et al. 2013). This was confirmed in our study by the fact that \( g_\text{s} \) reached higher values than \( g_\text{m} \) by the end of the growing season. The curvilinear relationship between \( g_\text{s} \) and \( g_{\text{s-sat}} \) substantiates the modelled relationship predicted by Tholen et al. (2012). Significant relationships between \( g_\text{s} \) and \( g_\text{m} \) have also been reported in other studies (Douthe et al. 2011, Egea et al. 2011, Buckley and Warren 2014).

Variations in WUE originate from variations in either \( A \), \( g_\text{s} \) or both (Farquhar and Richards 1984). Previous studies on poplars have suggested that variations among genotypes are generally driven by variations in \( g_\text{s} \) (Monclús et al. 2006, Fichot et al. 2011, Rasheed et al. 2011, Cao et al. 2012) although one opposite result has been reported (Rasheed et al. 2013). Our results suggest that variations in WUE across dates and genotypes were primarily driven by variations in \( g_{\text{s-sat}} \). This was supported by the fact that while WUE and \( g_{\text{s-sat}} \) were negatively related—as expected—WUE and \( A_{\text{sat}} \) were also negatively related which was at first counter-intuitive. This negative relationship can be explained by the fact that variations in \( A_{\text{sat}} \) were actually overridden by larger parallel variations in \( g_{\text{s-sat}} \). In addition, WUE and \( g_{\text{s-sat}} \) were negatively and positively related to \( \delta^{18}\text{O}_{\text{bi}} \) respectively. Our results suggest that variations in \( \delta^{18}\text{O}_{\text{bi}} \) reflected a significant part of variations in \( g_{\text{s-sat}} \). The oxygen in organic matter is derived from water and the \( \delta^{18}\text{O} \) of organic matter is primarily affected by source \( \delta^{18}\text{O} \) and by evaporative processes (Scheidegger et al. 2000, Roden and Farquhar 2012). As we did not measure the source \( \delta^{18}\text{O} \) in the present study, we have no evidence for differences in the source \( \delta^{18}\text{O} \). We know, however, that the different genotypes experienced different water table depths, which significantly and spatially varied throughout the plantation (L.S. Broeckx, unpublished data). So we hypothesize that the different genotypes acquired water from different soil horizons, considering the observed genotypic differences in plant size (Duursma et al. 2011) and assuming genotypic differences in rooting depth in response to the varying water table depths. The response of rooting depth to water table depth is a trait adaptive to the native riparian habitat of poplars (Rood et al. 2003).

As expected from the theory (Farquhar et al. 1982), the values of WUE₁ and \( \Delta^{13}\text{C}_{\text{bi}} \) were significantly and negatively related, which confirms previously reported observations for various crop species (Farquhar and Richards 1984, Meinzer et al. 1990) and for woody species (Guehl et al. 1995, Ponton et al. 2002, Ripulone et al. 2004). The significant scatter in and disturbance of the observed relationship may be explained by several things. Firstly, WUE values correspond to virtually instantaneous measurements, while \( \Delta^{13}\text{C}_{\text{bi}} \) reflects a temporal integration of WUE, over the course of leaf formation and recent photosynthetic activity. Secondly, \( A_{\text{sat}} \) and \( g_{\text{s-sat}} \) were measured under saturating conditions after the sampled leaf had acclimated to the chamber conditions, such that the values of WUE, reflected ‘maximal’ functioning. This optimal functioning is obviously not maintained during the entire leaf lifespan. Thirdly, finite but variable \( g_\text{m} \) can affect WUE₁ and influence the relationship between WUE₁ and \( \Delta^{13}\text{C}_{\text{bi}} \) (Warren and Adams 2006, Flexas et al. 2008, Seibt et al. 2008, Soolanayakanahally et al. 2009). The hyperbolic relationship
observed between WUE, and \( g_s/g_{s\text{-sat}} \) supports this line of reasoning and is consistent with both theory (Flexas et al. 2013) and data reported for different species (Galme et al. 2010, Flexas et al. 2013). In addition, the observed negative \( \Delta^{13}C_{bl}-WUE_{\text{sat}} \) relationship varied significantly among genotypes and with timing throughout the growing season. This observation confirms the effects of both species and water availability on the relationship between \( \Delta^{13}C_{bl} \) and WUE that were previously reported for poplar (DesRochers et al. 2007, Xu et al. 2008, Achache et al. 2011). The absence of a correlation in genotype Wolters is most likely explained by lower water availability experienced as a consequence of low(er) total LA (Broeckx et al. 2012a, 2012b), hence the lower transpiration and reduced soil water depletion. The lack of a significant correlation observed between \( \Delta^{13}C_{bl} \) and \( \delta^{18}O_{bl} \), although \( \delta^{18}O_{bl} \) was significantly related to \( g_{s\text{-sat}} \) and WUE (see the discussion above), also reinforces the idea that the WUE--\( \Delta^{13}C_{bl} \) relationship was partly influenced by \( g_m \).

The economics of N and water use during photosynthesis is primarily interlinked through their mutual dependence on stomatal conductance. Especially during drought stomatal closure contributes to increasing WUE, on the one hand, while decreasing PNUE on the other hand resulting in a trade-off between both traits (Field et al. 1983, Warren and Adams 2006). Our results were consistent with this concept. The reduced assimilation rate caused by a decrease in stomatal conductance with constant N allocation increased the N cost per unit of carbon gain, suggesting maximization of resource-use efficiency depending on the most limiting resource (Reich et al. 1989, Xu and Baldocchi 2003, Han 2011). However, when the early July data (i.e., when low soil water availability had the largest effect on leaf gas exchange) were discarded from the analysis, WUE, and PNUE were not significantly related. This suggests that WUE, and PNUE are uncoupled under optimal conditions, as already observed for other poplar species (Soolanayakanahally et al. 2009). As suggested by Soolanayakanahally et al. (2009), this might be expected if \( g_{s\text{-sat}}, g_m \) and other factors influencing net assimilation rate vary independently, as was apparently the case in our study under non-limiting conditions (Figure 3).

In conclusion, our results showed significant seasonal evolution in photosynthesis, in WUE—as quantified by \( \Delta^{13}C \) and \( \delta^{18}O \)—and in PNUE of poplars grown under a high-density SRC regime. The seasonal evolution was mostly explained by variations in soil water availability and by stomatal control, but was strongly genotype dependent. This study suggests taking genotypic differences in seasonal evolution into account in future modelling studies.

**Supplementary data**

Supplementary data are available at *Tree Physiology* online.

**Acknowledgments**

The authors gratefully acknowledge Joris Cools for excellent technical support, Kristof Mouton for logistic support at the field site, thesis student Elyne Horemans for statistical advice, as well as Gerrit Switsers and Nadine Calluy for laboratory analyses.

**Conflict of interest**

None declared.

**Funding**

This research has received funding from the European Research Council under the European Commission’s Seventh Framework Programme (FP7/2007–2013) as ERC grant agreement no. 233366 (POPFULL), as well as from the Flemish Hercules Foundation as Infrastructure contract ZW09-06. Further funding was provided by the Flemish Methusalem Programme and by the Research Council of the University of Antwerp. Funding to pay the Open Access publication charges for this article was provided by the European Commission’s Seventh Framework Programme (FP7/2007–2013) as ERC grant agreement no. 233366 (POPFULL).

**References**

AEBIOM (2012) European bioenergy outlook. Statistical Report, European Biomass Association, Brussels, November 2012, 123 p.

Alfas N, Pelis A, Niinemets U, Ceulemans R (2005) Growth and production of a short rotation coppice culture of poplar. II. Clonal and year-to-year differences in leaf and petiole characteristics and stand leaf area index. Biomass Bioenergy 28:536–547.

Allen SJ, Hall RL, Rosier PT (1999) Transpiration by two poplar varieties grown as coppice for biomass production. Tree Physiol 19:493–501.

Barbour MM (2007) Stable oxygen isotope composition of plant tissue: a review. Funct Plant Biol 34:83–94.

Bauerele WL, Weston DJ, Bowden JD, Dudley JB, Toier JE (2004) Leaf absorptance of photosynthetically active radiation in relation to chlorophyll meter estimates among woody plant species. Sci Hort 101:169–178.

Bernacchi CJ, Singsaas EL, Pimentel C, Portis AR, Long SP (2001) Improved temperature response functions for models of Rubisco-limited photosynthesis. Plant Cell Environ 24:253–259.

Bernacchi CJ, Calfapietra C, Davey PA, Wittig V, Scarascia-Mugnozza GE, Raines CA, Long SP (2003) Photosynthesis and stomatal conductance responses of poplars to free-air CO₂ enrichment (PopFACE) during the first growth cycle and immediately following coppice. New Phytol 159:609–621.

Berndes G (2002) Bioenergy and water—the implications of large-scale bioenergy production for water use and supply. Glob Environ Change 12:253–271.

Bonhomme L, Barbaroux C, Monclus R, Morabito D, Berthelot A, Villar M, Dreyer E, Brignolas F (2008) Genetic variation in productivity, leaf traits and carbon isotope discrimination in hybrid poplars cultivated on contrasting sites. Ann For Sci 65:503–511.
Bota J, Medrano H, Flexas J (2004) Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? New Phytol 162:671–681.

Broeckx LS, Verlinden MS, Ceulemans R (2012a) Establishment and two-year growth of a bio-energy plantation with fast-growing *Populus* trees in Flanders (Belgium): effects of genotype and former land use. Biomass Bioenergy 42:151–163.

Broeckx LS, Verlinden MS, Vangronsveld J, Ceulemans R (2012b) Importance of crown architecture for leaf area index of different *Populus* genotypes in a high-density plantation. Tree Physiol 32:1214–1226.

Broeckx LS, Verlinden MS, Berhongaray G, Zona D, Fichot R, Ceulemans R (2013) The effect of a dry spring on seasonal carbon allocation and vegetation dynamics in a poplar bioenergy plantation. Glob Change Biol Bioenerg. doi:10.1111/gcbb.12087.

Buckley TN, Warren CR (2014) The role of mesophyll conductance in the economics of nitrogen and water use in photosynthesis. Photosynth Res 119:77–88.

Cano FJ, Sanchez-Gomez D, Rodriguez-Calcerrada J, Warren CR, Gil L, Aranda I (2013) Effects of drought on mesophyll conductance and photosynthetic limitations at different tree canopy layers. Plant Cell Environ 36:1961–1980.

Cao X, Jia JB, Li H et al. (2012) Photosynthesis, water use efficiency and stable carbon isotope composition are associated with anatomical properties of leaf and xylem in six poplar species. Plant Biol 14:612–620.

Cernusak LA, Aranda J, Marshall JD, Winter K (2007) Large variation in whole-plant water-use efficiency among tropical tree species. New Phytol 173:294–305.

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osorio ML, Carvalho I, Faria T, Pinheiro C (2002) How plants cope with water stress in the field. Photosynthesis and growth. Ann Bot 89:907–916.

Craig H (1957) Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim Cosmochim Acta 12:133–149.

Demarez V, Gastellu-Etchegorry JP, Mougin E, Marty G, Proisy C, Demarez V, Gastellu-Etchegorry JP, Mougin E, Marty G, Proisy C (2007) The interaction between nitrogen source, soil pH, and drought in the growth and physiology of three poplar clones. Can J Bot 85:1046–1057.

DesRochers A, van den Driessche R, Thomas BR (2007) The interaction between nitrogen source, soil pH, and drought in the growth and physiology of three poplar clones. Can J Bot 85:1046–1057.

Diaz-Espejo A, Fernandez J, Nicolas E (2007) Seasonal evolution of leaf chlorophyll content of a temperate forest. Inversion of the PROSPECT model. Int J Remote Sens 20:879–894.

DesRochers A, van den Driessche R, Thomas BR (2007) The interaction between nitrogen source, soil pH, and drought in the growth and physiology of three poplar clones. Can J Bot 85:1046–1057.

Diaz-Espejo A, Fernandez J, Nicolas E (2007) Seasonal evolution of biochemical and diffusional limitations to and photosynthetic capacity in olive under drought. Plant Cell Environ 30:922–933.

Dillen SY, Marron N, Koch B, Ceulemans R (2008) Genetic variation of stomatal traits and carbon isotope discrimination in two hybrid poplar families (*Populus deltoides* ‘S9–2’ × *P. nigra* ‘Ghoy’ and *P. deltoides* ‘S9–2’ × *P. trichocarpa* ‘V24’). Ann Bot 102:399–407.

Dillen SY, Op de Beeck M, Hufkens K, Buonanduci M, Philips NG (2012) Seasonal patterns of foliar reflectance in relation to photosynthetic capacity and color index in two co-occurring tree species, *Quercus rubra* and *Betula papyrifera*. Agric For Meteorol 160:60–68.

Douthe C, Dreyer E, Epron D, Warren CR (2011) Mesophyll conductance to CO₂ assessed from online TDL-AS records of *C₃* plants. J Exp Bot 62:5335–5346.

Dunlap JM, Stettler RF (1998) Genetic variation and productivity of *Populus trichocarpa* and its hybrids. X. Trait correlations in young black cottonwood from four river valleys in Washington. Trees 13:28–39.

Duursma RA, Barton CVM, Eamus D, Medlyn BE, Ellsworth DS, Forster MA, Tissue DT, Linder S, McMurtrie RE (2011) Rooting depth explains [CO₂] × drought interaction in *Eucalyptus saligna*. Tree Physiol 31:922–931.

Easterling DR, Mehl G, Parmesan C, Changnon SA, Karl TR, Mearns LO (2000) Climate extremes: observations, modeling and impacts. Science 289:2068–2074.

Egea G, Gonzalez-Real MM, Baille A, Nortes PA, Diaz-Espejo A (2011) Disentangling the contributions of ontogeny and water stress to photosynthetic limitations in almond trees. Plant Cell Environ 34:962–979.

Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust J Plant Physiol 11:539–552.

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO₂ assimilation in leaves of *C₃* species. Planta 149:78–90.

Farquhar GD, Oleary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the inter-cellular carbon-dioxide concentration in leaves. Aust J Plant Physiol 9:121–137.

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Ann Rev Plant Phys 40:503–537.

Fichot R, Barigah TS, Chamaillard S, Le Thiec D, Laurans F, Cochard H, Brignolas F (2010) Common trade-offs between xylem resistance to cavitation and other physiological traits do not hold among unrelated *Populus deltoides* × *Populus nigra* hybrids. Plant Cell Environ 33:1553–1568.

Fichot R, Chamaillard S, Depardieu C, Le Thiec D, Cochard H, Barigah TS, Brignolas F (2011) Hydraulic efficiency and coordination with xylem resistance to cavitation, leaf function, and growth performance among eight unrelated *Populus deltoides* × *Populus nigra* hybrids. J Exp Bot 62:2093–2106.

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

Flexas J, Medrano H (2002) Energy dissipation in *C₃* plants under drought. Funct Plant Biol 29:1209–1215.

Flexas J, Both J, Loreto F, Cornic G, Sharkey TD (2004) Diffusive and metabolic limitations to photosynthesis under drought and salinity in *C₃* plants. Plant Biol 6:269–275.

Flexas J, Ribas-Carbo M, Diaz-Espejo A, Galmes J, Medrano H (2008) Mesophyll conductance to CO₂: current knowledge and future prospects. Plant Cell Environ 31:602–621.

Flexas J, Barbour MM, Brendel O et al. (2012) Mesophyll diffusion conductance to CO₂: an unappreciated central player in photosynthesis. Plant Sci 193–194:70–84.

Flexas J, Ninemets U, Gallé A et al. (2013) Diffusional conductances to CO₂ as a target for increasing photosynthesis and photosynthetic water-use efficiency. Photosynth Res 117:45–59.

Galmes J, Medrano H, Flexas J (2007) Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. New Phytol 175:81–93.

Galmes J, Conesa MA, Ochogavia JM et al. (2010) Physiological and morphological adaptations in relation to water use efficiency in Mediterranean accessions of *Salanum lycopersicum*. Plant Cell Environ 34:245–260.

Gilbert ME, Zwieniecki MA, Holbrook NM (2011) Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. J Exp Bot 62:2875–2887.

Gornall JM, Guy RD (2007) Geographic variation in ecophysiological traits of black cottonwood (*Populus trichocarpa*). Can J Bot 85:1202–1213.

Grassi G, Magnani F (2005) Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. Plant Cell Environ 28:834–849.

Grassi G, Vicinelli E, Ponti F, Canton L, Magnani F (2005) Seasonal and interannual variability of photosynthetic capacity in relation to...
leaf nitrogen in a deciduous forest plantation in northern Italy. Tree Physiol 25:349–360.

Guehl JM, Fort C, Ferhi A (1995) Differential response of leaf conductance, carbon-isotope discrimination and water-use efficiency to nitrogen deficiency in maritime pine and pedunculate oak plants. New Phytol 131:149–157.

Han Q (2011) Height-related decrease in mesophyll conductance, leaf photosynthesis and compensating adjustments associated with leaf nitrogen concentrations in Pinus densiflora. Tree Physiol 31:976–984.

Harley PC, Loreto F, Di Marco G, Sharkey TD (1992) Theoretical considerations when estimating the mesophyll conductance to CO₂ flux analysis of the response of photosynthesis to CO₂. Plant Physiol 98:1429–1436.

IPCC (2007) Fourth Assessment Report: Climate Change (AR4). The Physical Science Basis. Summary for Policymakers. http://www.ipcc.ch.

King JS, Ceulemans R, Albaugh JM et al. (2013) The challenge of long-term increased drought severity. Plant Cell Environ 36:87–102.

Kosugi Y, Matsuo N (2006) Seasonal fluctuations and temperature dependence of leaf gas exchange parameters of co-occurring evergreen and deciduous trees in a temperate broad-leaved forest. Tree Physiol 26:1173–1184.

Larchevêque M, Maurel M, DesRochers A, Larocque GR (2011) How does drought tolerance compare between two improved hybrids of balsam poplar and an unimproved native species. Tree Physiol 31:240–249.

Liang ZS, Yang HW, Shao HB, Han RL (2006) Investigation on water consumption characteristics and water use efficiency of poplar under soil water deficits on the Loess Plateau. Colloid Surf B 53:23–28.

Limonou J-M, Misson L, Lavois A-V, Martin NK, Rambal S (2010) Do photosynthetic limitations of evergreen Quercus ilex leaves change with long-term increased drought severity. Plant Cell Environ 33:863–875.

Linderson ML, Iritz Z, Lindroth A (2007) The effect of water availability on stand-level productivity, transpiration, water use efficiency and radiation use efficiency of field-grown willow clones. Biomass Bioenergy 31:460–468.

Lindroth A, Verwijst T, Halldin S (1994) Water-use efficiency of willow: variation with season, humidity and biomass allocation. J Hydrol 156:1–19.

Meinzer FC, Goldstein G, Grantz DA (1990) Carbon isotope discrimination in coffee genotypes grown under limited water-supply. Plant Physiol 92:130–135.

Meirsson D, Lazadzina H, Cermak J, Van Slycken J, Ceulemans R (1999) Measured sap flow and simulated transpiration from a poplar stand in Flanders (Belgium). Agric For Meteorol 96:165–179.

Misson L, Limouzin J-M, Rodriguez R, Letts MG (2010) Leaf physiological responses to extreme droughts in Mediterranean Quercus ilex forest. Plant Cell Environ 33:1898–1910.

Monclus R, Dreyer E, Villar M et al. (2006) Impact of drought on productivity and water use efficiency in 29 genotypes of Populus deltoides × Populus nigra. New Phytol 169:765–777.

Monclus R, Villar M, Barbaroux C et al. (2009) Productivity, water-use efficiency and tolerance to moderate water deficit correlate in 33 poplar genotypes from a Populus deltoides × Populus trichocarpa F1 progeny. Tree Physiol 29:1329–1339.

Montplied P, Granier A, Dreyer E (2009) Seasonal time-course of gradients of photosynthetic capacity and mesophyll conductance to CO₂ across a beech (Fagus sylvatica L.) canopy. J Exp Bot 60:2407–2418.

Niinemets U, Cescatti A, Rodeghiero M, Tosens T (2005) Leaf internal diffusion conductance limits photosynthesis more strongly in older leaves of Mediterranean evergreen broad-leaved species. Plant Cell Environ 28:1552–1566.

Paris P, Mareshli L, Sabatti M, Pisaneli A, Ecosse A, Nardin F, Scarsascia-Mugnozza GE (2011) Comparing hybrid Populus clones for SFR across northern Italy after two biennial rotations: survival, growth and yield. Biomass Bioenergy 35:1524–1532.

Pel C, Frak E, Le Roux X, Genty B (2002) Effect of local irradiance on CO₂ transfer conductance of mesophyll in walnut. J Exp Bot 53:2423–2430.

Pons TL, Westbeek MHM (2004) Analysis of differences in photosynthetic nitrogen-use efficiency between four contrasting species. Physiol Plant 122:68–78.

Pons TL, Flexas J, von Caemmerer S, Evans JR, Genty B, Ribas-Carbo M, Brugnoli E (2009) Estimating mesophyll conductance to CO₂: methodology, potential errors, and recommendations. J Exp Bot 60:2217–2234.

Ponson S, Dupoey J-L, Breda N, Dreyer E (2002) Comparison of water-use efficiency of seedlings from two sympatric oak species: genotype × environment interactions. Tree Physiol 22:413–422.

Rasheed F, Richard B, Le Thiec D, Montplied P, Paillassa E, Brignolas F, Dreyer E (2011) Time course of δ13C in poplar wood: genotype ranking remains stable over the life cycle in plantations despite some differences between cellulose and bulk wood. Tree Physiol 31:1183–1193.

Rasheed F, Dreyer E, Richard B, Brignolas F, Montplied P, Le Thiec D (2013) Genotype differences in δ13C discrimination between atmosphere and leaf matter match differences in transpiration efficiency at leaf and whole-plant levels in hybrid Populus deltoides × nigra. Plant Cell Environ 36:87–102.

Reich PB, Walters MB, Tabone TJ (1989) Response of Ulmus americana seedlings to varying nitrogen and water status. Water and nitrogen use efficiency in photosynthesis. Tree Physiol 5:173–184.

Richardson AD, Duigan SP, Berlyn GP (2002) An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytol 153:185–194.

Ripullone F, Lauteri M, Grassi G, Amato M, Borghetti M (2004) Variation in nitrogen supply changes water-use efficiency of Pseudotsuga menziesii and Populus × euroamericana; a comparison of three approaches to determine water-use efficiency. Tree Physiol 24:671–679.

Rodin JS, Farquhar GD (2012) A controlled test of the dual-isotope approach for the interpretation of stable carbon and oxygen isotope ratio variation in tree rings. Tree Physiol 32:490–503.

Rood SB, Braatne JH, Hughes FMR (2003) Ecophysiology of riparian cottonwoods: stream flow dependency, water relations and restoration. Tree Physiol 23:1113–1124.

Roupars O, Gross P, Dreyer E (1996) Limitation of photosynthetic activity by CO₂ availability in the chloroplasts of oak leaves from different species and during drought. Ann For Sci 53:243–254.

Scheidegger Y, Saurer M, Bahn M, Siegwolf R (2000) Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. Oecologia 125:350–357.

Seibt U, Rajabi A, Griffiths H, Berry JA (2008) Carbon isotopes and water use efficiency: sense and sensitivity. Oecologia 155:441–454.

Seneviratne SI, Corti T, Davin EL, Hirschi M, Jaeger EB, Lehner I, Orlowski B, Teuling AJ (2010) Investigating soil moisture–climate interactions in a changing climate: a review. Earth Sci Rev 99:125–161.

Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaaas EL (2007) Fitting photosynthetic carbon dioxide response curves for C₃ leaves. Plant Cell Environ 30:1035–1040.

Soolanayakanahally RY, Guy RD, Silim SN, Drewes EC, Schroeder WR (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 Environ 32:1821–1832.

Tholen D, Ethier G, Genty B, Pepin S, Zhu XG (2012) Variable mesophyll conductance revisited: theoretical background and experimental implications. Plant Cell Environ 35:2087–2103.
Toillon J, Fichot R, Dallé E, Berthelot A, Brignolas F, Marron N (2013) Planting density affects growth and water-use efficiency depending on site in *Populus deltoides* × *P. nigra*. For Ecol Manag 304:345–354.

Verbeke G, Molenerghs G (2000) Linear mixed models for longitudinal data. 1st edn. Springer, New York, NY, USA, 568 pp.

Wang Q, Tenhunen J, Falge E, Bernhofer C, Granier A, Vesala T (2004) Simulation and scaling of temporal variation in gross primary production for coniferous and deciduous temperate forests. Glob Change Biol 10:37–51.

Warren CR, Adams MA (2006) Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen use in photosynthesis. Plant Cell Environ 29:192–201.

Warren CR, Livingston NJ, Turpin DH (2004) Water stress decreases the transfer conductance of Douglas-fir (*Pseudotsuga menziesii*) seedlings. Tree Physiol 24:971–979.

Wilson KB, Baldocchi DD, Hanson PJ (2000a) Quantifying stomatal and non-stomatal limitations to carbon assimilation resulting from leaf aging and drought in mature deciduous tree species. Tree Physiol 20:787–797.

Wilson KB, Baldocchi DD, Hanson PJ (2000b) Spatial and seasonal variability of photosynthetic parameters and their relationship to leaf nitrogen in a deciduous forest. Tree Physiol 20:565–578.

Wilson KB, Baldocchi DD, Hanson PJ (2001) Leaf age affects the seasonal pattern of photosynthetic capacity and net ecosystem exchange of carbon in a deciduous forest. Plant Cell Environ 24:571–583.

Xu L, Baldocchi DD (2003) Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature. Tree Physiol 23:865–877.

Xu X, Peng G, Wu C, Korpelainen H, Li C (2008) Drought inhibits photosynthetic capacity more in females than in males of *Populus cathayana*. Tree Physiol 28:1751–1759.

Zhu G-F, Li X, Su Y-H, Lu L, Huang C-L (2011) Seasonal fluctuations and temperature dependence in photosynthetic parameters and stomatal conductance at the leaf scale of *Populus euphratica* Oliv. Tree Physiol 31:178–195.

Zona D, Janssens IA, Gioli B, Jungkunst HF, Camino Serrano M, Ceulemans R (2012) *N*₂*O* fluxes of a bioenergy poplar plantation during a 2 years rotation period. Glob Change Biol Bioenerg 5:536–547.

Zona D, Janssens IA, Aubinet M, Gioli B, Vicca S, Fichot S, Ceulemans R (2013) Fluxes of the greenhouse gases (*CO₂*, *CH₄* and *N*₂*O*) above a short-rotation poplar plantation after conversion from agricultural land. Agric For Meteorol 169:100–110.

Zsuffa L, Giordano E, Pryor LD, Stettler RF (1996) Trends in poplar culture: some global and regional perspectives. In: Stettler RF, Bradshaw HD, Heilman PE, Hinckley TM (eds) Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa, 515–539.