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Physiological and isotopic ($\delta^{13}C$ and $\delta^{18}O$) responses of three tropical tree species to water and nutrient availability

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ABSTRACT

Water-use efficiency and stable isotope composition were studied in three tropical tree species. Seedlings of *Tectona grandis*, *Swietenia macrophylla* and *Platymiscium pinnatum* were grown at either high or low water supply, and with or without added fertilizer. These three species previously exhibited low, intermediate and high whole-plant water-use efficiency (*TE*) when grown at high water supply in unfertilized soil. Responses of *TE* to water and nutrient availability varied among species. The *TE* was calculated as experiment-long dry matter production divided by cumulative water use. Species-specific offsets were observed in relationships between *TE* and whole-plant ^{13}C discrimination ($\Delta^{13}C_p$). These offsets could be attributed to a breakdown in the relationship between $\Delta^{13}C_p$ and the ratio of intercellular to ambient CO_2 partial pressures (c_i/c_a) in *P. pinnatum*, and to variation among species in the leaf-to-air vapour pressure difference (v). Thus, a plot of $v \cdot TE$ against c_i/c_a showed a general relationship among species. Relationships between $\delta^{18}O$ of stem dry matter and stomatal conductance ranged from strongly negative for *S. macrophylla* to no relationship for *T. grandis*. Results suggest interspecific variation among tropical tree species in relationships between stable isotope ratios ($\delta^{13}C$ and $\delta^{18}O$) and the gas exchange processes thought to affect them.

Key-words: carbon isotope ratio; leaf N concentration; oxygen isotope ratio; transpiration; tropical tree; water-use efficiency.

INTRODUCTION

Ever since photosynthetic organisms began to colonize the land surface nearly 500 million years ago, they have faced an inevitable dilemma: terrestrial plants must expose moist tissues to the atmosphere, risking desiccation and death, to absorb CO_2 for photosynthesis. Most terrestrial environments are subject to a limiting supply of water for plant transpiration in at least some part of the annual cycle. Thus, the efficiency with which terrestrial plants exchange water for CO_2 could have important implications for plant performance and distribution.

Plant physiologists have applied the term water-use efficiency to describe the rate of CO_2 uptake or plant dry matter production for a given rate of plant water loss (Bacon 2004). Water-use efficiency at the leaf level (A/E) can be expressed as the rate of diffusion of CO_2 into the leaf during photosynthesis for a given rate of diffusion of water vapour out of the leaf (Farquhar & Richards 1984):

$$\frac{A}{E} = \frac{g_c(c_a - c_i)}{g_s(e_i - e_a)} = \frac{c_a - c_i}{1.6v}, \quad (1)$$

where A is photosynthesis (μmol CO_2 m^{-2} s^{-1}), E is transpiration (mmol H_2O m^{-2} s^{-1}), g_c and g_s are stomatal conductances to CO_2 and water vapour, respectively (mol m^{-2} s^{-1}), c_a and c_i are CO_2 partial pressures in ambient air and leaf intercellular air spaces, respectively (Pa), e_i and e_a are water vapour partial pressures in the intercellular air spaces and ambient air, respectively (kPa), v is the leaf-to-air vapour pressure difference (kPa), defined as $e_i - e_a$, and 1.6 is the ratio of the diffusivity of CO_2 to that of water vapour in air.

Equation (1) can be scaled from the leaf to the whole plant by taking into account respiratory C use and water loss not associated with photosynthesis. Respiratory C use results in a net efflux of CO_2 from non-photosynthetic organs during the day and from the whole plant at night. Water loss not associated with photosynthesis can result from soil evaporation and transpiration at night. The whole-plant water-use efficiency, or transpiration efficiency of C gain (TE), can thus be defined as (Farquhar & Richards 1984; Hubick & Farquhar 1989):

$$TE = \frac{(1-\phi_c)(c_a - c_i)}{1.6v(1+\phi_w)} = \frac{(1-\phi_c)c_a\left(1 - \frac{c_i}{c_a}\right)}{1.6v(1+\phi_w)}, \quad (2)$$

where ϕ_c is the proportion of C taken up by photosynthesis that is subsequently lost to the atmosphere by respiration, and ϕ_w is unproductive water loss as a proportion of water loss associated with photosynthetic C uptake. If soil evaporation is factored out independently, the ϕ_w can be approximated as E_n/E_d, where E_n is nighttime transpiration and E_d

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is daytime transpiration. The second form of equation (2) is written as a function of c_i/c_a, because this term also relates to carbon isotope discrimination ($\Delta^{13}C$). The *TE* has units of mmol C mol^{-1} H_2O.

For some applications, it is convenient to describe the leaf-to-air vapour pressure difference, v, as a product of the air vapour pressure deficit (D), and a second term, f_v (Cernusak *et al.* 2008b). The f_v is a scaling factor relating the magnitude of v to that of D, such that $v = Df_v$. If leaf temperature is equal to air temperature, f_v will be unity, whereas if leaf temperature exceeds air temperature, f_v will exceed unity. Separating v into these two components allows equation (2) to be written as

$$D \cdot TE = \frac{(1-\phi_c)c_a\left(1-\frac{c_i}{c_a}\right)}{1.6 f_v (1+\phi_w)}. \quad (3)$$

Factoring D from the right side of equation (3) allows comparison of the transpiration efficiency of plants grown under different evaporative conditions (Tanner & Sinclair 1983; Hubick & Farquhar 1989). That is to say, weighting *TE* by D adjusts for variation in *TE* that is purely environmental. The term $D \cdot TE$ has units of Pa mol C mol^{-1} H_2O. For averaging purposes, the v in equation (2) and the D in equation (3) should be weighted by g_s integrated over the period during which the leaf is illuminated (Farquhar *et al.* 1989b). However, this would require that the diurnal course of g_s be known. In the absence of such information, we have calculated v and D in equations (2) and (3) as averages for daytime hours.

As noted above, photosynthetic discrimination against ^{13}C ($\Delta^{13}C$) relates independently to c_i/c_a. For C_3 plants, the relationship between the two can be described as follows (Farquhar, O'Leary & Berry 1982; Farquhar & Richards 1984; Hubick, Farquhar & Shorter 1986):

$$\Delta^{13}C = a - d + (b-a)\frac{c_i}{c_a}, \quad (4)$$

where a is discrimination against ^{13}C during diffusion of CO_2 through stomata (4.4‰), b is discrimination against ^{13}C by carboxylating enzymes (~29‰ for Rubisco), and d is a composite term that summarizes collectively discriminations associated with dissolution of CO_2, liquid phase diffusion, photorespiration and dark respiration (Farquhar, Ehleringer & Hubick 1989a). The d was estimated to have a mean value near 3‰ for 15 tropical tree and liana species (Cernusak *et al.* 2008b), and other estimates in the literature range from approximately 0 to 4‰ (Hubick *et al.* 1986; Hubick & Farquhar 1989; Hubick 1990; Cernusak *et al.* 2007b). The $\Delta^{13}C$ is defined with respect to CO_2 in air as $\Delta^{13}C = R_a/R_p - 1$, where R_a is $^{13}C/^{12}C$ of CO_2 in air and R_p is $^{13}C/^{12}C$ of plant C.

Measurements of $\Delta^{13}C$ have played an important role in water-use efficiency research (Farquhar & Richards 1984; Farquhar *et al.* 1989b; Ehleringer 1993; Brugnoli & Farquhar 2000; Seibt *et al.* 2008). The advantages of $\Delta^{13}C$ over conventional gas exchange measurements for determining c_i/c_a are that $\Delta^{13}C$ integrates approximately over the lifetime of the leaf or plant, and that samples can be collected and easily stored for analysis at a later date. It has further been suggested that measurement of the stable oxygen isotope ratio ($\delta^{18}O$) of the same plant material could provide additional insight into plant water-use efficiency (Farquhar *et al.* 1989b; Sternberg, Mulkey & Wright 1989; Farquhar, Condon & Masle 1994; Yakir & Israeli 1995; Scheidegger *et al.* 2000; Barbour 2007; Grams *et al.* 2007). For plants grown in a common environment, the $\delta^{18}O$ is expected to reflect variation in g_s (Barbour & Farquhar 2000), but not A, whereas $\Delta^{13}C$ is expected to vary as a function of either A or g_s. Thus, measurements of $\Delta^{13}C$ and $\delta^{18}O$ together could aid in identifying physiological mechanisms responsible for variation in water-use efficiency.

In the steady state, the $\delta^{18}O$ of water at the evaporative sites in leaves ($\delta^{18}O_e$) can be modeled as (Craig & Gordon 1965; Dongmann *et al.* 1974; Farquhar & Lloyd 1993),

$$\delta^{18}O_e = \delta^{18}O_s + \varepsilon^+ + \varepsilon_k + (\delta^{18}O_v - \delta^{18}O_s - \varepsilon_k)\frac{e_a}{e_i}, \quad (5)$$

where $\delta^{18}O_s$ is $\delta^{18}O$ of water taken up by roots from the soil, ε^+ and ε_k are equilibrium and kinetic fractionation factors, respectively, and $\delta^{18}O_v$ is $\delta^{18}O$ of atmospheric water vapour. The ε^+ can be modeled as a function of leaf temperature (Bottinga & Craig 1969), and ε_k as a function of diffusion resistance partitioning between stomata and boundary layer, with weighting by appropriate fractionation factors (Farquhar *et al.* 1989b; Cappa *et al.* 2003). The $\delta^{18}O$ of average lamina leaf water ($\delta^{18}O_L$) can then be described as (Farquhar & Lloyd 1993; Farquhar & Gan 2003),

$$\delta^{18}O_L = \delta^{18}O_s + (\delta^{18}O_e - \delta^{18}O_s)\left(\frac{1-e^{-\wp}}{\wp}\right). \quad (6)$$

The $\wp$ is a Péclet number, defined as $EL/(CD_{18})$, where E is transpiration rate (mol m^{-2} s^{-1}), L is a scaled effective path length (m), C is the molar concentration of water (mol m^{-3}), and D_{18} is the diffusivity of $H_2{}^{18}O$ in water, which can be predicted as a function of leaf temperature (Cuntz *et al.* 2007). The $\delta^{18}O$ of plant organic material ($\delta^{18}O_p$) is expected to vary as a function of $\delta^{18}O_L$ (Barbour & Farquhar 2000):

$$\delta^{18}O_p = \delta^{18}O_s + (\delta^{18}O_L - \delta^{18}O_s)(1 - p_{ex}p_x) + \varepsilon_{wc} + \varepsilon_{cp}, \quad (7)$$

where p_{ex} is the proportion of oxygen atoms that exchange with local water during cellulose synthesis, p_x is the proportion of un-enriched water at the site of tissue synthesis, ε_{wc} is the fractionation between organic oxygen and medium water, and ε_{cp} is the $\delta^{18}O$ difference between tissue dry matter and the cellulose component. The $p_{ex}p_x$, ε_{wc} and ε_{cp} have been observed to be reasonably constant for stem wood in trees (Cernusak, Farquhar & Pate 2005).

Although tropical trees grow in forests with relatively high annual precipitation, they are generally exposed to

intermittent drought, especially as seedlings and saplings, which can influence the distribution and abundance of species in the environment (Engelbrecht & Kursar 2003; Engelbrecht, Kursar & Tyree 2005; Engelbrecht *et al.* 2007). Water-use efficiency may therefore be an important functional trait for tropical trees, and large variation in whole-plant water-use efficiency has been observed among seedlings of several tropical tree species (Cernusak *et al.* 2007a, 2008b). These observations were carried out under conditions of approximately uniform water and nutrient availability. However, water-use efficiency is known to vary in response to the availability of both water (Hubick *et al.* 1986; Zhang & Marshall 1994; Sun *et al.* 1996) and nutrients (Toft, Anderson & Nowak 1989; Livingston *et al.* 1999; Cernusak *et al.* 2007b). A decreased supply of soil water would be expected to cause c_i/c_a to decrease due to lower g_s, whereas a higher nitrogen concentration in leaves would be expected to cause c_i/c_a to decrease due to higher A. Thus, either situation could lead to an increase in A/E and TE, as shown in equations (1) and (2). Our first objective in the present study was to test whether the ranking of TE previously established for three tropical tree species would be maintained under conditions of variable water and nutrient supply.

In our previous studies, we observed species-specific offsets in the relationship between whole-plant $\Delta^{13}C$ ($\Delta^{13}C_p$) and TE (Cernusak *et al.* 2007a, 2008b), suggesting a possible uncoupling between the two parameters at the species level. As indicated in equations (2) and (4), variation among species in the relationship between TE and $\Delta^{13}C$ could result from variable dependence among species of $\Delta^{13}C$ on c_i/c_a, or from variable dependence among species of TE on c_i/c_a. Separating these two possibilities would provide a valuable insight into the use of $\Delta^{13}C$ for inferring variation among species in TE. Our second objective in the present study was to investigate the physiological bases for offsets among species in the relationship between TE and $\Delta^{13}C$.

Equations (5) to (7) suggest that for plants grown in a common environment, $\delta^{18}O_p$ should decrease as a function of increasing g_s. This would be caused by a decrease in e_i associated with evaporative cooling of the leaf, a decrease in ε_k associated with a decrease in the proportion of total diffusive resistance accounted for by stomata, and an increase in the Pèclet number associated with an increase in E. However, recent reports have indicated that, in at least some instances, the relationship between $\delta^{18}O_p$ and g_s or transpiration rate did not match this expectation (Sheshshayee *et al.* 2005; Cernusak *et al.* 2007b, 2008a). Thus, our third objective in the present study was to examine relationships between organic material $\delta^{18}O$ and g_s in three tropical tree species.

METHODS

Plant material and study site

The three species employed were *Platymiscium pinnatum* (Jacq.) Dugand (Fabaceae), *Swietenia macrophylla* King (Meliaceae) and *Tectona grandis* Linn. f. (Verbenaceae). These three species exhibited highest, intermediate and lowest TE, respectively, of seven tropical tree species examined in a previous study (Cernusak *et al.* 2007a). Plants were grown at the Santa Cruz Experimental Field Facility of the Smithsonian Tropical Research Institute in Gamboa, Panama (9°07′N, 79°42′W). The altitude at the site is approximately 28 m above sea level. *T. grandis* and *S. macrophylla* were grown from seed collected in the Panama Canal watershed. Recently germinated seedlings of *P. pinnatum*, approximately two to four weeks old, were collected from the Azuero Peninsula, Panama and transplanted to the study site, due to difficulty in locating viable seed for this species. Meteorological conditions observed at the study site over the course of the experiment are presented in Table 1.

Experimental treatments

Twenty seedlings of each species were planted individually in 19 L pots, for a total of 60 pots. The pots were placed underneath a rain shelter with a glass roof on tables approximately 0.8 m above a concrete surface. The rain shelter reduced incoming photosynthetically active radiation (PAR) by about 20% compared to that observed outside the shelter under sunny conditions. The shelter had no sidewalls, such that air temperature, wind speed and relative humidity were similar to ambient conditions. Each pot contained 17.5 kg dry homogenized soil mixture.

Table 1. Mean monthly meteorological conditions for daytime hours through the course of the experiment. Daytime hours were defined as between 0700 and 1730 local time. We focused on daytime hours to characterize the environmental conditions under which photosynthesis took place

Month	Air temperature (°C)	Relative humidity (%)	Photosynthetically active radiation (μmol m^{-2} s^{-1})	Vapour pressure deficit (kPa)
November 2006	27.5	85.5	580	0.53
December 2006	29.4	80.8	679	0.79
January 2007	30.3	70.2	861	1.29
February 2007	29.9	69.4	966	1.29

The soil mixture comprised 80% by volume dark, air-dried topsoil and 20% by volume air-dried rice husks. The rice husks were added to improve soil structure and drainage. The pots were saturated with water and drained overnight to establish the pot water content at field capacity, which was determined to be 5.5 kg. The soil surface of each pot was then covered with 1.5 kg gravel to reduce soil evaporation. Twenty control pots with no plants were deployed along side the 60 pots containing plants to estimate pot water loss due to evaporation from the soil surface.

At the beginning the experiment, ten of the 20 pots for each species were randomly chosen to receive approximately 12 g Osmocote-Plus controlled-release fertilizer (Scotts-Sierra, Maryville, OH, USA). The fertilizer contained by weight 15% N, 9% P and 12% K, and had an estimated release time of five to six months. Five fertilized and five unfertilized pots from each species were then randomly allocated to receive reduced water supply. All pots started the experiment watered to field capacity. Those receiving the full water allocation were weighed each week and re-watered to near field capacity. Later in the experiment, pots were weighed and re-watered at shorter intervals, depending on water loss rates. Those receiving the reduced water allocation were allowed to dry down to pot water contents of less than 2.5 kg, or approximately 40% of field capacity, over several weeks. Thereafter they were weighed and re-watered to this pot water content each week, or at shorter intervals, as necessary. Pots were weighed to the nearest 5 g with a 64 kg capacity balance (Sartorius QS64B, Thomas, Swedesboro, NJ, USA). Drain holes at the bases of the pots were sealed for the duration of the experiment.

The experiment began on 27 October 2006. Initial plant dry weights were estimated by harvesting five representative individuals of each species. Mean values were 0.6, 0.4 and 0.7 g for *T. grandis*, *S. macrophylla* and *P. pinnatum*, respectively. The *T. grandis* plants grew considerably faster than those of *S. macrophylla* or *P. pinnatum*, and it was therefore necessary to harvest the former earlier than the latter. The *T. grandis* plants were harvested on 20 December 2006, and *S. macrophylla* and *P. pinnatum* were harvested on 1 March 2007.

Growth and transpiration measurements

Cumulative plant water use over the course of the experiment was calculated as the sum of pot water loss minus the average sum of water loss from the control pots. This calculation assumes that soil evaporation from pots without plants was the same as that from pots with plants. Half the control pots were allowed to dry down to pot water contents of 2.5 kg to estimate soil evaporation for the reduced water treatment. Prior to harvest, the pots were weighed at dawn and dusk for 2 d to partition diel transpiration into nighttime and daytime components. This allowed calculation of E_n/E_d. Immediately following harvest, total leaf area of each plant was measured with a LI-3100 Leaf Area Meter (Li-Cor, Lincoln, NE, USA). Harvested plants were dried to constant mass at 70 °C, and dry mass of leaves, stems and roots was determined separately for each plant to the nearest 0.02 g.

The mean relative growth rate (*RGR*) of each plant was calculated as $RGR = [\ln(m_2) - \ln(m_1)]/t$, where m_1 and m_2 are plant dry mass at the beginning and end of the experiment, respectively, and t is duration of the experiment (Blackman 1919). The mean transpiration rate (*MTR*) of each plant over the course of the experiment was calculated as $MTR = E_t/[(LA_1 + LA_2)0.5t]$, where E_t is cumulative transpiration, and LA_1 and LA_2 are leaf area at the beginning and end of the experiment (Sheshshayee *et al.* 2005). The *TE* of each plant was calculated as $TE = (m_{C2} - m_{C1} + l_C)/E_t$, where m_{C1} and m_{C2} are the plant C mass at the beginning and end of the experiment, and l_C is the C mass of leaves abscised over the course of the experiment.

To aid in comparison of plants that grew at different rates and over different time periods, we calculated growth-weighted averages of D, v and gravimetric soil water content (*SWC*) for each plant. Weekly dry matter increments were predicted from estimates of *RGR* (Cernusak *et al.* 2008b). Weekly averages of D were calculated from measurements of air temperature and relative humidity recorded by the weather station adjacent to the rain shelter (Winter *et al.* 2001; Winter, Aranda & Holtum 2005). We used data for the hours between 0700 and 1730 local time, the period during which most photosynthesis would have taken place. A leaf energy budget model developed by DGG de Pury and GD Farquhar (unpublished) and described by Barbour *et al.* (2000) was used to make predictions of average daytime leaf temperature. The model was parameterized with weekly averages of air temperature, relative humidity, wind speed and irradiance. Representative leaf area and stomatal conductance were additional input parameters. Weekly averages for v were calculated as the difference between predicted daytime e_i and daytime e_a. Weekly averages for *SWC* for each plant were calculated from the recorded pot weights. Average fresh mass for each plant for each week was calculated from the predicted dry mass at the beginning and end of the week and fresh mass to dry mass ratios measured at the end of the experiment. The weekly average *SWC* was calculated as the average water content of the pot for the week divided by the mass of air-dried soil mixture. Growth-weighted averages of D, v and *SWC*, over the course of the experiment were calculated for each plant as

$$x_g = \frac{\sum_{i=1}^{n} x_i m_i}{\sum_{i=1}^{n} m_i}, \quad (8)$$

where x_g is the growth-weighted average of either D, v, or *SWC*, x_i is the average value of the same parameter for week i, and m_i is the dry mass increment for week i.

Leaf gas exchange measurements

Photosynthesis (A), stomatal conductance (g_s) and c_i/c_a were measured on three to five fully expanded leaves per plant using an Li-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). Measurements were made at *PAR* of 1200 μmol m^{-2} s^{-1}, which was supplied by an artificial light source (Li-Cor). Mean v during measurements was 1.6 ± 0.3 kPa (mean ± 1 SD) and mean leaf temperature was 33 ± 1 °C (mean ± 1 SD). Measurements of *T. grandis* took place on 12 December 2006. These measurements were made approximately in the middle of a watering cycle. Measurements of *S. macrophylla* and *P. pinnatum* took place on 26 and 28 February 2007. Each plant of *S. macrophylla* and *P. pinnatum* was measured twice, once at the end of the watering cycle and once at the beginning. The two sets of measurements were averaged for each plant.

Stable isotope and elemental analyses

Leaves, stems and roots of each plant were ground separately to a fine, homogenous powder. Samples of approximately 3 mg were analysed for $\delta^{13}C$ and C and N concentrations with an elemental analyser (ECS 4010, Costech Analytical Technologies, Valencia, CA, USA) coupled to an isotope ratio mass spectrometer (Delta XP, Finnigan MAT, Bremen, Germany). Approximately 1 mg of stem dry matter of each plant was analysed for $\delta^{18}O$ on an isotope ratio mass spectrometer (Delta XP, Finnigan MAT) following pyrolysis in a high temperature furnace (Thermoquest TC/EA, Finnigan MAT). These analyses were carried out at the Stable Isotope Core Laboratory, Washington State University, Pullman, WA, USA. The $\delta^{13}C$ and $\delta^{18}O$ values have been expressed relative to standards of PeeDee Belemnite and Vienna Standard Mean Ocean Water, respectively. The $\Delta^{13}C$ of plant material was calculated as $\Delta^{13}C = (\delta_a - \delta_p)/(1 + \delta_p)$, where δ_a is $\delta^{13}C$ of CO_2 in air and δ_p is $\delta^{13}C$ of plant C. The δ_a was assumed to be –8‰. In a previous investigation at the same study site, the $\delta^{18}O$ of irrigation water was determined to be –4.0‰ (Cernusak *et al.* 2007b), although possible deviations from that value cannot be precluded for the present study.

Statistical analyses

Relationships between continuous variables were analysed by least-squares linear regression. Variation among species in relationships between continuous variables was analysed with analysis of covariance. Variation among species and treatments in physiological and isotopic parameters was assessed by analysis of variance. In these analyses, the number of observations was 60, the degrees of freedom for species was two, the degrees of freedom for nutrient and water treatments were one each, and the degrees of freedom error was 48. Pair-wise comparisons among species following analyses of variance were made according to Tukey's method. Results were considered statistically significant at $P < 0.05$. Statistical analyses were carried out in Systat 11.0 (SPSS, Chicago, IL, USA).

RESULTS

Growth, biomass allocation and elemental composition

Growth-weighted *SWC* as a proportion of field capacity varied between high and low water supply regimes, as intended in the experimental design. Mean values were 0.82 and 0.37 for *T. grandis*, 0.85 and 0.34 for *S. macrophylla*, and 0.92 and 0.41 for *P. pinnatum*, for high and low water supply, respectively. A value of one implies field capacity and zero implies air-dried soil.

A summary of growth parameter variation among species and treatments is given in Table 2. Mean *RGR* varied significantly with species ($P < 0.0001$), nutrient treatment ($P = 0.002$) and water treatment ($P = 0.0008$), and with the interaction between water treatment and species ($P = 0.0001$). Root/shoot ratio varied with species ($P < 0.0001$) and nutrient treatment ($P < 0.002$). Leaf area ratio (*LAR*) varied with species ($P < 0.0001$), nutrient treatment ($P < 0.0001$), and the interactions between water treatment and species ($P = 0.0006$) and nutrient and water treatments ($P = 0.02$). Specific leaf area (*SLA*) varied with species ($P < 0.0001$), and interactions between water treatment and species ($P = 0.02$) and nutrient and water treatments ($P = 0.02$). The mean *RGR* across all three species correlated significantly with *LAR* ($R^2 = 0.63$, $P < 0.0001$, $n = 60$), and with *SLA* ($R^2 = 0.38$, $P < 0.0001$, $n = 60$).

Whole-plant N concentration (N_p) varied significantly by species ($P < 0.0001$), by nutrient treatment ($P < 0.0001$) and by water treatment ($P = 0.001$). It was highest in *P. pinnatum*, lowest in *S. macrophylla* and intermediate in *T. grandis* (Table 2). The N_p was higher in fertilized than in unfertilized plants, and higher in plants at low than at high water supply (Table 2). There was a significant interaction between water treatment and species ($P = 0.02$). Patterns for leaf N concentration, both on a mass and an area basis, generally mirrored those for N_p (Table 2). Whole-plant C/N ratio varied among species and treatments in an inverse pattern to that for N_p (Table 2). Mean whole-plant C concentrations were 44.4, 45.4 and 45.1% for *T. grandis*, *S. macrophylla* and *P. pinnatum*, respectively.

Leaf gas exchange

Stomatal conductance to water vapour (g_s) varied significantly among species ($P < 0.0001$) and between water treatments ($P < 0.0001$), but not between nutrient treatments. Interaction terms between nutrient treatment and species ($P = 0.02$) and water treatment and species ($P = 0.0002$) were significant, indicating variation among species in the response of g_s to nutrient and water supply. *T. grandis* displayed the highest mean g_s, *S. macrophylla* the lowest and *P. pinnatum* an intermediate value (Table 2). The g_s of both *S. macrophylla* and *T. grandis* showed marked declines at low

Table 2. Morphological and physiological parameters for experimental plants. *Tectona grandis* plants were harvested on 20 December 2006, and those of *Swietenia macrophylla* and *Platymiscium pinnatum* on 1 March 2007. Values are presented as mean, with 1 *SD* in parentheses. For each treatment, $n = 5$. Treatments are as follows: –N–W (minus fertilizer, minus water), –N+W (minus fertilizer, plus water), +N–W (plus fertilizer, minus water) and +N+W (plus fertilizer, plus water)

	Tectona grandis				*Swietenia macrophylla*				*Platymiscium pinnatum*			
Parameter	–N–W	–N+W	+N–W	+N+W	–N–W	–N+W	+N–W	+N+W	–N–W	–N+W	+N–W	+N+W
Final dry mass (g)	63 (9)	99 (25)	66 (16)	149 (19)	63 (12)	83 (13)	58 (5)	131 (37)	42 (36)	18 (8)	56 (29)	70 (72)
Final leaf area (m^2)	0.48 (0.08)	0.66 (0.14)	0.52 (0.12)	1.33 (0.15)	0.30 (0.07)	0.33 (0.05)	0.35 (0.05)	0.65 (0.18)	0.11 (0.08)	0.06 (0.03)	0.16 (0.09)	0.30 (0.29)
Relative growth rate (mg g^{-1} d^{-1})	87.3 (2.7)	95.3 (4.6)	87.9 (4.6)	103.3 (2.4)	40.1 (1.5)	42.3 (1.3)	39.5 (0.7)	45.7 (2.6)	29.5 (9.1)	25.5 (4.1)	34.3 (4.8)	33.1 (9.2)
Root/shoot ratio (g g^{-1})	0.62 (0.10)	0.56 (0.04)	0.41 (0.06)	0.25 (0.05)	0.27 (0.04)	0.31 (0.04)	0.20 (0.04)	0.17 (0.02)	1.30 (0.54)	1.37 (0.95)	1.08 (0.37)	0.56 (0.15)
Leaf area ratio (m^2 kg^{-1})	7.6 (0.7)	6.8 (0.4)	8.0 (0.4)	8.9 (0.5)	4.8 (0.4)	4.1 (0.4)	6.0 (0.4)	5.0 (0.9)	3.1 (1.1)	3.6 (1.5)	2.9 (0.5)	5.3 (1.8)
Specific leaf area (m^2 kg^{-1})	17.3 (0.9)	15.5 (0.4)	15.8 (1.1)	16.8 (0.6)	14.1 (0.8)	13.0 (0.7)	14.6 (0.6)	13.4 (1.8)	12.6 (2.4)	12.7 (1.1)	11.1 (1.5)	14.5 (4.0)
Leaf [N] (mg g^{-1})	21.2 (3.4)	12.6 (0.4)	29.1 (1.5)	20.0 (2.3)	17.7 (0.9)	13.8 (0.9)	24.5 (1.5)	22.5 (1.7)	36.2 (7.0)	35.5 (4.9)	41.1 (6.6)	36.1 (6.3)
Whole-plant [N] (mg g^{-1})	13.2 (2.1)	8.3 (0.4)	20.3 (1.5)	14.2 (2.1)	9.6 (0.5)	7.5 (0.7)	15.0 (1.1)	12.8 (1.0)	18.9 (5.5)	19.6 (4.1)	23.0 (4.0)	21.6 (4.8)
Whole-plant C/N (g g^{-1})	34.4 (5.2)	53.3 (2.4)	22.1 (1.7)	31.6 (4.1)	47.3 (2.1)	60.4 (6.5)	30.8 (2.4)	35.6 (2.6)	25.7 (7.7)	23.8 (5.3)	20.0 (3.1)	21.5 (4.6)
TE (mmol C mol^{-1} H_2O)	2.97 (0.13)	2.46 (0.29)	3.01 (0.30)	2.63 (0.18)	2.58 (0.30)	1.62 (0.17)	2.98 (0.15)	2.41 (0.13)	2.00 (0.55)	2.03 (0.54)	2.39 (0.34)	2.39 (0.20)
Leaf $\delta^{13}C$ (‰)	–27.6 (0.3)	–29.0 (0.4)	–27.4 (0.2)	–28.4 (0.3)	–27.8 (0.7)	–30.9 (0.2)	–26.4 (0.3)	–29.2 (0.4)	–28.6 (0.7)	–28.5 (0.6)	–28.3 (0.2)	–27.9 (0.1)
Stem $\delta^{13}C$ (‰)	–26.4 (0.3)	–27.6 (0.4)	–26.2 (0.3)	–27.3 (0.4)	–25.6 (0.7)	–28.8 (0.4)	–24.5 (0.3)	–27.0 (0.4)	–26.8 (0.3)	–27.0 (0.6)	–26.2 (0.3)	–26.6 (0.4)
Root $\delta^{13}C$ (‰)	–26.0 (0.2)	–27.5 (0.5)	–26.0 (0.3)	–27.2 (0.4)	–25.4 (0.6)	–28.4 (0.3)	–24.6 (0.3)	–26.6 (0.3)	–26.0 (0.2)	–26.3 (0.7)	–25.2 (0.2)	–25.8 (0.3)
Photosynthesis (μmol m^{-2} s^{-1})	13.8 (1.2)	12.3 (1.2)	14.9 (2.1)	17.8 (1.1)	8.4 (1.7)	11.4 (0.8)	7.7 (0.3)	14.1 (1.1)	15.5 (3.1)	9.8 (2.3)	13.0 (4.0)	12.7 (2.6)
Conductance (mmol m^{-2} s^{-1})	291 (83)	452 (131)	335 (131)	617 (72)	145 (54)	395 (49)	101 (7)	355 (48)	423 (128)	338 (142)	281 (163)	340 (90)
Instantaneous c_i/c_a	0.72 (0.04)	0.82 (0.03)	0.73 (0.05)	0.82 (0.01)	0.66 (0.03)	0.83 (0.01)	0.61 (0.01)	0.76 (0.01)	0.77 (0.04)	0.81 (0.05)	0.71 (0.07)	0.79 (0.02)

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compared to high water supply, whereas that of *P. pinnatum* showed a slight increase at low compared to high water supply (Table 2).

Photosynthesis (A) varied significantly among species ($P < 0.0001$), and between nutrient treatments ($P = 0.006$), but not between water treatments. Interaction terms were significant between water treatment and species ($P < 0.0001$) and between nutrient and water treatments ($P = 0.0001$). Mean A was highest in *T. grandis*, lowest in *S. macrophylla* and intermediate in *P. pinnatum*, and it was higher in fertilized than in unfertilized plants (Table 2). The response of A to *SWC* varied among species, as evidenced by the significant water treatment by species interaction. The A of *S. macrophylla* showed a marked decline at low compared to high water supply, whereas that of *T. grandis* showed a smaller decline. In contrast, the A of *P. pinnatum* was higher at low than at high water supply (Table 2). The significant interaction between nutrient and water treatments resulted from a much stronger response of A to fertilizer addition at high compared to low water supply (Table 2).

Instantaneous measurements of c_i/c_a varied significantly among species ($P < 0.0001$), between nutrient treatments ($P = 0.0007$) and between water treatments ($P < 0.0001$). Additionally, interaction terms between nutrient treatment and species ($P = 0.03$) and between water treatment and species were significant ($P < 0.0001$). Mean values for c_i/c_a were significantly lower in *S. macrophylla* than in *T. grandis* or *P. pinnatum*, whereas they were similar between the latter two species (Table 2). The c_i/c_a was lower in fertilized than in unfertilized plants, and lower at low than at high water supply. The decrease in c_i/c_a in *S. macrophylla* at low water supply was much sharper than that in *T. grandis* or *P. pinnatum*, which lead to the strong water treatment by species interaction.

Whole-plant transpiration

Variation in measurements of whole-plant transpiration is shown in Fig. 1a–f. The E_d was based on pot weights taken shortly before harvest at dawn and dusk, and therefore represents an average transpiration rate over the day. The *MTR* represents an average daily transpiration rate over the full course of the experiment, calculated by employing the assumption that leaf area increased linearly during that period. Patterns among species and treatments for E_d and *MTR* were generally in good agreement with each other (Fig. 1), and the two independent estimates of whole-plant transpiration were closely correlated across the full data set ($r = 0.82$, $P < 0.0001$, $n = 59$).

The E_d varied significantly by species ($P = 0.03$) and by nutrient treatment ($P = 0.004$), but not by water treatment. There was a strong interaction between water treatment and species ($P < 0.0001$), as can be clearly seen in Fig. 1a–c. The E_d decreased at low compared to high *SWC* in *S. macrophylla* (Fig. 1a), did not change depending on *SWC* in

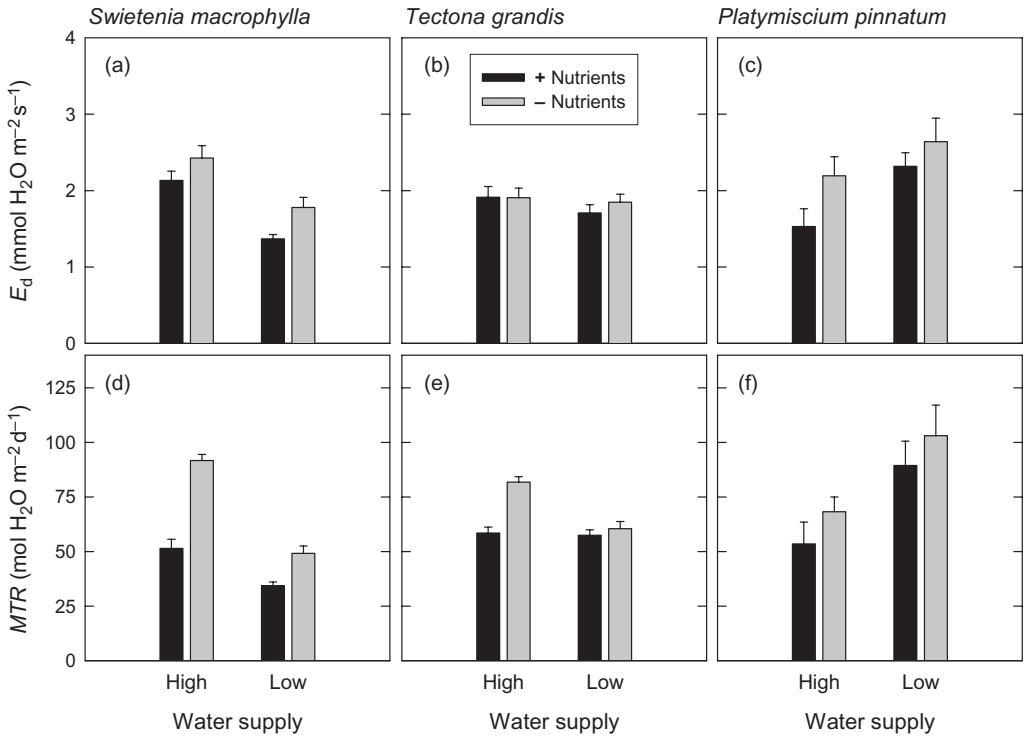

Figure 1. Two measurements of whole-plant transpiration rate for (a, d) *S. macrophylla*, (b, e) *T. grandis* and (c, f) *P. pinnatum*. The E_d is the average transpiration rate over a day calculated from pot weights taken at dawn and dusk shortly before harvest. The *MTR* is the mean daily transpiration rate over the full course of the experiment. Black bars refer to fertilized plants, and grey bars refer to unfertilized plants. Error bars represent one standard error. For each treatment combination, $n = 5$.

T. grandis (Fig. 1b), and increased at low compared to high *SWC* in *P. pinnatum* (Fig. 1c). Results for *MTR* were similar, with significant variation by species ($P = 0.0001$) and by nutrient treatment ($P < 0.0001$), but not by water treatment. Again, there was a significant interaction between water treatment and species ($P < 0.0001$), with patterns among species in response to decreasing *SWC* similar to those observed for E_d (Fig. 1d–f). For *MTR* there was also a moderately significant interaction between nutrient and water treatments ($P = 0.05$). Both E_d and *MTR* were significantly higher in unfertilized than in fertilized plants (Fig. 1).

The ratio of nighttime to daytime transpiration rates, E_n/E_d, varied significantly by species ($P < 0.0001$), but not by nutrient or water treatments. There was a significant interaction between water treatment and species ($P = 0.0008$). The mean E_n/E_d for *T. grandis* was 0.052, that for *S. macrophylla* was 0.032 and that for *P. pinnatum* was 0.012. The E_n/E_d was higher at low than at high water supply for *S. macrophylla* and *P. pinnatum*, but the converse was true for *T. grandis*.

Transpiration efficiency

The *TE*, calculated as experiment-long dry matter production divided by total water use for each plant, varied significantly by species ($P < 0.0001$), by nutrient treatment ($P < 0.0001$) and by water treatment ($P < 0.0001$). There were significant interactions between nutrient treatment and species ($P = 0.04$) and between water treatment and species ($P = 0.0009$), indicating variation among species in responses of *TE* to nutrient and water supply. Mean *TE* was significantly higher in *T. grandis* than in *S. macrophylla* or *P. pinnatum*, and similar in the latter two species (Table 2). The *TE* was higher in fertilized than in unfertilized plants, and higher at low than at high water supply. The *TE* of *S. macrophylla* and *P. pinnatum* increased in response to fertilizer addition, whereas that of *T. grandis* showed no response to fertilizer addition. On the other hand, *TE* of *S. macrophylla* and *T. grandis* increased with decreasing *SWC*, whereas that of *P. pinnatum* showed no response to *SWC* (Table 2).

Estimation of the growth-weighted vapour pressure deficit, *D*, showed that the higher *TE* of *T. grandis* resulted from a reduced air vapour pressure deficit for the period during which this species grew. The *T. grandis* plants were harvested in late December, whereas those of *S. macrophylla* and *P. pinnatum* were harvested at the beginning of March, due to variation among species in *RGR*. The vapour pressure deficit in January and February was about twice that in November and December (Table 1), as would be expected with the progression of the dry season at the study site. The *D·TE* showed a similar pattern of variation to *TE* within species (Fig. 2a–c); however, the ranking among the three species was different than for *TE*. The mean values of *D·TE* were 3.00, 2.66 and 2.07 Pa mol C

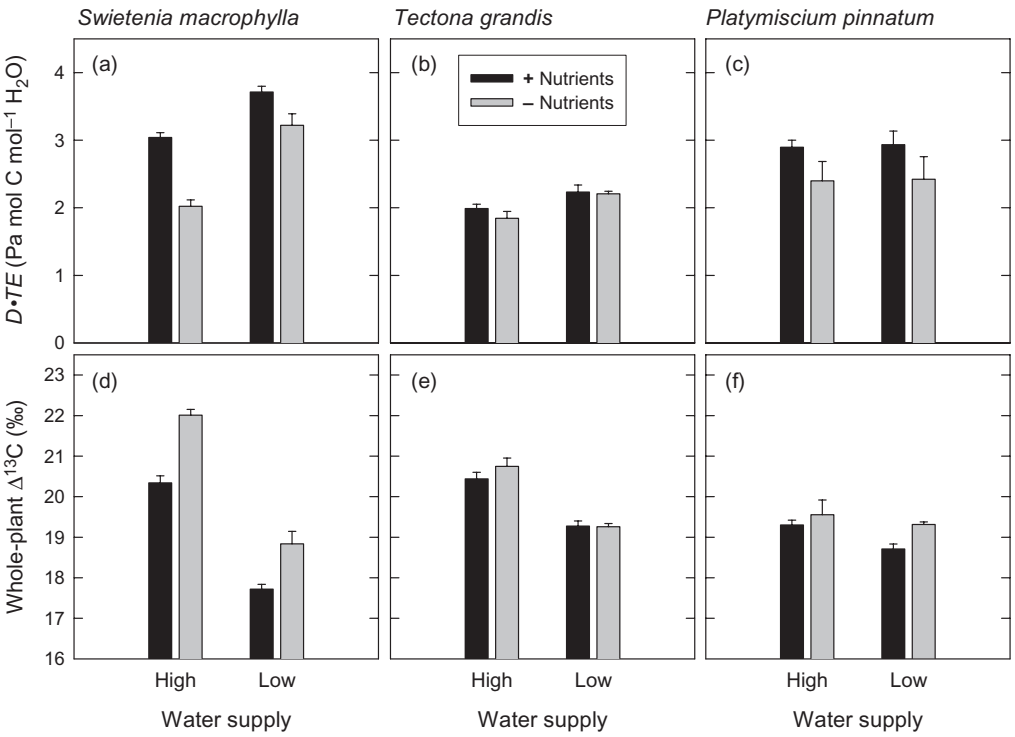

Figure 2. Upper panels show transpiration efficiency weighted by air vapour pressure deficit (*D·TE*) for (a) *S. macrophylla*, (b) *T. grandis* and (c) *P. pinnatum*. The lower panels show whole-plant $\Delta^{13}C$ for (d) *S. macrophylla*, (e) *T. grandis* and (f) *P. pinnatum*. Black bars refer to fertilized plants, and grey bars refer to unfertilized plants. Transpiration efficiency was calculated from experiment-long dry mass increments and cumulative water use. Error bars represent one standard error. For each treatment combination, $n = 5$.

mol^{-1} H_2O for *S. macrophylla*, *P. pinnatum* and *T. grandis*, respectively.

Stable isotope composition

Mean $\delta^{13}C$ for leaves, stems and roots for each treatment within each species is given in Table 2. Leaf $\delta^{13}C$ was consistently more negative than that of stems or roots. Mean values across the full data set were –28.3, –26.7 and –26.2‰, for leaves, stems and roots, respectively.

Whole-plant $\Delta^{13}C$ ($\Delta^{13}C_p$) varied by species ($P < 0.0001$), by nutrient treatment ($P < 0.0001$) and by water treatment ($P < 0.0001$). Interactions between nutrient treatment and species ($P < 0.0001$) and between water treatment and species ($P < 0.0001$) were significant, indicating variation among species in responses of $\Delta^{13}C_p$ to nutrient and water availability. The $\Delta^{13}C_p$ of *S. macrophylla* showed the strongest response to both fertilizer addition and decreasing *SWC* (Fig. 2d). That of *T. grandis* showed no response to fertilizer addition, and an intermediate response to decreasing *SWC* (Fig. 2e). Finally, $\Delta^{13}C_p$ of *P. pinnatum* showed only weak responses to either fertilizer addition or decreasing *SWC* (Fig. 2f).

Slopes of the relationships between $\Delta^{13}C_p$ and instantaneous c_i/c_a varied among species ($P < 0.0001$), as shown in Fig. 3. Regression equations indicated slope estimates of 18.0, 10.6 and 2.7‰ for *S. macrophylla*, *T. grandis* and *P. pinnatum*, respectively. The instantaneous c_i/c_a explained 96% of variation in $\Delta^{13}C_p$ for *S. macrophylla* ($P < 0.0001$, $n = 20$), 67% for *T. grandis* ($P < 0.0001$, $n = 20$) and 10% for *P. pinnatum* ($P = 0.18$, $n = 20$).

Relationships between $D{\cdot}TE$ and $\Delta^{13}C_p$ varied among species (Fig. 4a). The slopes of the relationships were not significantly different; however, there was significant variation in the intercepts ($P < 0.0001$). Thus, we observed species-specific offsets in the relationship between $D{\cdot}TE$ and $\Delta^{13}C_p$. Variation in the relationships for *S. macrophylla* and *T. grandis* could be reconciled by taking into account modeled differences between leaf temperature and air temperature. Thus, relationships between $v{\cdot}TE$ and $\Delta^{13}C_p$ for *S. macrophylla* and *T. grandis* were very similar (Fig. 4c). However, the intercept of the relationship between $v{\cdot}TE$ and $\Delta^{13}C_p$ for *P. pinnatum* still differed significantly from those for *S. macrophylla* and *T. grandis* ($P < 0.0001$). On the other hand, relationships for *S. macrophylla* and *P. pinnatum* could be reconciled by replacing $\Delta^{13}C_p$ with instantaneous c_i/c_a (Fig. 4b & d). Accordingly, a plot of $v{\cdot}TE$ against instantaneous c_i/c_a (Fig. 4d) produced the greatest homogeneity among species for the four sets of relationships shown in Fig. 4. There still existed a small but significant variation in slopes among species in the relationships between $v{\cdot}TE$ and instantaneous c_i/c_a ($P = 0.02$). However, the vast majority of variation in $v{\cdot}TE$ across the full data set could be accounted for by considering only instantaneous c_i/c_a. Thus, a general linear model, taking as independent variables c_i/c_a, species, and the species by c_i/c_a interaction, explained 89% of variation in $v{\cdot}TE$. On the other hand, c_i/c_a on its own explained 82% of variation in $v{\cdot}TE$.

Relationships between the $\delta^{18}O$ of stem dry matter ($\delta^{18}O_{st}$) and g_s varied among species ($P < 0.0001$), as shown in Fig. 5. The $\delta^{18}O_{st}$ showed a strong dependence on g_s for *S. macrophylla* ($R^2 = 0.80$, $P < 0.0001$, $n = 20$), a weak dependence for *P. pinnatum* ($R^2 = 0.19$, $P = 0.05$, $n = 20$), and no dependence for *T. grandis* ($R^2 = 0.05$, $P = 0.37$, $n = 20$). Analyses of the dependence of $\delta^{18}O_{st}$ on three independent estimates of transpiration rate are shown in Table 3. The $\delta^{18}O_{st}$ was strongly correlated with transpiration for *S. macrophylla*, weakly correlated for *P. pinnatum*, and not correlated for *T. grandis* (Table 3).

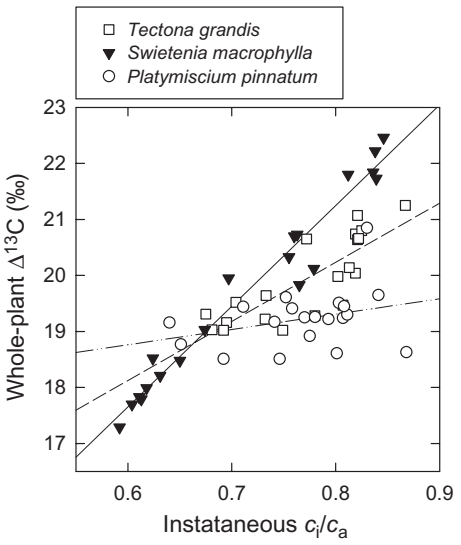

Figure 3. Whole-plant $\Delta^{13}C$ plotted against instantaneous c_i/c_a. The solid line refers to *S. macrophylla*, the dashed line to *T. grandis* and the dash-dotted line to *P. pinnatum*.

DISCUSSION

Ranking of TE among species

We used $D{\cdot}TE$ in our analysis to account for differences in *TE* that resulted from variation in *D*, the growth-weighted air vapour pressure deficit. Variation in *D* resulted primarily from the need to harvest the *T. grandis* plants at an earlier date than the other two species, due to faster *RGR* in the former (Table 2). The *TE* was calculated as dry matter production over the full course of the experiment divided by cumulative plant water use. Results of the present study add to a growing body of evidence showing that $D{\cdot}TE$ of *S. macrophylla* is higher than that of *T. grandis*. Both *S. macrophylla* (mahogany) and *T. grandis* (teak) are commercial timber species that are widely planted in the tropics. The mean $D{\cdot}TE$ of *S. macrophylla* across all treatments in the present study was 3.00 Pa mol C mol^{-1} H_2O, whereas that of *T. grandis* was 2.07 Pa mol C mol^{-1} H_2O; thus that of *S. macrophylla* was 45% higher compared to that of *T. grandis*. The ranking between these two species did not change among water and nutrient treatments. Differences

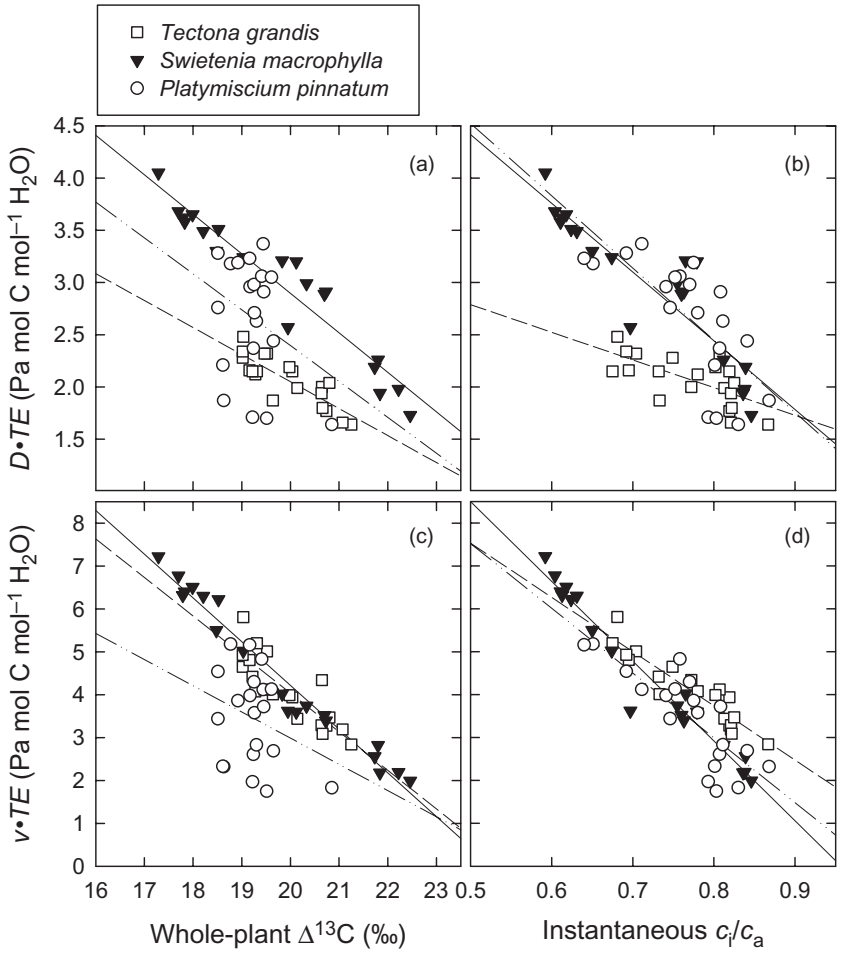

Figure 4. Upper panels show transpiration efficiency weighted by air vapour pressure deficit ($D{\cdot}TE$) plotted against (a) whole-plant $\Delta^{13}C$, and (b) instantaneous c_i/c_a. Lower panels show transpiration efficiency weighted by leaf-to-air vapour pressure difference ($v{\cdot}TE$) plotted against (c) whole-plant $\Delta^{13}C$, and (d) instantaneous c_i/c_a. Transpiration efficiency was calculated from measurements of dry matter production and cumulative water use over the full course of the experiment. Solid lines refer to *S. macrophylla*, dashed lines to *T. grandis* and dash-dotted lines to *P. pinnatum*.

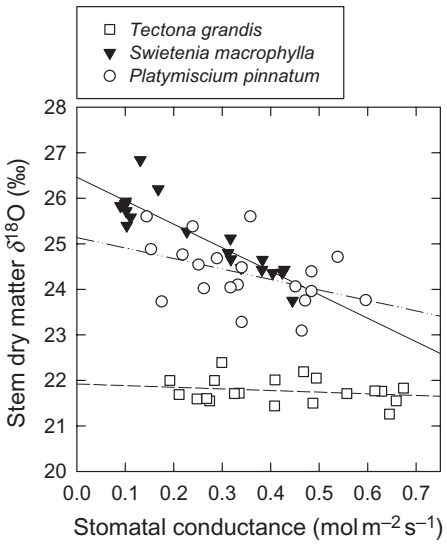

Figure 5. The $\delta^{18}O$ of stem dry matter plotted against stomatal conductance to water vapour. The solid line refers to *S. macrophylla*, the dashed line to *T. grandis* and the dash-dotted line to *P. pinnatum*.

between *TE* of *S. macrophylla* and *T. grandis* of a similar magnitude have been observed in previous experiments (Winter *et al.* 2005; Cernusak *et al.* 2007a, 2008b).

In contrast to the stability in the ranking of $D{\cdot}TE$ among treatments between *S. macrophylla* and *T. grandis*, that of *P. pinnatum* was variable. *P. pinnatum* was previously observed to have the highest $D{\cdot}TE$ among the three study species under high water supply in unfertilized soil (Cernusak *et al.* 2007a, 2008b). Under similar conditions in the present experiment, *P. pinnatum* also had the highest $D{\cdot}TE$, consistent with the previous results. However, $D{\cdot}TE$ of *P. pinnatum* showed no response to decreasing *SWC* and only a weak response to fertilizer addition (Fig. 3c). Thus, under conditions of low water supply, or in fertilized soil, the $D{\cdot}TE$ of *P. pinnatum* was intermediate between that of *S. macrophylla* and *T. grandis*. *P. pinnatum* is a leguminous tree capable of forming N_2-fixing root nodules. The leaf N concentration was higher in *P. pinnatum* than in *S. macrophylla* or *T. grandis* in all treatments; however, *P. pinnatum* enjoyed its greatest advantage in leaf N concentration over the other two species under conditions of high water supply in unfertilized soil (Table 2). Thus, the ability to symbiotically fix N_2 from the atmosphere may have contributed toward higher water-use efficiency under conditions of relatively low soil N availability and high water supply.

Table 3. Results of linear regression analyses with stem dry matter $\delta^{18}O$ as the dependent variable and three different measures of transpiration rate alternately taken as independent variables. The E_i is the instantaneous transpiration rate (mmol m^{-2} s^{-1}) measured with the portable photosynthesis system; E_d is the average transpiration rate over a day determined gravimetrically (mmol m^{-2} s^{-1}); and *MTR* is the mean transpiration rate over the course of the experiment (mol m^{-2} d^{-1}). Asterisks indicate statistical significance as follows: *($P < 0.05$), **($P < 0.01$) and ***($P < 0.001$); $n = 20$ for all analyses

Independent variable	Intercept	Regression coefficient with stem $\delta^{18}O$ as dependent variable			Model R^2
		Tectona grandis	*Swietenia macrophylla*	*Platymiscium pinnatum*	
E_i	21.9	–0.02			0.01
E_d	21.6	0.08			0.01
MTR	21.5	0.00			0.03
E_i	26.8		–0.52		0.73***
E_d	27.4		–1.16		0.49***
MTR	26.5		–0.02		0.42**
E_i	25.7			–0.34	0.20*
E_d	24.7			–0.17	0.03
MTR	24.6			–0.00	0.01

Relationships between *TE*, $\Delta^{13}C_p$ and c_i/c_a

We observed significant offsets among the three species in relationships between $D \cdot TE$ and $\Delta^{13}C_p$ (Fig. 4a), consistent with previous results (Cernusak *et al.* 2007a, 2008b). In the present study, it could be demonstrated that the variation among species in the relationship between $D \cdot TE$ and $\Delta^{13}C_p$ resulted from both an uncoupling of $\Delta^{13}C_p$ from c_i/c_a in *P. pinnatum*, and from larger f_v in *T. grandis* than in the other two species, where f_v is defined as v/D. Thus, plotting $v \cdot TE$ against instantaneous c_i/c_a resulted in a generally uniform relationship among the three species (Fig. 4d). Data collected previously for these species (Cernusak *et al.* 2008b) can be added to the analysis. For the combined dataset, instantaneous c_i/c_a explained 87% of variation in $v \cdot TE$ ($R^2 = 0.87$, $P < 0.0001$, $n = 79$). Adding species and a species by c_i/c_a interaction term resulted in only a very slight increase in the proportion of variation in $v \cdot TE$ that was explained ($R^2 = 0.89$, $P < 0.0001$, $n = 79$), providing further evidence of a common relationship between $v \cdot TE$ and c_i/c_a among the three species.

These results suggest that in addition to c_i/c_a, f_v can be an important source of variation in $D \cdot TE$. Mean values of f_v among the three species were 2.0, 1.4 and 1.3 for *T. grandis*, *S. macrophylla* and *P. pinnatum*, respectively. These values of f_v correspond to mean predicted differences between leaf and air temperature of 2.9, 2.1 and 1.3 °C for the three species, respectively. The larger f_v for *T. grandis* resulted from a combination of a larger representative leaf area employed in the leaf energy budget model (325 cm^2 compared to 50 cm^2 for *S. macrophylla* and *P. pinnatum*), and lower wind speeds during the growth period of *T. grandis* compared to that of *S. macrophylla* and *P. pinnatum*. The $v \cdot TE$ did not differ significantly between *T. grandis* and *S. macrophylla* in the present experiment ($P = 0.25$), supporting the suggestion that variation in *TE* between these two species, when grown under similar environmental conditions, results at least partly from differences in v, caused by differences in leaf temperature (Winter *et al.* 2005).

Equation (2) suggests that ϕ_c, the fraction of C taken up by photosynthesis that is subsequently lost to the atmosphere by respiration, can be estimated by plotting $v \cdot TE$ against $c_a(1 - c_i/c_a)/1.6$. Figure 6 shows such a plot, and includes data from previous measurements on the same three species (Cernusak *et al.* 2008b). The slope of a relationship between $v \cdot TE$ and $c_a(1 - c_i/c_a)/1.6$, with the intercept forced through the origin, is thus equal to

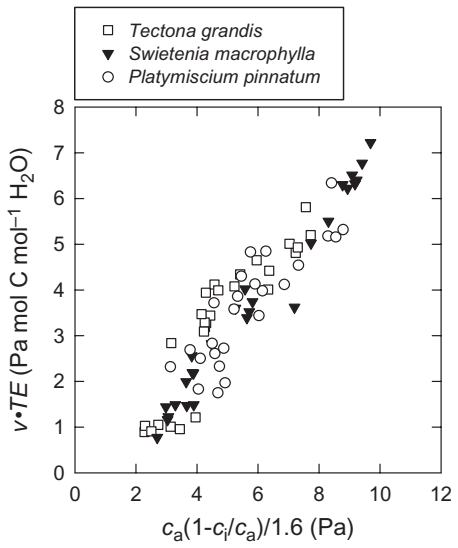

Figure 6. Transpiration efficiency weighted by leaf-to-air vapour pressure difference ($v \cdot TE$) plotted against $c_a(1 - c_i/c_a)/1.6$. The c_i/c_a was determined from instantaneous gas exchange measurements. Transpiration efficiency was determined from experiment-long measurements of dry matter production and cumulative water use. The c_a was assumed to be 38 Pa. The figure contains previously published data for 19 plants (Cernusak *et al.* 2008b).

$(1 - \phi_c)/(1 + \phi_w)$. Assuming c_a of 38 Pa, slope estimates for *T. grandis*, *S. macrophylla* and *P. pinnatum* are 0.705, 0.657 and 0.642, respectively. Combining these with estimates of ϕ_w, calculated as E_n/E_d (0.052, 0.032 and 0.012, respectively), results in estimates for ϕ_c of 0.258, 0.322 and 0.350 for *T. grandis*, *S. macrophylla* and *P. pinnatum*. This suggests a modest variation among species in ϕ_c. This method of estimating ϕ_c is rather indirect, and it would be helpful to confirm the results with direct measurements of whole-plant gas exchange. Nonetheless, it suggests that ϕ_c and ϕ_w are unlikely to cause large variations in *TE*, whereas c_i/c_a and v are primary controls. This agrees with some previous analyses (Cernusak *et al.* 2007b, 2008b), but contradicts others (Guehl, Fort & Ferhi 1995; Hobbie & Colpaert 2004).

We observed strong variations among the three species in the dependence of $\Delta^{13}C_p$ on c_i/c_a (Fig. 3). In the case of *P. pinnatum*, the slope of the relationship between the two parameters did not differ significantly from zero. On the one hand, the timescales of measurement for $\Delta^{13}C_p$ and c_i/c_a were very different, with c_i/c_a integrating over minutes and $\Delta^{13}C_p$ integrating over months. On the other hand, the strong correlation between $v \cdot TE$ and c_i/c_a (Fig. 5d) suggests that the measured c_i/c_a values were generally representative of those experienced throughout the experiment. Unfortunately, our dataset provides no insight into whether the uncoupling between $\Delta^{13}C_p$ and c_i/c_a in *P. pinnatum* occurred during photosynthesis, or as the result of a post-photosynthetic process (Hobbie & Werner 2004; Badeck *et al.* 2005; Bowling, Pataki & Randerson 2008; Cernusak *et al.* 2009). Simultaneous measurements of $\Delta^{13}C$ and c_i/c_a during photosynthetic gas exchange would be helpful in this respect.

Dependence of $\delta^{18}O_{st}$ on g_s

There is growing interest in using measurements of $\delta^{18}O$ in plant organic material as a proxy indicator for variation in g_s (Farquhar & Lloyd 1993; Barbour *et al.* 2000; Barbour 2007). Such an application would be particularly useful in water-use efficiency research, as it would allow variation in $\Delta^{13}C$ to be attributed to variation in either A or g_s. The expectation of a negative relationship between $\delta^{18}O$ of plant organic material and g_s is theoretically based in steady-state leaf water ^{18}O enrichment (Farquhar & Lloyd 1993; Farquhar, Cernusak & Barnes 2007; Ripullone *et al.* 2008), and the expected relationship has been observed in a number of species (Barbour & Farquhar 2000; Barbour *et al.* 2000, 2004; Grams *et al.* 2007; Sullivan & Welker 2007). In the present study, we observed a strong negative relationship between $\delta^{18}O_{st}$ and g_s in *S. macrophylla*, a weak negative relationship in *P. pinnatum*, and no relationship in *T. grandis* (Fig. 5). Correlations between $\delta^{18}O_{st}$ and transpiration rate followed a similar pattern (Table 3). All species covered a similarly large range of g_s, and we are therefore unable to explain the presence of a negative relationship in one species and not another.

It was previously observed in *Fagus sylvatica* and *Picea abies* that $\delta^{18}O$ of leaf dry matter did not correlate with g_s, whereas the $\delta^{18}O$ of leaf cellulose did (Grams *et al.* 2007). The difference between $\delta^{18}O$ of leaf dry matter and leaf cellulose is known to be somewhat variable (Farquhar, Barbour & Henry 1998; Cernusak, Pate & Farquhar 2004; Cernusak *et al.* 2005), whereas the difference between $\delta^{18}O$ of wood dry matter and wood cellulose is generally stable (Borella, Leuenberger & Saurer 1999; Barbour, Andrews & Farquhar 2001; Cernusak *et al.* 2005). We chose to analyse the $\delta^{18}O$ of stem dry matter rather than leaf dry matter with this consideration in mind. Nonetheless, we cannot rule out the possibility that relationships between $\delta^{18}O$ and g_s could differ between stem cellulose and stem dry matter in our dataset.

The mean $\delta^{18}O_{st}$ of *T. grandis* was less than that of *S. macrophylla* and *P. pinnatum* by approximately 3‰ (Fig. 5). This partly reflected the higher relative humidity during November and December, compared to that during January and February (Table 1). *T. grandis* was harvested on 20 December, whereas *S. macrophylla* and *P. pinnatum* were harvested on 1 March. The growth-weighted estimate of $\delta^{18}O_e$, calculated as described previously (Cernusak *et al.* 2008b), was lower by 3.0‰ for *T. grandis* compared to the average for *S. macrophylla* and *P. pinnatum*. Across the full data set, the $\delta^{18}O_e$ explained 67% of variation in $\delta^{18}O_{st}$ ($R^2 = 0.67$, $P < 0.0001$, $n = 60$), suggesting a reasonable agreement between predicted variation in leaf water ^{18}O enrichment and observed variation in stem dry matter $\delta^{18}O$.

Whole-plant transpiration

Whole-plant transpiration was higher in unfertilized than in fertilized plants when expressed on a leaf area basis, and this pattern was consistent across the dataset (Fig. 1). Assuming that fertilization generally functioned to increase the N concentration of the soil solution, this result is consistent with previous results indicating increased plant transpiration in response to low N availability (Guehl *et al.* 1995; Livingston *et al.* 1999; Cernusak *et al.* 2007b; Cramer, Hoffmann & Verboom 2008). An increased transpiration rate in this case would serve to increase concentrations of nitrogenous solutes at the root surface due to convective transport (mass flow) and thus increase N absorption compared to that at a lower transpiration rate (Barber 1995). Interestingly, in our dataset, g_s did not vary by nutrient treatment, suggesting that modulation of whole-plant transpiration in response to N availability did not occur through variation in g_s. Canopy architecture may have played a more important role in coordinating whole-plant transpiration with N availability through increased self-shading and canopy boundary layer development in fertilized plants, caused by larger leaf areas (Table 2).

The response of whole-plant transpiration to decreased *SWC* varied among the three species (Fig. 1). The response of *P. pinnatum* was difficult to explain, in that it increased at low compared to high *SWC* (Fig. 1c & f). Measurements of g_s were consistent with this response. For *P. pinnatum*, g_s tended to increase with declining *SWC* from near field capacity to about one-third field capacity. At *SWC* less than

about one-third field capacity, g_s of *P. pinnatum* decreased linearly. In general, variation in growth and water use was more variable within treatments for *P. pinnatum* than for the other two species (Table 2). This could have related to the fact that the *P. pinnatum* seedlings were collected from the field and transplanted to the study site, whereas the other two species were germinated from seed at the study site under controlled conditions. Thus, it is possible that the higher transpiration rates at low compared to high *SWC* for *P. pinnatum* were coincidental, resulting from large background variation in physiological performance among the study population for this species. Nevertheless, a diverse range of responses of g_s and whole-plant transpiration to declining *SWC* has been observed in other tropical tree species (Bonal *et al.* 2000; Bonal & Guehl 2001), and the apparent increase in transpiration in *P. pinnatum* in response to low water supply that we observed is therefore worthy of further investigation.

Conclusions

The response of $D{\cdot}TE$ to variable water and nutrient supply did indeed differ among the three species studied (Fig. 2a–c). Thus, their relative ranking was not maintained across treatments. Offsets were observed among species in relationships between $D{\cdot}TE$ and $\Delta^{13}C_p$ (Fig. 4a). For *P. pinnatum*, this offset resulted from a breakdown in the dependence of $\Delta^{13}C_p$ on c_i/c_a (Fig. 3). In the case of *T. grandis*, the offset resulted from variation in f_v, such that it could be reconciled by substituting $v{\cdot}TE$ for $D{\cdot}TE$ (Fig. 4c). Thus, species-specific offsets in relationships between $D{\cdot}TE$ and $\Delta^{13}C_p$ resulted from both variable dependence of $\Delta^{13}C$ on c_i/c_a and variable dependence of $D{\cdot}TE$ on c_i/c_a. Additionally, we observed a clear variation among species in the dependence of stem dry matter $\delta^{18}O$ on stomatal conductance (Fig. 5).

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REFERENCES

Bacon M.A. (2004) Water use efficiency in plant biology. In *Water Use Efficiency in Plant Biology* (ed. M.A. Bacon), pp. 1–26. Blackwell Publishing, Oxford, UK.

Badeck F.W., Tcherkez G., Nogues S., Piel C. & Ghashghaie J. (2005) Post-photosynthetic fractionation of stable carbon isotopes between plant organs – a widespread phenomenon. *Rapid Communications in Mass Spectrometry* **19,** 1381–1391.

Barber S.A. (1995) *Soil Nutrient Bioavailability: A Mechanistic Approach*, 2nd edn. John Wiley & Sons, New York, USA.

Barbour M.M. (2007) Stable oxygen isotope composition of plant tissue: a review. *Functional Plant Biology* **34,** 83–94.

Barbour M.M. & Farquhar G.D. (2000) Relative humidity- and ABA-induced variation in carbon and oxygen isotope ratios of cotton leaves. *Plant, Cell & Environment* **23,** 473–485.

Barbour M.M., Fischer R.A., Sayre K.D. & Farquhar G.D. (2000) Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and grain yield in irrigated wheat. *Australian Journal of Plant Physiology* **27,** 625–637.

Barbour M.M., Andrews J.T. & Farquhar G.D. (2001) Correlations between oxygen isotope ratios of wood constituents of *Quercus* and *Pinus* samples from around the world. *Australian Journal of Plant Physiology* **28,** 335–348.

Barbour M.M., Roden J.S., Farquhar G.D. & Ehleringer J.R. (2004) Expressing leaf water and cellulose oxygen isotope ratios as enrichment above source water reveals evidence of a Péclet effect. *Oecologia* **138,** 426–435.

Blackman V.H. (1919) The compound interest law and plant growth. *Annals of Botany* **33,** 353–360.

Bonal D. & Guehl J.M. (2001) Contrasting patterns of leaf water potential and gas exchange responses to drought in seedlings of tropical rainforest species. *Functional Ecology* **15,** 490–496.

Bonal D., Barigah T.S., Granier A. & Guehl J.M. (2000) Late-stage canopy tree species with extremely low $\delta^{13}C$ and high stomatal sensitivity to seasonal soil drought in the tropical rainforest of French Guiana. *Plant, Cell & Environment* **23,** 445–459.

Borella S., Leuenberger M. & Saurer M. (1999) Analysis of $\delta^{18}O$ in tree rings: wood-cellulose comparison and method dependent sensitivity. *Journal of Geophysical Research* **104,** 19267–19273.

Bottinga Y. & Craig H. (1969) Oxygen isotope fractionation between CO_2 and water, and the isotopic composition of marine atmospheric CO_2. *Earth and Planetary Science Letters* **5,** 285–295.

Bowling D.R., Pataki D.E. & Randerson J.T. (2008) Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes. *New Phytologist* **178,** 24–40.

Brugnoli E. & Farquhar G.D. (2000) Photosynthetic fractionation of carbon isotopes. In *Photosynthesis: Physiology and Metabolism* (eds R.C. Leegood, T.C. Sharkey & S. von Caemmerer), pp. 399–434. Kluwer, Dordrecht, the Netherlands.

Cappa C.D., Hendricks M.B., DePaulo D.J. & Cohen R.C. (2003) Isotopic fractionation of water during evaporation. *Journal of Geophysical Research* **108,** 4525.

Cernusak L.A., Pate J.S. & Farquhar G.D. (2004) Oxygen and carbon isotope composition of parasitic plants and their hosts in southwestern Australia. *Oecologia* **139,** 199–213.

Cernusak L.A., Farquhar G.D. & Pate J. (2005) Environmental and physiological controls over oxygen and carbon isotope composition of Tasmanian blue gum, *Eucalyptus globulus*. *Tree Physiology* **25,** 129–146.

Cernusak L.A., Aranda J., Marshall J.D. & Winter K. (2007a) Large variation in whole-plant water-use efficiency among tropical tree species. *New Phytologist* **173,** 294–305.

Cernusak L.A., Winter K., Aranda J., Turner B.L. & Marshall J.D. (2007b) Transpiration efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility. *Journal of Experimental Botany* **58,** 3549–3566.

Cernusak L.A., Mejia-Chang M., Winter K. & Griffiths H. (2008a) Oxygen isotope composition of CAM and C_3 *Clusia* species: non-steady-state dynamics control leaf water ^{18}O enrichment in succulent leaves. *Plant, Cell & Environment* **31,** 1644–1662.

Cernusak L.A., Winter K., Aranda J. & Turner B.L. (2008b) Conifers, angiosperm trees, and lianas: growth, whole-plant water and nitrogen use efficiency, and stable isotope composition ($\delta^{13}C$ and $\delta^{18}O$) of seedlings grown in a tropical environment. *Plant Physiology* **148,** 642–659.

Cernusak L.A., Tcherkez G., Keitel C., *et al.* (2009) Why are non-photosynthetic tissues generally ^{13}C enriched compared to leaves in C_3 plants? Review and synthesis of current hypotheses. *Functional Plant Biology* **36,** 199–213.

Craig H. & Gordon L.I. (1965) Deuterium and oxygen-18 variations in the ocean and the marine atmosphere. In *Proceedings of a Conference on Stable Isotopes in Oceanographic Studies and Palaeotemperatures* (ed. E. Tongiorgi), pp. 9–130. Lischi and Figli, Pisa, Italy.

Cramer M.D., Hoffmann V. & Verboom G.A. (2008) Nutrient availability moderates transpiration in *Ehrharta calycina*. *New Phytologist* **179,** 1048–1057.

Cuntz M., Ogée J., Farquhar G.D., Peylin P. & Cernusak L.A. (2007) Modelling advection and diffusion of water isotopologues in leaves. *Plant, Cell & Environment* **30,** 892–909.

Dongmann G., Nurnberg H.W., Förstel H. & Wagener K. (1974) On the enrichment of $H_2^{18}O$ in the leaves of transpiring plants. *Radiation and Environmental Biophysics* **11,** 41–52.

Ehleringer J.R. (1993) Gas-exchange implications of isotopic variation in arid-land plants. In *Plant Responses to Water Deficit* (eds H. Griffiths & J. Smith), pp. 265–284. BIOS Scientific Publishers, London, UK.

Engelbrecht B.M.J. & Kursar T.A. (2003) Comparative drought-resistance of seedlings of 28 species of co-occurring tropical woody plants. *Oecologia* **136,** 383–393.

Engelbrecht B.M.J., Kursar T.A. & Tyree M.T. (2005) Drought effects on seedling survival in a tropical moist forest. *Trees-Structure and Function* **19,** 312–321.

Engelbrecht B.M.J., Comita L.S., Condit R., Kursar T.A., Tyree M.T., Turner B.L. & Hubbell S.P. (2007) Drought sensitivity shapes species distribution patterns in tropical forests. *Nature* **447,** 80–82.

Farquhar G.D. & Gan K.S. (2003) On the progressive enrichment of the oxygen isotopic composition of water along leaves. *Plant, Cell & Environment* **26,** 801–819.

Farquhar G.D. & Lloyd J. (1993) Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In *Stable Isotopes and Plant Carbon–Water Relations* (eds J.R. Ehleringer, A.E. Hall & G.D. Farquhar), pp. 47–70. Academic Press, San Diego, CA, USA.

Farquhar G.D. & Richards R.A. (1984) Isotopic composition of plant carbon correlates with water-use efficiency in wheat genotypes. *Australian Journal of Plant Physiology* **11,** 539–552.

Farquhar G.D., O'Leary M.H. & Berry J.A. (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* **9,** 121–137.

Farquhar G.D., Ehleringer J.R. & Hubick K.T. (1989a) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40,** 503–537.

Farquhar G.D., Hubick K.T., Condon A.G. & Richards R.A. (1989b) Carbon isotope fractionation and plant water-use efficiency. In *Stable Isotopes in Ecological Research* (eds P.W. Rundel, J.R. Ehleringer & K.A. Nagy), pp. 21–46. Springer-Verlag, New York, USA.

Farquhar G.D., Condon A.G. & Masle J. (1994) Use of carbon and oxygen isotope composition and mineral ash content in breeding for improved rice production under favorable, irrigated conditions. In *Breaking the Yield Barrier* (ed. K.G. Cassman), pp. 95–101. International Rice Research Institute, Manila, Philippines.

Farquhar G.D., Barbour M.M. & Henry B.K. (1998) Interpretation of oxygen isotope composition of leaf material. In *Stable Isotopes: Integration of Biological, Ecological, and Geochemical Processes* (ed. H. Griffiths), pp. 27–48. BIOS Scientific Publishers Ltd., Oxford, UK.

Farquhar G.D., Cernusak L.A. & Barnes B. (2007) Heavy water fractionation during transpiration. *Plant Physiology* **143,** 11–18.

Grams T.E.E., Kozovits A.R., Haberle K.H., Matyssek R. & Dawson T.E. (2007) Combining $\delta^{13}C$ and $\delta^{18}O$ analyses to unravel competition, CO_2 and O_3 effects on the physiological performance of different-aged trees. *Plant, Cell & Environment* **30,** 1023–1034.

Guehl J.M., 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 Phytologist* **131,** 149–157.

Hobbie E.A. & Colpaert J.V. (2004) Nitrogen availability and mycorrhizal colonization influence water use efficiency and carbon isotope patterns in *Pinus sylvestris*. *New Phytologist* **164,** 515–525.

Hobbie E.A. & Werner R.A. (2004) Intramolecular, compound-specific, and bulk carbon isotope patterns in C_3 and C_4 plants: a review and synthesis. *New Phytologist* **161,** 371–385.

Hubick K.T. (1990) Effects of nitrogen-source and water limitation on growth, transpiration efficiency and carbon-isotope discrimination in peanut cultivars. *Australian Journal of Plant Physiology* **17,** 413–430.

Hubick K.T. & Farquhar G.D. (1989) Carbon isotope discrimination and the ratio of carbon gained to water lost in barley cultivars. *Plant, Cell & Environment* **12,** 795–804.

Hubick K.T., Farquhar G.D. & Shorter R. (1986) Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. *Australian Journal of Plant Physiology* **13,** 803–816.

Livingston N.J., Guy R.D., Sun Z.J. & Ethier G.J. (1999) The effects of nitrogen stress on the stable carbon isotope composition, productivity and water use efficiency of white spruce (*Picea glauca* (Moench) Voss) seedlings. *Plant, Cell & Environment* **22,** 281–289.

Ripullone F., Matsuo N., Stuart-Williams H., Wong S.-C., Borghetti M., Tani M. & Farquhar G.D. (2008) Environmental effects on oxygen isotope enrichment of leaf water in cotton leaves. *Plant Physiology* **146,** 729–736.

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 J.A. (2008) Carbon isotopes and water use efficiency: sense and sensitivity. *Oecologia* **155,** 441–454.

Sheshshayee M.S., Bindumadhava H., Ramesh R., Prasad T.G., Lakshminarayana M.R. & Udayakumar M. (2005) Oxygen isotope enrichment ($\Delta^{18}O$) as a measure of time-averaged transpiration rate. *Journal of Experimental Botany* **56,** 3033–3039.

Sternberg L.S.L., Mulkey S.S. & Wright S.J. (1989) Oxygen isotope ratio stratification in a tropical moist forest. *Oecologia* **81,** 51–56.

Sullivan P.F. & Welker J.M. (2007) Variation in leaf physiology of *Salix arctica* within and across ecosystems in the High Arctic: test of a dual $\delta^{13}C$ and $\delta^{18}O$ conceptual model. *Oecologia* **151,** 372–386.

Sun Z.J., Livingston N.J., Guy R.D. & Ethier G.J. (1996) Stable carbon isotopes as indicators of increased water use efficiency and productivity in white spruce (*Picea glauca* (Moench) Voss) seedlings. *Plant, Cell & Environment* **19,** 887–894.

Tanner C.B. & Sinclair T.R. (1983) Efficient water use in crop production: research or re-search. In *Limitations to Efficient Water Use in Crop Production* (ed. H. Taylor), pp. 1–28. ASA-CSSA-SSSA, Madison, WI, USA.

Toft N.L., Anderson J.E. & Nowak R.S. (1989) Water-use efficiency and carbon isotope composition of plants in a cold desert environment. *Oecologia* **80,** 11–18.

Winter K., Aranda J., Garcia M., Virgo A. & Paton S.R. (2001) Effect of elevated CO_2 and soil fertilization on whole-plant growth and water use in seedlings of a tropical pioneer tree, *Ficus insipida* Willd. *Flora* **196,** 458–464.

Winter K., Aranda J. & Holtum J.A.M. (2005) Carbon isotope composition and water-use efficiency in plants with crassulacean acid metabolism. *Functional Plant Biology* **32,** 381–388.

Yakir D. & Israeli Y. (1995) Reduced solar irradiance effects on net primary productivity (NPP) and the $\delta^{13}C$ and $\delta^{18}O$ values in plantations of *Musa* sp. *Musaceae*. *Geochimica et Cosmochimica Acta* **59,** 2149–2151.

Zhang J.W. & Marshall J.D. (1994) Population differences in water-use efficiency of well-watered and water-stressed western larch seedlings. *Canadian Journal of Forest Research* **24,** 92–99.

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