Response of Internal Conductance to Soil Drought in Sun and Shade Leaves of Adult *Fagus crenata*

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Abstract – The internal conductance to CO_2 (g_i) is an important constraint of photosynthesis, and its acclimation to environmental factors varies widely within plant functional groups, genera and even species. We measured photosynthesis and chlorophyll fluorescence response curves to intercellular CO_2 concentrations simultaneously in attached sun and shade leaves of 90-year-old *Fagus creneta* Blume trees to determine (1) how g_i varies within the crown and (2) whether soil drought affects g_i. Internal conductance to CO_2 was found to be 0.058 and 0.185 mol m^{-2} s^{-1} in lower and upper crowns, respectively, resulting in a decrease of about 70 µmol mol^{-1} in CO_2 concentration from the intercellular space (C_i) to the site of carboxylation inside the chloroplast stroma (C_c). The results suggest that g_i is as important as stomatal conductance for photosynthetic efficiency in *F. crenata*. If this large decrease from C_i to C_c was not accounted for, the maximum rate of carboxylation (V_{cmax}) in sun and shade leaves was underestimated ca 36% and 24%, respectively. When soil water supply was sufficient, leaf water potential dropped to a daily minimum in early morning, facilitating CO_2 transfer and thus photosynthesis. When soil water potential at 25 cm depth fell to –0.015 MPa, g_i and stomatal conductance decreased by 20–40% in comparison with their respective values under sufficient soil water supply. In contrast, V_{cmax} decreased by 7% in sun leaves, but there was no change in this parameter in shade leaves. Ignoring the effect of g_i on C_c under stressed conditions would lead to up to 22% underestimates of V_{cmax}, and consequently overestimates of biochemical limitations. These results suggest that CO_2 diffusional limitations have more significant effects than biochemical limitations on the rate of photosynthesis in *F. crenata* during soil drought.

light acclimation / Japanese beech / maximum rate of carboxylation / mesophyll conductance / photosynthesis / stomatal conductance

1 INTRODUCTION

Ongoing increases in the atmospheric CO_2 concentration and associated climate changes have promoted a growing interest in the function of forest ecosystems as potential carbon sinks (Baldocchi – Harley 1995). In order to evaluate these effects a number of models have been developed to aid the study of physiological processes affecting forest carbon fixation. Many investigations of carbon fixation have employed the biochemical model of Farquhar et al. (1980) at the leaf level. This model uses two key parameters: the maximum rate of

carboxylation (V_{cmax}) and the maximum rate of electron transport (J_{max}). Variations in V_{cmax} and J_{max} within canopies are strongly correlated with their growth irradiance via the allocation of leaf nitrogen (Wilson et al. 2000a; Meir et al. 2002; Han et al. 2003). These associations between irradiance, nitrogen and V_{cmax} within canopies are widely used in scaling estimates of leaf photosynthesis to canopy level (Baldocchi – Harley 1995). The models that use these parameters have typically assumed an infinite internal conductance to CO_2 (g_i), which describes the draw-down in CO_2 concentration between intercellular spaces (C_i) and the site of carboxylation inside the chloroplast stroma (C_c) (Harley et al. 1992b). However, it is becoming apparent from recent studies that finite g_i alone are sufficient to limit the rate of photosynthesis (Harley et al. 1992a; Flexas et al. 2008). Recent studies have demonstrated that g_i also acclimates and responds both in the long term (weeks and days) and short term (hours and minutes) to environmental factors, including light, temperature, water and CO_2 concentrations (Flexas et al. 2008). For example, g_i has been found to be two-fold lower in shade leaves than in sun leaves of *Fagus sylvatica* L. crowns (Warren – Adams 2006; Montpied et al. 2009). One consequence of finite g_i is that V_{cmax} values calculated from C_i are not influenced solely by biochemical factors, but also by g_i. Therefore, these changes may be important in regulating photosynthesis in response to changes in environmental factors. Further, recent studies have indicated that variations in g_i are associated with variations in leaf forms and plant functional groups, hence there are large variations in g_i within groups, genera and even species (Hanba et al. 2002; Terashima et al. 2006; Flexas et al. 2008). This suggests that g_i is a rapidly adapting trait that may contribute to the differences in photosynthetic efficiency found among different species. Therefore, further studies are needed to fully characterize the responses of g_i to environmental factors, especially since g_i and related processes are likely to be heavily influenced by anticipated global climate changes (Flexas et al. 2008).

The most extensive deciduous broad-leaved forests in Japan are the beech (*Fagus crenata* Blume) forests, distributed from Kuromatsunai, Hokkaido (42^oN, 140^oE) to Takakuma, Kyushu (31^oN, 130^oE). These beech forests are important for wildlife and protection of water catchments, hence their ecological features and physiological traits have been extensively studied (Hashizume - Fukutomi 1978; Nakashizuka 1983; Kakubari 1991; Hiura 1998; Han et al. 1999). The cited studies have found (*inter alia*) that the photosynthetic capacity of *F. crenata* responds highly plastically to irradiance (Uemura et al. 2000; Iio et al. 2005), it is highly sensitive to drought stress (Maruyama - Toyama 1987; Uemura et al. 2004), and varies amongst ecotypes (Koike – Maruyama 1998; Bayramzadeh et al. 2008; Tateishi et al. 2010). However, g_i and the constraints it imposes on photosynthesis in this species have not been examined previously. Therefore, in this study, photosynthesis and chlorophyll fluorescence were measured simultaneously in attached sun and shade leaves of 90-year-old Japanese beech trees to determine (1) how g_i varies between sun and shade leaves and (2) the effect of soil drought on g_i.

2   MATERIALS AND METHODS

2.1 Study site

The study area was located in the Naeba Mountains in southern Niigata Prefecture, Japan (36°51' N, 138°46' E), where *F. crenata* forests are found over an altitudinal range from 550 to 1500 m. Eight permanent plots along the altitudinal gradient were established in 1970 for long-term ecological monitoring within the framework of the International Biological Program (Kakubari 1977). The bedrock is predominantly andesite and basalt, on which moderately water-retentive brown forest soil has formed. During the period 1976–2009, the

mean annual precipitation and temperature were recorded as 2,214 mm and 11.5 °C, respectively, at a nearby meteorological station (36°57' N, 138°49' E, 340m asl., Japanese Bureau of Meteorology). Snow accumulates to depths up to about 3-4 m during the winter. One of eight permanent plots at the ridgeline of a south facing slope with elevation of 950 m was chosen for this study. The chosen plot was dominated by *F. crenata* (87%), with sporadic occurrence of *Quercus crispula* Blume, *Magnolia obovata* Thunb., *Prunus grayana* Maxim., *Acer rufinerve* Sieb. et Zucc.*, A. japonicum* Thunb.*, A. tschonoskii* Maxim.*, A. palmatum* Thunb.*, Kolopanax pictus* Thunb. and *Acanthopanax sciadophylloides* Franch. et Savat. The average age of the *F. crenata* trees in the chosen plot was about 90 years and the stand stem basal area was about 45.6 $m^2\,ha^{-1}$.

2.2 Soil water measurement

Soil volumetric water content (SWC) was measured near the selected trees at 10 and 25 cm depths with time-domain reflectometry (TDR) sensors (ML2, Delta-T Devices, Cambridge, UK). Soil water potential at 25 cm depth was measured simultaneously with a tensiometer (KDC-55, Kona, Sapporo, Japan) inserted near the TDR sensors to convert SWC measurements to soil water potential during the period 2006 to 2008. These measurements were made at 10 s intervals, and data were stored as 30 min means using a data logger (DL2e, Delta-T, Cambridge, UK).

2.3 Measurements of gas exchange and chlorophyll fluorescence

Three trees with heights between 19.5 and 22 m were accessed with the aid of a research tower. Gas exchange and chlorophyll fluorescence were measured simultaneously *in situ* for leaves in both the upper and lower crowns (hereafter referred to as sun and shade leaves, respectively), between 18 August and 20 August 2009. The photosynthesis and fluorescence response curves to intercellular CO_2 concentration (A/C_i) were measured with two inter-calibrated portable gas exchange systems (LI-6400, LI-COR, Lincoln, USA) with an integrated fluorescence chamber (LI-6400-40). Dark respiration and the minimum and maximum fluorescence of dark-adapted leaves were measured at 22:00 on the day prior to the measurements of A/C_i curves. For each A/C_i curve, leaves were exposed to 360 $\mu mol\,mol^{-1}$ CO_2 until rates of photosynthesis and transpiration were steady. Then, the CO_2 concentration in the chamber was adjusted stepwise to 300, 250, 200, 150, 100, 50, 360, 450, 600, 800, 1000, 1300, 1500 and 1800 $\mu mol\,mol^{-1}$, allowing at least four minutes for adjustment and stabilization at each step. Photosynthetic photon flux density was set at photosynthesis-saturated points which were 1000 and 700 $\mu mol\,m^{-2}\,s^{-1}$ for sun leaves and shade leaves, respectively (Iio et al. 2005). Leaf temperatures were generally controlled at 25.0 °C, but on warm afternoons they increased to a maximum of 28.1 °C owing to the limited cooling capacity of the LI-6400. For individual leaves the variation in leaf temperature was less than 1.0 °C during the measurement periods. The vapor pressure deficit in the chamber was lower than 1.45 kPa for all measurements. Each leaf was revisited 2–3 times a day between 6:30 to 14:00 to investigate the effects of water stress on photosynthetic parameters.

2.4 Measurement of leaf water potential

After the completion of measurements for each A/C_i curve, a leaf on a neighboring shoot exposed to a similar light intensity was removed, placed in a sealable plastic bag with a small piece of humid paper towel and dropped down to the forest floor. Leaf water potential was measured immediately using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, USA).

2.5 Measurement of leaf nitrogen concentration

Leaves were harvested after the gas exchange measurements and their projected leaf areas were measured using a scanner (LiDE200, Canon, Tokyo, Japan) and image analysis software (LIA32, K. Yamamoto of Nagoya University, Nagoya, Japan). They were then dried at 70 °C for 48 hours, combusted with circulating O_2 using an NC analyzer (Sumigraph NC-900, SCAS, Osaka, Japan), and their nitrogen concentration per unit area (N_a) was determined using a gas chromatograph (GC-8A, Shimadzu, Kyoto, Japan).

2.6 Calculation of photosynthetic parameters

Photosynthetic electron transport (J) was calculated according to the default program installed in the Li-6400 assuming a leaf absorptance of 0.85. Internal conductance (g_i) was estimated using the "variable J method" of Harley et al (1992a):

$$g_i = \frac{A}{C_i - \dfrac{\Gamma^*[J + 8(A + R_d)]}{J - 4(A + R_d)}} \tag{1}$$

where the CO_2 compensation point in the absence of mitochondrial respiration (Γ^*) was taken from Bernacchi (2002), and the rate of non-photorespiratory respiration continuing in the light (R_d) was taken as half of the rate of respiration measured in the dark (Niinemets et al. 2005). Then g_i values were calculated for every step of the A/C_i curves, and C_c was calculated as:

$$C_c = C_i - A/g_i \tag{2}$$

Since the CO_2 concentration may affect g_i (Flexas et al. 2007), data in the range of $10 < dC_c/dA < 50$ were averaged to estimate a constant g_i value (Harley et al. 1992a):

$$dC_c/dA = 12 \times \Gamma^* \times J / [J - 4 \times (A + R_d)]^2 \tag{3}$$

From this constant g_i, we estimated V_{cmax} and J_{max} from A/C_c curves based on the model of Farquhar et al. (1980) using a non-linear curve-fitting routine available in Microsoft Excel (Sharkey et al. 2007). Limiting factors (the properties of ribulose 1·5-biosphosphate carboxylase/oxygenase, the rate of ribulose 1·5-biosphosphate regeneration and the rate of use of triose phosphates) were determined from J obtained by fluorescence measurements. All V_{cmax} and J_{max} values were standardized to a common temperature of 25°C. We also compared the difference in V_{cmax} and J_{max} when draw-down between C_i and C_c was, and was not, taken into account.

2.7 Data analyses

The significance of the differences in leaf traits between sun and shade leaves was tested by Tukey-Kramer's test, using StatView (SAS Institute Inc., Cary, NC).

3   RESULTS

3.1 Soil water conditions

Soil water is usually not limiting in the Naeba Mountains because of the high mean annual precipitation. However, the annual precipitation in 2009, the year in which this study was conducted, was 1902 mm (*Figure* 1b), 312 mm lower than the 2214 mm average for the period 1976-2009, and the second lowest among all years in this period. The lower than

average rainfall led to prolonged soil water deficits during the summer of 2009 (*Figure* 1c). Daily average soil water potentials during the measurements of gas exchange were –0.020, –0.026, and –0.032 MPa at 10 cm depth, and –0.012, –0.013, and –0.015 MPa at 25cm depth on the 18th, 19th and 20th of August 2009, respectively. The atmospheric vapor pressure deficit during the measurements varied between 0.32–1.75, 0.35–1.26, and 0.43–1.09 kPa on the 18th, 19th and 20th August 2009, respectively (data not shown).
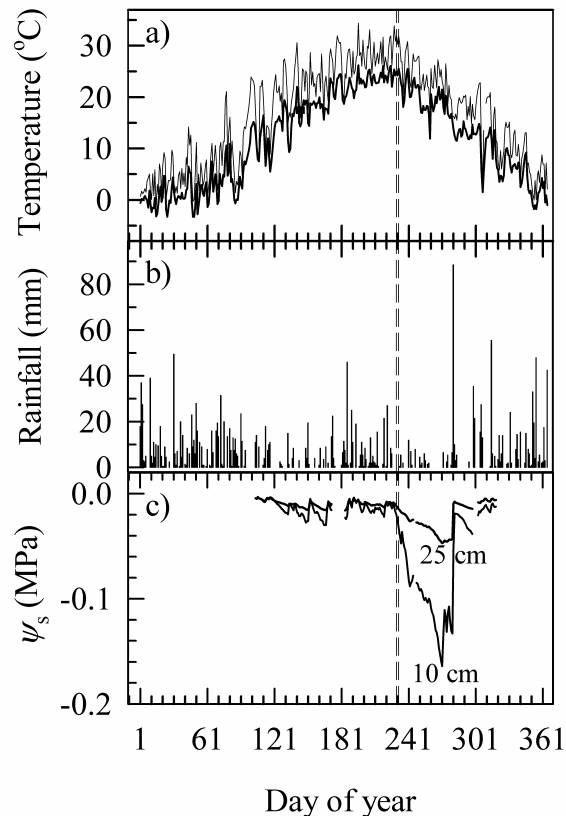

Figure 1. Seasonal course of local environmental factors during 2009.
(a) Daily mean (bold line) and maximum (thin line) air temperatures, (b) daily rainfall, and
(c) daily mean soil water potential (Ψ_s) at depths of 10 cm and 25 cm. Vertical dashed lines
indicate dates of gas exchange measurement campaigns

3.2 Photosynthetic plasticity within the crowns

The light-saturated photosynthetic rate at ambient CO_2 concentrations (A_{max}), g_i, g_s, V_{cmax} and J_{max} were all approximately two-fold higher in sun leaves compared to shade leaves (*Table* 1). Internal conductance to CO_2 was 0.166 mol m^{-2} s^{-1}, a similar level to g_s (0.185 mol m^{-2} s^{-1}) in sun leaves ($P = 0.541$). In contrast, in shade leaves, g_i was 0.058 mol m^{-2} s^{-1}, significantly lower than their g_s (0.100 mol m^{-2} s^{-1}) ($P = 0.019$). The decrease in CO_2 concentration from ambient to C_i varied between 76–132 µmol mol^{-1} within the crowns, and was about 56 µmol mol^{-1} higher in sun leaves than in shade leaves (*Table* 1). In contrast, the draw-down in CO_2 concentration from C_i to C_c was, on average, about 70 µmol mol^{-1} in both sun and shade leaves. The maximum rate of carboxylation estimated from A/C_i curves ($V_{cmax, Ci}$) was underestimated ca 36% and 24% than the value estimated from A/C_c curves ($V_{cmax, Cc}$) in sun and shade leaves, respectively. In contrast, estimates of J_{max} derived from the A/C_i curves and A/C_c curves did not differ significantly, for either sun or shade leaves.

Table 1. Comparison of photosynthetic parameters between sun and shade leaves of 90-year-old Fagus crenata trees without water stress

	A_{max}	g_s	g_i	C_i	C_c	$V_{cmax, Ci}$	$V_{cmax, Cc}$	$J_{max, Ci}$	$J_{max, Cc}$
Sun	12.6 ± 0.9	0.185 ± 0.024	0.166 ± 0.029	228 ± 8	158 ± 10	62 ± 5[a]	95 ± 2[b]	121 ± 6[a]	129 ± 6[a]
Shade	3.7 ± 0.3	0.100 ± 0.006	0.058 ± 0.009	284 ± 4	215 ± 5	20 ± 2[a]	26 ± 2[b]	44 ± 4[a]	46 ± 4[a]
P value	0.0006	0.027	0.024	0.004	0.007	0.00004	0.001	0.0004	0.0005

Measurements were made under light-saturated photosynthesis. Parameters determined are the rate of net photosynthesis (A_{max}, µmol m^{-2} s^{-1}) at an ambient CO_2 concentration of 360 µmol mol^{-1}, stomatal conductance (g_s, mol m^{-2} s^{-1}), internal conductance (g_i, mol m^{-2} s^{-1}), intercellular CO_2 concentration (C_i, µmol mol^{-1}), chloroplast CO_2 concentration (C_c, µmol mol^{-1}), maximum rate of carboxylation (V_{cmax}, µmol m^{-2} s^{-1}) and electron transport (J_{max}, µmol m^{-2} s^{-1}) estimated from A/C_i or A/C_c ($V_{cmax, Ci}$ and $V_{cmax, Cc}$, $J_{max, Ci}$ and $J_{max, Cc}$, respectively). Data are means ± SE obtained from measurements on two leaves from each of three *F. crenara* trees. Significance was tested by Tukey-Kramer's test. Different superscript letters within rows indicate significant differences between the corresponding parameters estimated from A/C_i and A/C_c ($P < 0.05$).

There was no significant difference in leaf nitrogen concentration per unit dry mass between sun and shade leaves (*Table* 2). However, leaf dry mass per unit area (LMA) was 3-fold greater in sun leaves than shade leaves and thus N_a was 3-fold higher in sun leaves than in shade leaves.

Table 2 Comparison of structural parameters between sun and shade leaves

	LMA (g m^{-2})	N_a (g m^{-2})	N_m (mg g^{-1})
Sun	94.94 ± 5.44	2.29 ± 0.12	24.30 ± 1.75
Shade	31.33 ± 2.42	0.74 ± 0.04	23.56 ± 0.66
P value	0.0006	0.010	0.700

Parameters determined are leaf dry mass per unit area (LMA), leaf nitrogen concentration per unit area (N_a) and per unit dry mass (N_m). The data presented are the means ± SE of two leaves from each of three *F. crenara* trees. Significance was tested by Tukey-Kramer's test.

3.3 Responses to changes in leaf water potentials and soil drought stress

Leaf water potential at predawn was about –0.40 MPa and –0.50 MPa in shade and sun leaves, respectively (data not shown). It decreased quickly to about –1.50 MPa in the early morning and reached its daily minimum value of –2.60 MPa in mid-morning in sun leaves (refer to *Figure* 2). Leaf water potential followed a similar diurnal course in shade leaves, but with a minimum value of about –1.40 MPa, which was higher than in sun leaves. A_{max} did not decrease in either sun or shade leaves when soil water potential at 25 cm depth was higher than –0.013 MPa (*Figure* 2a). Neither g_s nor g_i was affected by leaf water potentials at this threshold of soil water potential (*Figures* 2b and 2c). When drought was prolonged and soil water potential fell below –0.015 MPa at 25 cm depth, g_i and g_s decreased by 20–40% in both sun and shade leaves in comparison with their respective values under unstressed soil water conditions (*Figure* 3). In contrast, $V_{cmax, Cc}$ was less affected by soil drought in shade leaves, and decreased by about 7% in sun leaves. The decrease in $V_{cmax, Ci}$ would be up to 22% in sun leaves, ignoring the effect of g_i on C_c. However, no significant difference was found between estimates of J_{max} obtained from A/C_i curves and A/C_c curves in either sun or shade leaves.
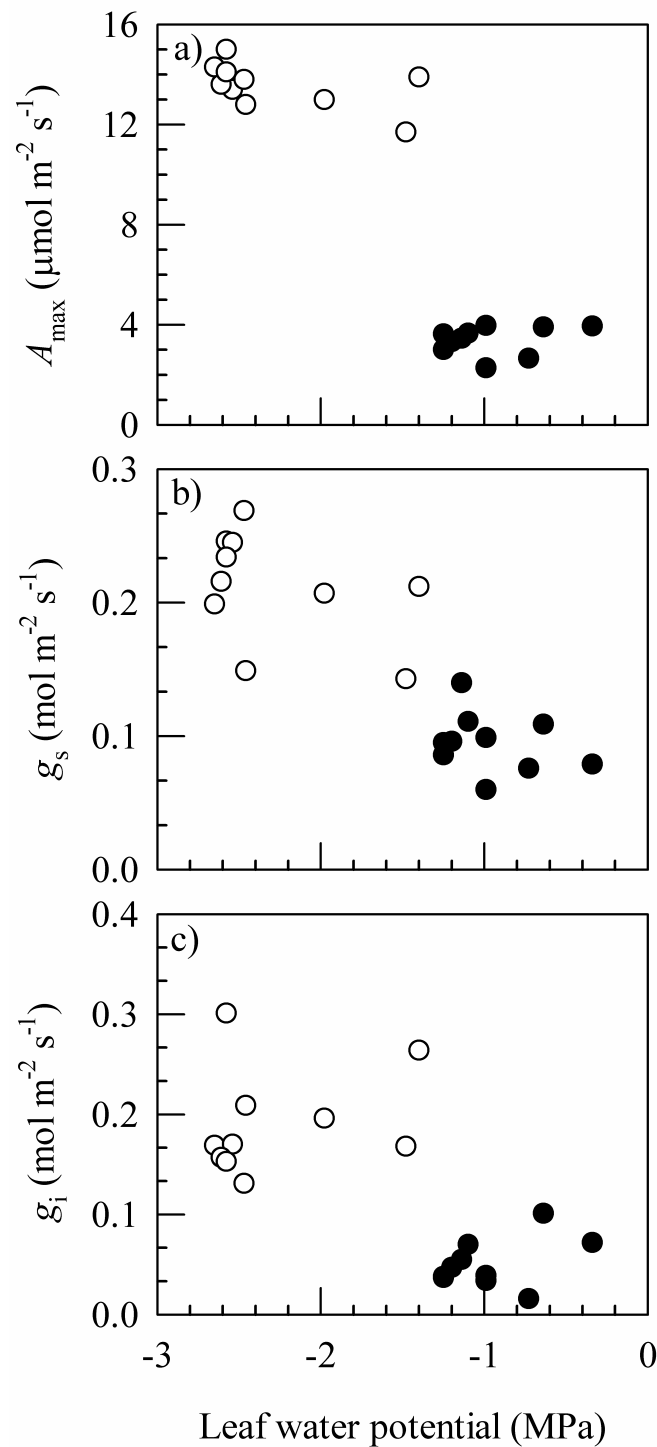
*Figure 2. The relationships between leaf water potential and
(a) light-saturated photosynthetic rate (A_{max}), (b) stomatal conductance (g_s) and (c) internal
conductance (g_i) in sun (open circles) and shade (filled circles) leaves of three mature Fagus
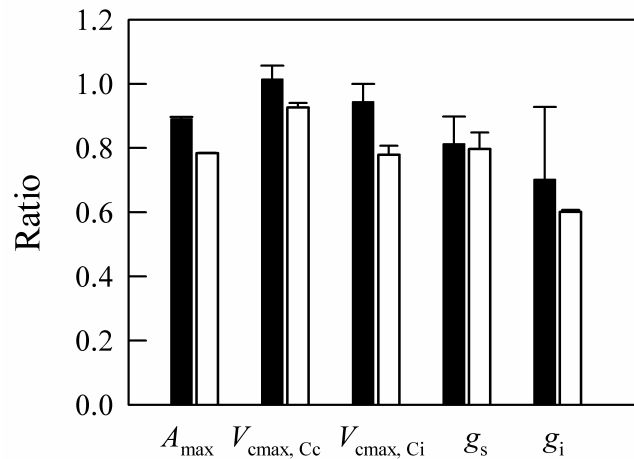crenata trees when soil water potential at 25 cm depth was higher than – 0.013 MPa*

Figure 3. Effects of soil water stress on photosynthetic parameters of both sun (open bars) and shade (filled bars) leaves of three mature Fagus crenata trees when soil water potential at 25 cm depth was – 0.015 MPa (ratios between measurements on stressed leaves and unstressed leaves, measured in the preceding two days). The determined parameters are light-saturated photosynthetic rate (A_{max}), maximum rate of carboxylation estimated from A/C_c curves ($V_{cmax, Cc}$) and A/C_i curves ($V_{cmax, Ci}$), stomatal conductance (g_s) and internal conductance (g_i)

4   DISCUSSION AND CONCLUSIONS

4.1 Internal conductance of Fagus crenata

Structural (LMA, N_a) and photosynthetic (A_{max}, V_{cmax}, J_{max}) plasticity to local irradiance was observed within the *F. creneta* crown, indicating that there is great plasticity in responses to light intensity in this species. These responses have been previously documented in both *F. crenata* (Han et al. 1999; Iio et al. 2005) and *F. sylvatica* (Warren et al. 2007; Montpied et al. 2009). The internal conductance to CO_2 varied between 0.058 and 0.166 mol m^{-2} s^{-1} in lower and upper crowns of 90-year old *F. crenata* trees and resulted in a large decrease in CO_2 concentration from C_i to C_c of approximately 70 µmol mol^{-1}. To the best of our knowledge, this is the first report of measurements of g_i in *F. crenata*, although g_i has been investigated previously in *F. sylvatica* (Warren et al. 2007; Montpied et al. 2009). Internal conductance has been found to vary between 0.14 and 0.21 mol m^{-2} s^{-1} within the crown of 60-year old *F. sylvatica* trees in south-western Germany (Warren et al. 2007), and between 0.02 and 0.40 mol m^{-2} s^{-1} within the crowns of 35-year old *F. sylvatica* trees in north-eastern France (Montpied et al. 2009). These findings suggest that the magnitude of g_i is related to species ecotype, tree age and study site, as has been described for other photosynthetic parameters (Koike and Maruyama 1998; Peuke et al. 2002). In addition, assuming infinite g_i affects estimates of V_{cmax} and the interpretation of V_{cmax} in response to environmental stress. Most published estimates of V_{cmax} underestimate the true V_{cmax} by excluding the effect of draw-down from C_i to C_c (Wullschleger 1993). In *F. crenata*, use of C_i instead of C_c in modeling leads to V_{cmax} being underestimated by 24–36 %, similar to reported underestimates of this parameter in *F. sylvatica* (Warren et al. 2007).

4.2 Variation in gi between sun and shade leaves

For *F. crenata*, g_i was approximately three-fold higher in sun leaves than in shade leaves, similar to the two-fold difference found in previous comparisons between sun and shade

leaves of *F. sylvatica* (Warren et al. 2007) and *Acer palmatum* (Hanba et al. 2002). Internal conductance is determined by cell wall thickness of mesophyll, the surface area of chloroplasts facing the intercellular airspace per unit leaf area (S_c), and the abundance and/or conductivity of aquaporins (Terashima et al. 2006; Flexas et al. 2008). In comparison with shade leaves of *F. crenata*, sun leaves haves larger S_c values (Oguchi et al. 2005), thicker palisade tissue (Uemura et al. 2000), and greater LMA. These structural differences may explain the difference in g_i between sun and shade leaves in *F. crenata*.

4.3 Water stress affects gi and photosynthesis

Leaf water potentials dropped to a diurnal minimum level of –2.60 MPa in sun leaves in the early morning, in accordance with previous observations at another site (550 m asl.) in the Naeba Mountains (Iio et al. 2004). In contrast, in another study of water potential in a beech forest the minimum leaf water potential was found to be about –1.50 MPa (Uemura et al. 2004). Although the reasons for such significant differences in leaf water potential are not fully understood, they may correlate with ecotypes (Bayramzadeh et al. 2008); *F. crenata* and *F. sylvatica* are known to have different characteristics that could be attributed to the climatic conditions in their original habitats, particularly to the amount of rainfall (Koike – Maruyama 1998; Peuke et al. 2002). The beech forests examined in this study are located at the side of the Sea of Japan with an average annual rainfall of 2214 mm for the period 1976–2009, whereas the study by Uemura et al. (2004) was carried out at the Pacific Ocean side with an average annual rainfall of 1408 mm for the same period.

In this study we found that g_i, g_s and A_{max} did not respond to decreasing leaf water potentials when there was sufficient soil water. Together with their rapid decline in the early morning, these results suggest that leaf water potentials fell in an adaptive response that promotes CO_2 diffusion into the carboxylation site when the soil water supply is sufficient and thus helps to maintain high photosynthetic rates. On the other hand, when soil water potential fell below a certain threshold, g_i and g_s decreased significantly in both sun and shade leaves, whereas the minimum daily leaf water potentials did not decline further. Reductions in g_i in response to soil drought rather than to atmospheric vapor pressure deficits have also been previously observed (Warren 2008). Considering this threshold of –0.015 MPa at 25cm depth for reductions in g_i, we concluded that *F. crenata* is sensitive to drought stress, as previously observed in assessments of factors affecting photosynthetic parameters of several *Fagus* species, e.g. *F. sylvatica* (Raftoyannis - Radoglou 2002), *F. crenata* (Maruyama – Toyama 1987; Uemura et al. 2004), and *F. grandifolia* (Tschaplinski et al. 1998).

Ignoring the effect of g_i on C_c resulted in an underestimation of V_{cmax}. The underestimation of V_{cmax} with water stress, found also previously (Wilson et al. 2000b), would suggest a predominant role of biochemical limitation to photosynthesis during drought. Thus, our results confirm that neglecting g_i may lead to an important overestimation of biochemical limitations. Nevertheless, V_{cmax} decreased 7% in sun leaves, even taking g_i into account, implying that the decrease in g_i due to soil drought was not fully responsible for the decrease in V_{cmax}. This finding is consistent with previous observations in *Quercus robur* L. and *Fraxinus oxyphylla* Bieb. (Grassi - Magnani 2005). In conclusion, the results of this study indicate that diffusional limitations due to both g_s and g_i affect photosynthetic rates in *F. crenata* under sufficient soil water supply, whereas biochemical limitations (i.e. V_{cmax}) are quantitatively important only during prolonged soil droughts.

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