Large variation in whole-plant water-use efficiency among tropical tree species

Lucas A. Cernusak¹, Jorge Aranda¹, John D. Marshall² and Klaus Winter¹

¹Smithsonian Tropical Research Institute, PO Box 0843-03092, Balboa, Ancon, Republic of Panama; ²Department of Forest Resources, University of Idaho, Moscow, ID 83844-1133, USA

Summary

• It is well known that whole-plant water-use efficiency (transpiration efficiency of carbon gain, $TE_C$) varies among plant species with different photosynthetic pathways. However, less is known of such variation among tree species within the $C_3$ group. Here we measured the $TE_C$ of seven $C_3$ tropical tree species. Isotopic analyses ($\delta^{13}C$, $\delta^{18}O$, and $\delta^{15}N$) and elemental analyses (carbon and nitrogen) were undertaken to provide insight into sources of variation in $TE_C$.

• Plants were grown over several months in approx. 80% full sunlight in individual 38-l containers in the Republic of Panama. Soil moisture content was nonlimiting.

• Significant variation was observed in $TE_C$ among the $C_3$ tree species. Values ranged from 1.6 mmol C mol$^{-1}$ H$_2$O for teak (Tectona grandis) to 4.0 mmol C mol$^{-1}$ H$_2$O for a legume, Platymiscium pinnatum.

• Variation in $TE_C$ was correlated with both leaf N concentration, a proxy for photosynthetic capacity, and oxygen-isotope enrichment, a proxy for stomatal conductance. The $TE_C$ varied with C-isotope discrimination within species, but the relationship broke down among species, reflecting the existence of species-specific offsets.

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


Introduction

The term water-use efficiency describes a plant's photosynthetic production rate relative to the rate at which it transpires water to the atmosphere. It is a measure of plant performance that has long been of interest to agronomists, foresters and ecologists (Bacon, 2004). In cropping systems, improving water-use efficiency presents a means of increasing crop production in the face of finite water supplies (Richards et al., 2002). In forestry systems, water-use efficiency is a critical link between wood production and water management. In global-change research, water-use efficiency links the carbon and water cycles of terrestrial vegetation, and is expected to increase in a future, high-CO$_2$ world (Guehl et al., 1994; Farquhar, 1997; Winter et al., 2001a).

Marked differences in water-use efficiency occur among plants employing the three photosynthetic pathways: $C_3$, $C_4$ and crassulacean acid metabolism (CAM). The $C_4$ and CAM pathways were derived from the ancestral $C_3$ pathway, and plants exhibiting $C_4$ and CAM photosynthesis are more water-use efficient than those exhibiting $C_3$ photosynthesis (Briggs & Shantz, 1914; Shantz & Piemeisel, 1927; Fischer & Turner, 1978; Winter et al., 2005). Tropical tree species that use the $C_4$ and CAM photosynthetic pathways are found in the genera Euphorbia (Pearcy & Troughton, 1975) and Clusia (Holm et al., 2004; Lütge, 2006), respectively. Such species are expected to have markedly higher whole-plant water-use efficiencies than those that rely on the $C_3$ photosynthetic pathway. By contrast, little is known of variation in whole-plant water-use efficiency among tropical tree species within
the $C_3$ group, which comprises the overwhelming majority of tropical tree species.

Based on the correlation between C-isotope discrimination ($\Delta$) and photosynthetic water-use efficiency in $C_3$ plants (Farquhar et al., 1982), many authors have inferred variation in whole-plant water-use efficiency of $C_3$ plants because of variation in the balance of $CO_2$ supply and demand during photosynthesis. Comparisons have been made across environmental gradients, for example altitude (Körner et al., 1991; Marshall & Zhang, 1994; Hultine & Marshall, 2000); drought (Zhang & Marshall, 1994; McNulty & Swank, 1995; Livingston & Spittlehouse, 1996); and soil fertility (Toft et al., 1989; Livingston et al., 1999; Duursma & Marshall, 2006). Fertility effects could be especially important for tropical forest trees because of intense competition for soil resources and the capacity of many species to fix atmospheric nitrogen through root symbioses. A species that is able to accumulate more N in its leaves for a given transpiration rate may benefit by having a higher photosynthetic capacity and a higher leaf-level photosynthetic water-use efficiency. In this way, the water-use efficiencies of N acquisition and C uptake may be intertwined.

In this study we aimed to determine how much variation exists in whole-plant water-use efficiency among a suite of $C_3$ tropical tree species. For comparison, a $C_4$ grass was also grown with the $C_3$ trees. Additionally, we measured the growth rates, morphology, and stable isotope ($\delta^{13}C$, $\delta^{18}O$, $\delta^{15}N$) and elemental (C, N) composition of the experimental plants to gain insight into the mechanisms responsible for observed variation in whole-plant water-use efficiency.

Theory

Four definitions of water-use efficiency are applied in this paper: photosynthetic water-use efficiency (PWUE), transpiration efficiency of C gain ($TE_C$), transpiration efficiency of N acquisition ($TE_N$), and transpiration ratio (TR). The PWUE describes the relative rates of exchange of $CO_2$ and water vapour between photosynthesizing leaves and the surrounding atmosphere (mmol $CO_2$ mol$^{-1}$ H$_2$O). The $TE_C$ describes water-use efficiency at the whole-plant level as the rate of C accumulation in dry matter relative to cumulative water loss (mmol C mol$^{-1}$ H$_2$O). Determinations of $TE_C$ usually integrate over several weeks to months, and are based on gravimetric measurements of dry matter accumulation and plant water loss, in combination with elemental analyses of the C mass fraction of plant dry matter. The $TE_N$ is determined in the same way, but using plant N content rather than plant C content. The TR is a measurement of water-use efficiency that has been employed since early research on the topic (Briggs & Shantz, 1914; Shantz & Piemeisel, 1927), and expresses the cumulative amount of water transpired by a plant during the production of a given amount of dry matter. Mathematically, it is the reciprocal of $TE_C$, but taking dry matter accumulation as the measure of plant production, rather than C accumulation. Typical units for TR are g H$_2$O g$^{-1}$ DM.

The PWUE can be expressed as the quotient of the diffusive fluxes of $CO_2$ into the leaf and water vapour out of the leaf during photosynthesis (Farquhar & Richards, 1984):

$$PWUE = A/E = \left[ \frac{g_C(p_a - p_t)}{g_w(w_1 - w_u)} \right] = \frac{(p_s - p_t)/1.6v}{(p_a - p_t)}$$

Equation 1 can be expanded from leaf level to whole-plant level by including terms for respiratory C use and water loss not associated with photosynthesis (Farquhar & Richards, 1984; Hubick & Farquhar, 1989). Thus the transpiration efficiency of C gain ($TE_C$) can be defined as:

$$TE_C = \left[ \frac{(1 - \phi)g_C(p_a - p_t)}/(1.6v(1 + \phi)) \right] = \frac{p_s(1 - \phi)(1 - p_t/p_s)}{1.6v(1 + \phi)}$$

Equation 2

where $\phi$ is the proportion of C fixed during photosynthesis that is subsequently lost by respiration from roots and stems during the day, and from roots, stems and leaves during the night; and $\phi_w$ is the proportion of water lost from the plant that is not associated with C uptake, that is, water lost by transpiration through partially open stomata at night, cuticular transpiration, and transpiration from stems and branches.

The second form of equation 2 is a slight modification of the first, which is written to emphasize the term $p_s/p_a$, because this term also relates independently to C-isotope discrimination ($\Delta$). Farquhar et al. (1982) derived an expression relating $\Delta$ to $p_s/p_a$ for $C_3$ photosynthesis:

$$\Delta = a - d + (b - d)p_s/p_a$$

Equation 3

where $a$ is the $^{13}C/^{12}C$ fractionation caused by gaseous diffusion through stomata (4.4%), and $b$ is the effective fractionation caused by the major carboxylating enzymes in $C_3$ plants (approx. 27%). The term $d$ summarizes collectively the fractionations caused by dissolution of $CO_2$, liquid phase diffusion, photorespiration and dark respiration (Farquhar et al., 1989a). The effects on the overall $\Delta$ of fractionations associated with these processes are thought to be small, but significant (Brugnoli & Farquhar, 2000; Ghashghaie et al., 2003). $\Delta$ is defined with respect to atmospheric $CO_2$ as $\Delta = R/R_a - 1$, where $R_A$ is $^{13}C/^{12}C$ of atmospheric $CO_2$ and $R_p$ is $^{13}C/^{12}C$ of plant material (Farquhar & Richards, 1984).
Equations 2 and 3 suggest that both $TE_C$ and $A$ share a mutual dependence on $p_i/p_a$. Combining these two equations yields:

$$TE_C = \left[ p_i (1 - \phi_v) (b - d - \Delta) \right] / \left[ 1.6 (1 + \phi_v) (b - d) \right] \quad \text{Eqn 4}$$

Because of the relationship between $TE_C$ and $A$, as shown in equation 4, $A$ has been relied on extensively to assess variation in water-use efficiency under a variety of experimental and natural conditions (Farquhar et al., 1989b; Ehleringer, 1993; Brugnoli & Farquhar, 2000). The advantage of measuring $A$ is that it provides a time-integrated, rather than instantaneous, estimate of $p_i/p_a$.

Finally, the transpiration ratio (TR) relates to $TE_C$ in the following way:

$$TR = (3/2)m_C/TE_C \quad \text{Eqn 5}$$

where $3/2$ is the molar weight ratio of water vapour and $C$, and $m_C$ is the mass fraction of $C$ in plant dry matter. Note that TR would then be multiplied by the scaling factor 1000 to give units of g H$_2$O g$^{-1}$ DM.

Materials and Methods

Plant growth and water use

The experiment took place at the Smithsonian Tropical Research Institute’s Santa Cruz Experimental Field Facility in Gamboa, Republic of Panama. The species grown for the experiment and their taxonomic families are given in Table 1. Most are tree species used in reforestation projects in Panama. The experiment started at the end of April 2004 and lasted until mid-September 2004. Seedlings were grown either from seed collected in the Panama Canal watershed, or from seedlings obtained from the native species reforestation project (PRORENA) based at the Smithsonian Tropical Research Institute. Initial seedling dry weights ranged from 0.1 to 0.6 g.

Seedlings were grown in 38-l plastic containers (Rubbermaid Round Brute, Consolidated, Twinsburg, OH, USA). The pots had a height of 44 cm, upper diameter of 36.5 cm, and lower diameter of 32 cm. They contained 1.8 kg charcoal at the base to improve drainage. Each pot contained 35 kg soil, which comprised a mixture of 60% (v/v) dark, air-dried topsoil and 40% leaf litter. All pots were filled with the same topsoil/leaf litter mixture, carefully homogenized to ensure a uniform rooting environment for all plants. Pots were not fertilized during the experiment. The soil surface was covered with 2 kg gravel to minimize evaporation. The water content of the soil was brought to field capacity by adding 12 kg of water to each pot. The side walls of the pots were covered with reflective insulation to prevent excessive heating in direct sunlight. The pots were situated under a transparent rain shelter with a glass roof, which typically reduced the incoming photon flux density by approx. 20%. In addition to the pots containing plants, eight control pots without plants were also deployed; these were used to estimate evaporation from the soil surface independently of plant transpiration.

The date of initiation and duration of gravimetric measurements for each species are presented in Table 1. At the beginning of the experiment, each pot was weighed once per week (Sartorius Balance QS64B, Thomas, Swedesboro, NJ, USA). The amount of water lost during each measuring cycle was replaced, bringing the pots back to their initial weight of 52 kg. As plant water use increased during the experiment, pots were weighed more frequently to ensure the amount of water lost during each measuring cycle did not exceed 3 kg. Total plant water use during the experiment was calculated by subtracting the cumulative water loss of control pots from that of pots with plants, corrected for the fresh weight increment of the plants during the experiment. At the conclusion of the experiment, plants were divided into leaves, stems and roots; these were dried to a constant weight at 70°C and weighed. Leaves that abscised during the course of the gravimetric measurements were collected and added to the plant dry weight for $TE_C$ calculations. Total area of fresh leaves was

Table 1 Descriptive data for the eight species grown for assessment of transpiration efficiency

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Start date</th>
<th>End date</th>
<th>No. plants</th>
<th>Final DW (g)</th>
<th>Final/initial DW</th>
<th>Mean VPD (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalbergia retusa Hemsl.</td>
<td>Fabaceae</td>
<td>26 Apr 2004</td>
<td>4 Aug 2004</td>
<td>7</td>
<td>63.8 (8.1)</td>
<td>709</td>
<td>0.62</td>
</tr>
<tr>
<td>Ficus insipida Wild.</td>
<td>Moraceae</td>
<td>26 Apr 2004</td>
<td>20 Aug 2004</td>
<td>6</td>
<td>43.6 (2.5)</td>
<td>436</td>
<td>0.59</td>
</tr>
<tr>
<td>Pachira quinata (Jacq.) W.S. Alverson</td>
<td>Bombacaceae</td>
<td>26 Apr 2004</td>
<td>9 Jul 2004</td>
<td>8</td>
<td>53.1 (3.2)</td>
<td>295</td>
<td>0.64</td>
</tr>
<tr>
<td>Platymiscium pinnatum (Jacq.) Dugand</td>
<td>Fabaceae</td>
<td>26 Apr 2004</td>
<td>27 Aug 2004</td>
<td>7</td>
<td>87.2 (12.8)</td>
<td>224</td>
<td>0.63</td>
</tr>
<tr>
<td>Pseudobombax septenatum (Jacq.) Dugand</td>
<td>Bombacaceae</td>
<td>26 Apr 2004</td>
<td>4 Sep 2004</td>
<td>6</td>
<td>68.4 (9.6)</td>
<td>684</td>
<td>0.59</td>
</tr>
<tr>
<td>Swietenia macrophylla King</td>
<td>Meliaceae</td>
<td>10 May 2004</td>
<td>15 Sep 2004</td>
<td>4</td>
<td>71.3 (12.9)</td>
<td>713</td>
<td>0.60</td>
</tr>
<tr>
<td>Tectona grandis Linn. f.</td>
<td>Verbenaceae</td>
<td>17 May 2004</td>
<td>14 Jul 2004</td>
<td>7</td>
<td>47.0 (5.8)</td>
<td>86</td>
<td>0.62</td>
</tr>
</tbody>
</table>

All are $C_3$ tree species with the exception of S. spontaneum, a $C_4$ grass. Values for final dry weight and final/initial dry weight are means; values in parentheses are 1 SE. Mean VPD for each species is the average midday vapour pressure deficit over the 45 d preceding plant harvest, when the majority of plant growth took place.
determined with a LI-3100 leaf-area meter (Li-Cor, Lincoln, NE, USA). Leaf-area data were not taken for *Saccharum spontaneum*. This species is therefore excluded from analyses involving leaf area.

### Meteorological conditions

Meteorological conditions during the experiment were recorded every 15 min by an automated weather station (Campbell Scientific, Logan, UT, USA) situated in an open area adjacent to the rain shelter. During postprocessing of the data we discovered that temperature and relative humidity measurements were excessively affected by rain events, probably caused by a leaky housing for the temperature/humidity probe; therefore temperature and humidity data were replaced with data recorded by the Panama Canal Authority for Gamboa, Panama (http://striweb.si.edu/esp/physical_monitoring/download_acp.htm). Free water evaporation was measured under the rain shelter using three Etgage evaporimeters (Etgage, Loveland, CO, USA).

Mean monthly meteorological conditions over the course of the experiment are given in Table 2. We focus on mean midday values for air temperature, relative humidity and air vapour pressure deficit (VPD) to provide an index of the daytime conditions under which photosynthesis took place. We observed a strong correlation between mean monthly midday VPD and mean monthly free water evaporation \( R^2 = 0.98, P = 0.0009, n = 5 \), whereby the former explained 98% of variation in the latter. Moreover, the intercept of the regression equation did not differ from zero \( P = 0.28, n = 5 \), predicting no evaporation for a saturated atmosphere. Thus the two independent measures of variation in the evaporative demand of the atmosphere were in good agreement.

Figure 1 shows the measured variation in free water evaporation over the course of the experiment. Values are average measurements made with three Etgage evaporimeters located under the rain shelter alongside the experimental plants. Day 1 of the experiment was 26 April 2004; it concluded on 15 September 2004 (day 142).

![Fig. 1 Variation in mean daily free water evaporation over the course of the experiment. Values are average measurements made with three Etgage evaporimeters located under the rain shelter alongside the experimental plants. Day 1 of the experiment was 26 April 2004; it concluded on 15 September 2004 (day 142).](image)

### Elemental and isotopic analyses

Plant dry matter was ground to a fine powder for measurements of elemental and isotopic composition. The C- and N-isotope ratios were measured on samples of approx. 3 mg on a Delta Plus isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) following combustion in an NC2500 elemental analyser (CE Instruments, Milan, Italy). The O-isotope ratios were measured on samples of approx. 1 mg on a Delta XP isotope ratio mass spectrometer (Finnigan MAT) following pyrolysis in a high-temperature furnace (Thermoquest TC/EA, Finnigan MAT). The C and N concentrations in

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**Table 2** Average meteorological conditions for the 5 months in 2004 during which the experiment took place

<table>
<thead>
<tr>
<th>Parameter</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean midday air temperature (°C)</td>
<td>28.8</td>
<td>29.3</td>
<td>28.8</td>
<td>28.4</td>
<td>29.2</td>
</tr>
<tr>
<td>Mean midday relative humidity (%)</td>
<td>80.9</td>
<td>83.8</td>
<td>84.4</td>
<td>85.7</td>
<td>82.4</td>
</tr>
<tr>
<td>Mean midday air vapour pressure deficit (kPa)</td>
<td>0.76</td>
<td>0.66</td>
<td>0.62</td>
<td>0.55</td>
<td>0.71</td>
</tr>
<tr>
<td>Mean midday wind speed (m s⁻¹)</td>
<td>0.63</td>
<td>0.69</td>
<td>0.67</td>
<td>0.65</td>
<td>0.80</td>
</tr>
<tr>
<td>Mean photosynthetic photon flux density (mol ITT⁻² d⁻¹)</td>
<td>25.7</td>
<td>23.0</td>
<td>22.3</td>
<td>20.4</td>
<td>28.3</td>
</tr>
<tr>
<td>Mean free water evaporation (mm d⁻¹)</td>
<td>3.96</td>
<td>3.26</td>
<td>3.14</td>
<td>2.74</td>
<td>3.56</td>
</tr>
</tbody>
</table>

Values for September are averages for 1–15 September (which was the final day of the experiment).
We observed significant variation among species in TE\(_C\) (Fig. 2a). As expected, the species with the highest TE\(_C\) was the C\(_4\) grass \(S.\) spontaneum. Surprisingly, however, mean TE\(_C\) for the C\(_3\) tree \(P.\) pinnatum was not significantly different from that of \(S.\) spontaneum. Among the seven C\(_3\) tree species, there was large variation in mean values for TE\(_C\). Species means ranged from 4.0 mmol C mol\(^{-1}\) H\(_2\)O for \(P.\) pinnatum to 1.6 mmol C mol\(^{-1}\) H\(_2\)O for \(T.\) grandis.

We also observed significant variation among species in TE\(_N\) (Fig. 2b). The amount of N taken up for a given amount of water transpired to the atmosphere was significantly higher in \(S.\) spontaneum and in the two leguminous trees, \(P.\) pinnatum and \(D.\) retusa, than in the other five tree species. Among the other five, \(T.\) grandis had the lowest TE\(_N\). There was a nearly sixfold variation in TE\(_N\) among species, with \(T.\) grandis having a mean value of 21 \(\mu\)mol N mol\(^{-1}\) H\(_2\)O, and \(D.\) retusa having a mean value of 121 \(\mu\)mol N mol\(^{-1}\) H\(_2\)O.

Mean relative growth rate (RGR) varied significantly among species (Fig. 2c). This was largely caused by two species, \(D.\) retusa and \(P.\) quinata, which had higher RGR than the other six. Of these two, the RGR of \(P.\) quinata was significantly higher than that of \(D.\) retusa. Across the C\(_3\) tree species, variation in RGR was significantly correlated with variation in specific leaf area, SLA (m\(^2\) kg\(^{-1}\)). The relationship between the two was RGR = 1.98SLA + 23.1 \((R^2 = 0.37, P < 0.0001, n = 46)\). The RGR was also correlated with leaf area ratio, LAR (m\(^2\) kg\(^{-1}\)), according to the relationship RGR = 4.23LAR + 31.4 \((R^2 = 0.27, P = 0.0001, n = 46)\). There was no significant correlation between RGR and TE\(_C\) \((P = 0.09, n = 53)\), or RGR and TE\(_N\) \((P = 0.91, n = 53)\).

### Carbon and nitrogen concentrations

Whole-plant C concentration varied among species (Table 3). The highest values, at approx. 45%, were observed in the two legume species \(D.\) retusa and \(P.\) pinnatum, and the lowest values, at approx. 41%, in \(F.\) insipida and \(S.\) spontaneum. Across all species, C concentration was higher in leaves than in stems \((P < 0.0001, n = 46)\) or roots \((P = 0.0003, n = 53)\). Mean values for leaves, stems and roots were 44.4, 42.2 and 42.8%, respectively.

Whole-plant N concentration also varied among species (Table 3). Values ranged from 2.1% for the legume \(D.\) retusa to 0.7% for \(T.\) grandis. Values for leaves ranged from 3.3% for \(D.\) retusa to 1.0% for \(T.\) grandis. Across all species, leaves had...
higher N concentrations than either stems (P<0.0001, n = 46) or roots (P<0.0001, n = 53). Mean values for leaves, stems and roots were 1.9, 0.7 and 0.8%, respectively.

The TEC was linearly related to leaf N concentration expressed on a leaf-area basis (Fig. 3a). The equation relating the two parameters was TEC = 0.014NA + 1.62 (R² = 0.40, P<0.0001, n = 46), where TEC is in mmol C mol⁻¹ H₂O and leaf N per unit leaf area (NA) is in mmol N m⁻². The NA in turn, was linearly related to TEC (Fig. 4a). The equation relating the two was NA = 0.80TEC + 26.0 (R² = 0.73, P<0.0001, n = 46), where TEC is in mmol N mol⁻¹ H₂O. Similarly, TEC was positively correlated with TE₂ (Fig. 4b). The TEC appeared to be a saturating function of TEC. The relationship could be described by the nonlinear equation TEC = 4.24(1 - e⁻⁻⁰.₀₂TE) (R² = 0.61, n = 53).

Stable isotope compositions

There was variation among species in whole-plant C-isotope discrimination, Δ (Table 4). Mean values for the C₃ tree species ranged from 20.3% for T. grandis and P. pinnatum to 21.7% for D. retusa. The mean value for S. spontaneum was 3.0%, reflecting the large variation in Δ between the C₃ and C₄ photosynthetic pathways. Across all C₃ species, leaf Δ was larger than that of both stems (P<0.0001, n = 46) and roots (P<0.0001, n = 46); mean values were 21.9, 20.5 and 20.6% for leaves, stems and roots, respectively. We calculated the difference between leaf Δ and that for heterotrophic tissues (stems plus roots) for each species (Table 4). This difference varied significantly among species, with values for the C₃ trees ranging from 2.6% for Swietenia macrophylla to 0.2% for F. insipida. The value for S. spontaneum, -1.2%, differed significantly from those of all C₃ species.

![Fig. 3 Transpiration efficiency of carbon gain plotted against (a) leaf nitrogen concentration per unit leaf area; (b) whole-plant carbon-isotope discrimination; (c) stem 8^18O.](image)

*Table 3 Concentrations of carbon and nitrogen in the dry matter of leaves, stems and roots for each species*

<table>
<thead>
<tr>
<th>Species</th>
<th>Carbon concentration (%)</th>
<th></th>
<th>Nitrogen concentration (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Stems</td>
<td>Roots</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Dalbergia retusa</td>
<td>47.6 (0.7)</td>
<td>43.1 (0.2)</td>
<td>44.8 (0.4)</td>
<td>45.2 (0.3) a</td>
</tr>
<tr>
<td>Ficus insipida</td>
<td>39.7 (0.2)</td>
<td>40.5 (0.3)</td>
<td>43.2 (0.5)</td>
<td>41.0 (0.1) d</td>
</tr>
<tr>
<td>Pachira quinata</td>
<td>44.1 (0.3)</td>
<td>41.1 (0.5)</td>
<td>42.4 (0.5)</td>
<td>42.6 (0.1) b</td>
</tr>
<tr>
<td>Platymiscium pinnatum</td>
<td>46.5 (0.7)</td>
<td>43.9 (0.5)</td>
<td>43.9 (0.5)</td>
<td>44.8 (0.3) a</td>
</tr>
<tr>
<td>Pseudobombax septenatum</td>
<td>43.3 (0.8)</td>
<td>41.6 (0.6)</td>
<td>41.1 (0.4)</td>
<td>42.0 (0.5) b,d</td>
</tr>
<tr>
<td>Swietenia macrophylla</td>
<td>45.1 (0.4)</td>
<td>43.2 (0.4)</td>
<td>44.2 (0.4)</td>
<td>44.1 (0.2) a,c</td>
</tr>
<tr>
<td>Tectona grandis</td>
<td>43.6 (0.3)</td>
<td>41.6 (0.4)</td>
<td>42.8 (0.4)</td>
<td>42.9 (0.2) b,c</td>
</tr>
<tr>
<td>Saccharum spontaneum</td>
<td>41.4 (2.1)</td>
<td>na</td>
<td>40.1 (1.8)</td>
<td>41.1 (1.5) d</td>
</tr>
</tbody>
</table>

Values are means (SD). Whole-plant values were calculated by mass balance in conjunction with the dry weight of each plant organ. Values within a column followed by different letters are statistically different at P<0.05.
Values are means (SD). Whole-plant values were calculated by mass balance using the dry mass and mass fraction of the element of interest in each plant organ. C-isotope composition was expressed as discrimination by assuming a value for atmospheric CO₂ of −8‰. δ¹⁵N values are referenced to air as a standard; those for δ¹⁸O to Vienna Standard Mean Ocean Water. For C-isotope discrimination, the difference between leaves and heterotrophic plant tissues (sum of stems and roots) is also given. For δ¹⁸O of Saccharum spontaneum, roots were analysed in place of stems as this grass species does not have a morphologically distinct stem. Values within a column followed by different letters are statistically different at \( P < 0.05 \).
between species and Δ was not significant (P = 0.98, n = 46), suggesting that the slopes of the relationships between \( \text{TE}_C \) and Δ did not differ among species. The next step of the analysis indicated that there was significant variation in \( \text{TE}_C \) associated with both species (\( P < 0.0001 \)) and with Δ (\( P = 0.0004 \)), suggesting that \( \text{TE}_C \) varied significantly with Δ, and that there was variation among species in the intercept of the relationship between \( \text{TE}_C \) and Δ. Overall, the model accounted for 88% of variation in \( \text{TE}_C \). As further evidence of uncoupling between \( \text{TE}_C \) and Δ at the species level, a linear regression of species means for the two parameters was not significant (\( P = 0.71, n = 7 \)).

Whole-plant δ\(^{18}\)O varied significantly among species (Table 4). Values ranged from 0.8% for the legume species D. retusa to 3.0% for S. macrophylla. The other legume species, P. pinnatum, had an intermediate value of 1.9%. Differences between plant tissues appeared to vary depending on species. For example, in D. retusa, roots were heavier by about 2.7% compared with stems or leaves, whereas in P. pinnatum leaves were heavier by 2.6% compared with roots or stems. A visual examination of the root systems of the two legume species showed D. retusa to be well nodulated, whereas P. pinnatum had only a few conspicuous nodules.

We observed significant variation among species in the δ\(^{18}\)O of both leaves and stems (Table 4). For all species combined, leaves were enriched by 1.2% compared with stems (\( P < 0.0001, n = 52 \)). The \( \text{TE}_C \) was significantly correlated with the δ\(^{18}\)O of both stems (Fig. 3c) and leaves; however, the relationship with stem δ\(^{18}\)O was much stronger than that with leaf δ\(^{18}\)O. The regression relating \( \text{TE}_C \) to stem δ\(^{18}\)O was \( \text{TE}_C = 0.68 \delta^{18}O_{\text{stem}} - 11.9 \) (\( R^2 = 0.50, P < 0.0001, n = 52 \)); that relating \( \text{TE}_C \) to leaf δ\(^{18}\)O was \( \text{TE}_C = 0.17 \delta^{18}O_{\text{leaf}} - 0.9 \) (\( R^2 = 0.08, P = 0.03, n = 53 \)).

We performed regression analyses to determine whether the patterns observed for species-level variation in \( \text{TE}_C \), Δ and δ\(^{18}\)O resulted from small differences in environmental conditions experienced by each species, caused by variation in harvest dates (Table 1). The mean \( \text{TE}_C \) for the C₃ tree species showed no correlation with mean midday VPD. This was true regardless of whether mean midday VPD was calculated over the 45 d preceding plant harvest (\( P = 0.61, n = 7 \)), or over the entire experimental period for each species (\( P = 0.65, n = 7 \)). Similarly, mean Δ for the C₃ tree species showed no correlation with mean midday VPD, whether calculated over the 45 d preceding plant harvest (\( P = 0.97, n = 7 \)) or over the entire experimental period (\( P = 0.38, n = 7 \)). Mean species δ\(^{18}\)O showed no correlation with mean midday relative humidity calculated over the 45 d preceding plant harvest (\( P = 0.77, n = 8 \)), and no correlation with that calculated over the entire experimental period for each species (\( P = 0.73, n = 8 \)). Relative humidity is the environmental parameter most likely to cause variation in δ\(^{18}\)O in this context. We conclude that the species-level trends that we observed in \( \text{TE}_C \), Δ and δ\(^{18}\)O were not caused by variation in the environmental conditions experienced by each species during growth.

**Discussion**

We observed large variation in \( \text{TE}_C \) under well watered conditions among a suite of tropical tree species employing the C₃ photosynthetic pathway. This variation in \( \text{TE}_C \) among species could not be accounted for by considering subtle differences in environmental conditions experienced during growth by each species caused by different harvest dates. The range of values was striking, with \( \text{TE}_C \) of P. pinnatum approaching that achieved by a concurrently grown C₄ grass (Fig. 2a). In contrast, the \( \text{TE}_C \) of T. grandis was about one-third that of the C₄ grass. We did not observe a correlation between RGR and \( \text{TE}_C \), suggesting that high water-use efficiency need not come at the expense of slow growth. Across species, \( \text{TE}_C \) was positively correlated with \( N_v \) (Fig. 3a), negatively correlated with whole-plant Δ (Fig. 3b), and positively correlated with stem dry matter δ\(^{18}\)O (Fig. 3c). In the following discussion, we first relate our results to observations elsewhere in the literature of whole-plant water-use efficiency for C₃ trees; we then rely on the above-stated correlations to draw inferences about the physiological mechanisms driving species-level variation in \( \text{TE}_C \).

The variations in whole-plant water-use efficiency that we observed are consistent with previous results that are available for some of the same species. Winter et al. (2005) reported mean values for TR of S. macrophylla and T. grandis of 256 and 373 g H₂O g⁻¹ DM, respectively. If we convert data from the current study to TR to match their data, we obtain mean values of 230 and 396 g H₂O g⁻¹ DM, respectively, in close agreement with the previous results. For the C₄ grass Z. mays, Winter et al. (2005) reported a mean TR of 132 g H₂O g⁻¹ DM, whereas we observed a mean value for S. spontaneum, also a C₄ grass, of 136 g H₂O g⁻¹ DM. For the C₃ tree F. insipida, Winter et al. (2001a) observed TR ranging from 229 to 309 g H₂O g⁻¹ DM for plants grown under conditions comparable with those of the current study (ambient CO₂, unfertilized soil, no open-top chamber). In comparison, we observed a mean value of 332 g H₂O g⁻¹ DM for this species. Thus both the relative ranking and absolute values for whole-plant water-use efficiency observed in this study agree well with previous results.

There are few other reports of variation among C₃ tree species in whole-plant water-use efficiency. Variation was observed between Q. robur and P. pinaster, but only at low nutrient availability (Guehl et al., 1995). Q. petraea was observed to consistently have a higher \( \text{TE}_C \) than P. pinaster, even under varying atmospheric CO₂ concentration and soil moisture (Guehl et al., 1994). Data presented by Pate & Dawson (1999) suggest variation in TR between mallee eucalypts (species names not provided) and E. globulus.
Examination of equation 2 suggests that variation in $TE_C$, for plants grown in a common environment, can occur during leaf-level gas exchange, because of variation in $p/p_a$ and/or $v$. Other potential sources of variation are $\Phi$, the proportion of C fixed during photosynthesis that is subsequently lost by respiration, and $\Phi_s$, the proportion of water lost from the plant that is not associated with photosynthesis. Variation in $p/p_a$ is often identified as a primary source of variation in $TE_C$ (Farquhar & Richards, 1984; Farquhar et al., 1989b; Hubick & Farquhar, 1989). In some studies with trees, $\Phi_s$ has also been suggested to be an important determinant (Guehl et al., 1994; Osório et al., 1998; Matzner et al., 2001); and in one case $\Phi_v$ was suggested to have influenced $TE_C$ significantly (Hobie & Colpaert, 2004). While we did not quantify these processes in our experiment, the measurements of elemental and isotopic composition allow us to draw some inferences about the processes responsible for the variation that we observed in $TE_C$.

The observed positive correlations between $TE_C$ and $N_A$ (Fig. 3a) and between $TE_C$ and $\delta^{18}O$ (Fig. 3c) suggest that $p/p_a$ was an important control on $TE_C$, but they address different modes of control. The $N_A$ generally shows a positive correlation with leaf photosynthetic capacity (Field & Mooney, 1986). Higher photosynthetic capacity is expected to result in lower $p/p_a$, all else being equal; similar responses have frequently been observed (Toft et al., 1989; Hultine & Marshall, 2000; Duursma & Marshall, 2006). For plants grown with the same source water and under the same atmospheric conditions, $\delta^{18}O$ of plant organic material is expected to show a negative correlation with stomatal conductance (Conata & Lloyd, 1993; Farquhar et al., 1998; Barbour & Farquhar, 2000; Babour et al., 2000, 2005; Cernusak et al., 2003). At a given photosynthetic capacity, lower stomatal conductance is expected to result in a lower $p/p_a$. Thus the correlations between $TE_C$ and both $N_A$ and $\delta^{18}O$ suggest that both influenced $p/p_a$, yielding the observed variation in $TE_C$.

We observed a stronger relationship between stem $\delta^{18}O$ and $TE_C$ than between leaf $\delta^{18}O$ and $TE_C$. Theory relating stomatal conductance to oxygen isotope enrichment in plant organic material is usually expressed in terms of plant cellulose, because the $\delta^{18}O$ of cellulose has a known relationship to the $\delta^{18}O$ of the water in which it forms (Farquhar et al., 1998; Cernusak et al., 2004, 2005); however, for stem wood this difference tends to be rather more constant among and within species (Borella et al., 1999; Babour et al., 2001; Cernusak et al., 2005). Thus species-specific variation in the difference between leaf dry matter $\delta^{18}O$ and leaf cellulose $\delta^{18}O$ may have weakened the relationship between leaf $\delta^{18}O$ and $TE_C$, whereas stem $\delta^{18}O$ probably would not have suffered from this complication.

If, as we suggest, variation in $p/p_a$ played an important role in determining variation in $TE_C$, one would expect a close relationship across species between $TE_C$ and $\Delta$. We observed significant correlation within species between $TE_C$ and $\Delta$, but found that the intercept of the relationship differed among species. Equation 4 can be rearranged to give:

$$ TE_C = -\Delta [p_a(1 - \phi_v)/[1.6v(1 + \phi_v)(b - d)] + [p_a(1 - \phi_v)(b - d)]/[1.6v(1 + \phi_v)(b - d)]] $$

Expressed in this way, the first term following $\Delta$ on the right side of the equation becomes the slope coefficient of the linear relation between $TE_C$ and $\Delta$, and the second term becomes the intercept. The ANCOVA indicated that, in our data set, the slope of the relationship between $TE_C$ and $\Delta$ did not vary among the $C_3$ species, whereas the intercept did. The only term in equation 6 that appears in the intercept term that does not appear in the slope term is $d$.

A mathematical definition of $d$ was given by Farquhar et al. (1989a). In addition to $^{13}C/^{12}C$ fractions caused by ‘dark’ respiration and photorespiration, $d$ also includes the effect on predicted $\Delta$ of the drawdown in $CO_2$ concentration between the leaf intercellular air spaces and the sites of carboxylation in chloroplasts. Accordingly, it has been suggested that leaf internal resistance to $CO_2$ diffusion may vary among species in such a way as to cause variation in $\Delta$ independently of variation in $p/p_a$ (Lloyd et al., 1992; Warren & Adams, 2006). Additionally, there have recently been several reports of $^{13}C$ discrimination during dark respiration, and there appears to be variation in this parameter among species (Duranceau et al., 1999; Ghashghaie et al., 2001; Ocheltree & Marshall, 2004; Xu et al., 2004). We observed variation among species in the $\Delta$ difference between leaves and heterotrophic plant tissues (Table 4), which may be indicative of variation among species respiratory $C$ isotope discrimination (Hobie & Werner, 2004).

Significant uncoupling between transpiration efficiency and $\Delta$ at the species level has been observed before: $Q. robur$ and $P. piniaster$ had whole-plant $\Delta$ that differed by 2.6%, whereas no difference was observed in transpiration efficiency (Guehl et al., 1995); similarly, $Pseudotsuga menziesii$ and $Populus x eurameriaca$ had a leaf $\Delta$ that differed by 3%, whereas there was no difference between the two in transpiration efficiency (Ripullone et al., 2004). Although we have argued that, in our experiment, significant variation in $TE_C$ among species was probably driven by variation in $p/p_a$, and that uncoupling between $TE_C$ and $\Delta$ therefore resulted from uncoupling between $p/p_a$ and $\Delta$, we cannot exclude the possibility that other terms in equation 2, such as $v$, $\phi_v$ and $\phi_s$, may also have contributed to such variation.

We observed variation among species in dry matter $\delta^{15}N$ (Table 4). The range of species means that we observed for whole-plant $\delta^{15}N$ (0.7 to 3.0%) was slightly less than the
corresponding range of values for leaf $\delta^{15}N$ (0.4 to 3.7‰). This range, in turn, is similar to the range of leaf $\delta^{15}N$ observed among 21 species in an Amazonian rainforest in French Guiana ($-$0.3 to 3.5‰; Guehl et al., 1998), and to that observed for 32 species in lowland tropical forest in Panama ($-$1 to 5% with 88% of values between 0 and 4‰; Santiago et al., 2004). Given the homogeneity of the plant culture conditions in our experiment, this amount of variation is surprising. One of the legume species in our study, *D. retusa*, had clearly visible, abundant bacterial nodules on its roots, whereas the other legume species, *P. pinnatum*, had far fewer visible nodules. *Dalbergia retusa* had leaf $\delta^{15}N$ nearest to 0‰ among all of the species, whereas *P. pinnatum* had the second highest leaf $\delta^{15}N$ at 3.0‰. Based on these data, one might suggest that *D. retusa* obtained a much greater proportion of biologically fixed, atmospheric N than *P. pinnatum*. However, the two species displayed similarly high values for the transpiration efficiency of N acquisition (Fig. 2b), which set them clearly apart from the other C$_3$ tree species. The $\delta^{15}N$ data are difficult to reconcile with this observation. Moreover, $\delta^{15}N$ values for the nonleguminous species covered essentially the same range as those for the legumes. As in the study in French Guiana (Guehl et al., 1998), we conclude that dry matter $\delta^{15}N$ did not provide a straightforward indication of biological fixation in our experiment.

We observed that mean RGR correlated with both SLA and LAR among the C$_3$ tree species. This result is consistent with observations among herbaceous plant species (Poorter & Remkes, 1990) and observations of two tropical tree species when exposed to variable environmental conditions (Winter et al., 2000; Winter et al., 2001b). The lack of correlation that we observed between RGR and $T_{E_C}$ is also similar to previous results comparing RGR and PWUE (Poorter & Farquhar, 1994).

We observed a positive relationship between $T_{E_C}$ and $T_{E_N}$ (Fig. 4b). The relationship appeared to be such that $T_{E_C}$ was a saturating function of $T_{E_N}$ with considerably more scatter in the relationship at high than at low $T_{E_N}$. It may be that $T_{E_N}$ sets an upper limit on the potential value of $T_{E_C}$, which can then be reduced by other processes at the high range of $T_{E_N}$, such as C allocation to symbiotic bacteria in root nodules. We suggest that the relationship between leaf N concentration per unit leaf area, $N_A$, and $T_{E_N}$ (Fig. 4a) provides a mechanistic link between $T_{E_N}$ and $T_{E_C}$. Increasing $T_{E_N}$ leads to increasing $N_A$, which in turn leads to lower $p/p_a$ and therefore higher $T_{E_C}$.

We observed marked variation in whole-plant water-use efficiency among tropical tree species grown as seedlings and saplings. It is still unknown whether such variation persists during ontogeny and is therefore expressed in mature trees growing in tropical forest stands (Holmton & Winter, 2005). If, as we have suggested, the variation among species is largely driven by variation in leaf-level gas-exchange characteristics, it seems reasonable to expect that such variation may be carried forward through the life cycle of the trees. If this is the case, it could have important implications for tropical forest management, particularly where manipulation of species composition is possible and both biomass production and control of water use are management objectives.

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