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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

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ABSTRACT

Seedlings of several species of gymnosperm trees, angiosperm trees, and angiosperm lianas were grown under tropical field conditions in the Republic of Panama; physiological processes controlling plant C and water fluxes were assessed across this functionally diverse range of species. Relative growth rate, $r$, was primarily controlled by the ratio of leaf area to plant mass, of which specific leaf area was a key component. Instantaneous photosynthesis, when expressed on a leaf-mass basis, explained 69% of variation in $r$ ($P<0.0001$, $n=94$). Mean $r$ of angiosperms was significantly higher than that of the gymnosperms; within angiosperms, mean $r$ of lianas was higher than that of trees. Whole-plant N use efficiency was also significantly higher in angiosperm than in gymnosperm species, and was primarily controlled by the rate of photosynthesis for a given amount of leaf N. Whole-plant water use efficiency, $TE_c$, varied significantly among species, and was primarily controlled by $c_i/c_a$, the ratio of intercellular to ambient CO2 partial pressures during photosynthesis. Instantaneous measurements of $c_i/c_a$ explained 51% of variation in $TE_c$ ($P<0.0001$, $n=94$). Whole-plant $^{13}$C discrimination also varied significantly as a function of $c_i/c_a$ ($R^2=0.57$, $P<0.0001$, $n=94$), and was, accordingly, a good predictor of $TE_c$. The $^{18}$O enrichment of stem dry matter was primarily controlled by the predicted $^{18}$O enrichment of evaporative sites within leaves ($R^2=0.61$, $P<0.0001$, $n=94$), with some residual variation explained by mean transpiration rate. Measurements of C and O stable isotope ratios could provide a useful means of parameterizing physiological models of tropical forest trees.
INTRODUCTION

Tropical forest ecosystems have been subject to extensive perturbations associated with anthropogenic activity in recent decades, and such perturbations will likely continue into the foreseeable future (Laurance et al., 2004; Wright, 2005). Effective environmental management requires knowledge of how such perturbations impact upon cycling of C and water between forest trees and the atmosphere, and how these C and water fluxes relate to plant nutrient status. A sound, mechanistic understanding of the physiological processes that control photosynthesis and transpiration in tropical trees is therefore essential for understanding and managing the human impact upon tropical forests. In this study, we analyzed the physiological controls over growth (the relative rate of C accumulation), N use efficiency (the rate of C accumulation for a given N content), water use efficiency (the ratio of whole-plant C gain to water loss), and stable isotope composition ($\delta^{13}$C and $\delta^{18}$O) in seedlings of a diverse suite of species grown side by side in a tropical environment.

Conifers dominated the world’s forests prior to the Cretaceous radiation in angiosperm diversity. However, conifers are largely absent from the lowland tropical and subtropical forests of today. It has been suggested that one means by which angiosperm tree species are able to out-compete gymnosperm tree species in tropical environments is through faster seedling growth caused by improved hydraulic efficiency (Bond, 1989; Brodribb et al., 2005). Angiosperm xylem tissue contains vessels, specialized water conducting cells that are generally larger in diameter, and therefore more conductive to water, than conifer tracheids (Sperry et al., 2006). Conifer tracheid diameters are biomechanically constrained, because these cells must perform the dual function of conducting water and providing structural support to woody tissues, whereas vessels need not perform the latter function in angiosperm wood. Lianas are large woody vines that occur predominantly in tropical forests; by attaching themselves to neighboring trees, they have evolved an additional means of freeing their xylem tissues from structural constraints. Thus, angiosperm lianas may achieve further increases in hydraulic efficiency compared to angiosperm trees (Gartner et al., 1990).

In this study, we grew seedlings of several species of gymnosperm trees, angiosperm trees, and angiosperm lianas in a tropical environment. We used this functionally diverse range of species to quantify the physiological controls over their C and water fluxes. We also took
advantage of the contrasting physiology of the study species to test the theoretical basis for variation in the C and O stable isotope composition of plant dry matter.

THEORY

Growth

Following Masle and Farquhar (1988), and based on earlier treatments (Blackman, 1919; Evans, 1972), we write the following expression to describe factors that influence the relative rate of C accumulation of a plant:

\[
\frac{\rho}{\phi_c} = \frac{Al(1 - \phi_c)}{\rho},
\]

where \( r \) is relative growth rate (mol C mol\(^{-1}\) C s\(^{-1}\)), \( m_c \) is plant C mass (mol C), \( t \) is time (s), \( A \) is leaf photosynthetic rate (mol C m\(^{-2}\) s\(^{-1}\)), \( l \) is the light period as a fraction of 24 hr, \( \phi_c \) is the proportion of C gained in photosynthesis that is subsequently used for respiration by leaves at night and by roots and stems during day and night, and \( \rho \) is the ratio of plant C mass to leaf area (mol C m\(^{-2}\)). Equation (1) provides a useful tool for examining sources of variation in \( r \) among plant species and individuals within a species. It is similar to the classical decomposition of \( r \) into net assimilation rate (NAR; g m\(^{-2}\) s\(^{-1}\)) and leaf area ratio (LAR; m\(^{2}\) g\(^{-1}\)), but allows the assimilation term to be expressed as a net photosynthetic rate, such as would be measured using standard gas exchange techniques (Long et al., 1996). Table I provides definitions of all abbreviations and symbols used in this paper.

Nitrogen use efficiency

Multiplying both sides of equation (1) by the molar ratio of plant C to N yields an expression for the N use efficiency of C accumulation:

\[
NUE = \frac{1}{m_n} \cdot \frac{dm_c}{dt} = A_n l(1 - \phi_c) n_l,
\]

where \( NUE \) is whole-plant N use efficiency (mol C mol\(^{-1}\) N s\(^{-1}\)), \( m_n \) is plant N mass (mol N), \( A_n \) is photosynthetic N use efficiency (mol C mol\(^{-1}\) N s\(^{-1}\)), and \( n_l \) is the proportion of plant N allocated to leaves. Equation (2) provides a basis for linking \( A_n \), a trait often quantified in ecophysiological investigations, with \( NUE \), an integrated measure of N use efficiency at the whole-plant level.
Transpiration efficiency and C isotope discrimination

The ratio of C gain to water loss at the leaf level during photosynthesis can be expressed as the ratio of the diffusive fluxes of CO₂ and water vapor into and out of the leaf, respectively (Farquhar and Richards, 1984):

\[
\frac{A}{E} = \frac{c_a - c_i}{1.6v},
\]

(3)

where \(E\) is transpiration (mol H₂O m⁻² s⁻¹), \(c_a\) and \(c_i\) are CO₂ partial pressures in ambient air and leaf intercellular air spaces, respectively, \(v\) is the leaf-to-air vapor pressure difference, and 1.6 is the ratio of diffusivities of CO₂ and H₂O in air. The \(v\) is defined as \(e_i-e_a\), where \(e_i\) and \(e_a\) are the intercellular and ambient vapor pressures, respectively. The ratio of C gain to water loss can be scaled to the whole-plant level by taking into account respiratory C use and water loss not associated with photosynthesis (Farquhar and Richards, 1984; Hubick and Farquhar, 1989):

\[
TE_c = \frac{(1-\phi_w)c_a\left(1 - \frac{c_i}{c_a}\right)}{1.6v(1+\phi_w)},
\]

(4)

where \(TE_c\) is the transpiration efficiency of C gain, and \(\phi_w\) is unproductive water loss as a proportion of water loss associated with C uptake, the former mainly comprising water loss at night through partially open stomata. Thus, \(\phi_w\) can be approximated as \(E_n/E_d\), where \(E_n\) is nighttime transpiration and \(E_d\) is daytime transpiration.

We suggest that the leaf to air vapor pressure difference, \(v\), can be written as the product of the air vapor pressure deficit (\(D\)), and a second term, \(\phi_v\), which describes the magnitude of \(v\) relative to \(D\), such that \(v=D\phi_v\). This allows equation (3) to be written as

\[
D \cdot TE_c = \frac{(1-\phi_w)c_a\left(1 - \frac{c_i}{c_a}\right)}{1.6\phi_v(1+\phi_w)}.
\]

(5)

Weighting \(TE_c\) by \(D\) facilitates comparison of the transpiration efficiency of plants grown under different environmental conditions by accounting for variation due to differences in atmospheric vapor pressure deficit (Tanner and Sinclair, 1983; Hubick and Farquhar, 1989). Thus, it accounts for variation in \(TE_c\) that is purely environmental. The \(D \cdot TE_c\) has units of Pa mol C mol⁻¹ H₂O.
Photosynthetic discrimination against $^{13}$C ($\Delta^{13}$C) shares a common dependence with $TE_c$ on $c_i/c_a$. The $\Delta^{13}$C in $C_3$ plants relates to $c_i/c_a$ according to the following equation (Farquhar et al., 1982; Farquhar and Richards, 1984; Hubick et al., 1986):

$$\Delta^{13}C = a - d + (b - a) \frac{c_i}{c_a}, \quad (6)$$

where $a$ is the discrimination against $^{13}$C during diffusion through stomata (4.4‰), $b$ is discrimination against $^{13}$C during carboxylation by Rubisco (29‰), and $d$ is a composite term that summarizes collectively the discriminations associated with dissolution of CO$_2$, liquid phase diffusion, photorespiration, and dark respiration (Farquhar et al., 1989a). The term $d$ may be excluded from equation (5), in which case the reduction in $\Delta^{13}$C caused by $d$ is often accounted for by taking a lower value for $b$. 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. Combining equations (5) and (6) gives

$$D \cdot TE_c = \frac{c_i(1 - \phi_v)(b - d - \Delta^{13}C)}{1.6\phi_v(1 + \phi_v)(b - a)} \cdot (7)$$

Equation (7) suggests a negative linear dependence of $TE_c$ (or $D \cdot TE_c$) on $\Delta^{13}$C, although it can be seen that there are many other terms in equation (7) that have potential to influence the relationship between the two.

**O isotope enrichment**

It has been suggested that measurements of the O isotope enrichment of plant organic material ($\Delta^{18}$Op) can provide complimentary information to that inferred from $\Delta^{13}$C in analyses of plant water-use efficiency (Farquhar et al., 1989b; Sternberg et al., 1989; Farquhar et al., 1994; Yakir and Israeli, 1995). Specifically, $\Delta^{18}$Op could provide information about the ratio of ambient to intercellular vapor pressures, $e_a/e_i$, and thus about the leaf-to-air vapor pressure difference, $e_i-e_a$, during photosynthesis. Note that $e_i-e_a$ is equal to $v$ in equation (3). In the steady state, water at the evaporative sites in leaves becomes enriched in $^{18}$O relative to water entering the plant from the soil, according to the following relationship (Craig and Gordon, 1965; Dongmann et al., 1974; Farquhar and Lloyd, 1993):

$$\Delta^{18}O_e = \varepsilon^+ + \varepsilon_k + \left(\Delta^{18}O_v - \varepsilon_k\right)\frac{e_a}{e_i}, \quad (8)$$
where $\Delta^{18}O_e$ is the $^{18}O$ enrichment of evaporative site water relative to source water, $\varepsilon^+$ is the equilibrium fractionation between liquid water and vapor, $\varepsilon_k$ is the kinetic fractionation that occurs during diffusion of water vapor out of the leaf, and $\Delta^{18}O_v$ is the discrimination of ambient vapor with respect to source water. The $\Delta^{18}O$ of any water or dry matter component is defined with respect to source water (water entering the roots from the soil) as $\Delta^{18}O = R/R_s - 1$, where $\Delta^{18}O$ is the $^{18}O$ enrichment of the component of interest and $R$ and $R_s$ are the $^{18}O/^{16}O$ ratios of the component of interest and source water, respectively. The $\varepsilon^+$ can be calculated as a function of leaf temperature (Bottinga and Craig, 1969), and $\varepsilon_k$ can be calculated by partitioning the resistance to water vapor diffusion between stomata and boundary layer, with the two weighted by appropriate fractionation factors (Farquhar et al., 1989b; Cappa et al., 2003). The $\Delta^{18}O_v$ can be calculated from measurements of the $\delta^{18}O$ of ambient vapor and source water. If such data are not available, a reasonable approximation is to estimate $\Delta^{18}O_v$ as $-\varepsilon^+$, which means that ambient vapor is assumed to be in isotopic equilibrium with soil water. An up-to-date summary of equations necessary for parameterization of equation (8) can be found in Cernusak et al. (2007b).

Average lamina leaf water $^{18}O$ enrichment ($\Delta^{18}O_L$) is generally less than that predicted for evaporative site water (Yakir et al., 1989; Flanagan, 1993; Farquhar et al., 2007), and carbohydrates exported from leaves have been observed to carry the signal of $\Delta^{18}O_L$ rather than $\Delta^{18}O_e$ (Barbour et al., 2000b; Cernusak et al., 2003; Cernusak et al., 2005; Gessler et al., 2007). The $\Delta^{18}O_L$ has been suggested to relate to $\Delta^{18}O_e$ according to the following relationship (Farquhar and Lloyd, 1993; Farquhar and Gan, 2003):

$$\Delta^{18}O_L = \Delta^{18}O_e \left(1 - e^{-\varphi E L}ight),$$

where $\varphi$ 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 (m$^2$ s$^{-1}$). The $C$ is a constant, and $D_{18}$ can be calculated from leaf temperature (Cuntz et al., 2007). The constancy of $L$, or otherwise, is currently under investigation (Barbour and Farquhar, 2004; Barbour, 2007; Kahmen et al., 2008; Ripullone et al., 2008). If $L$ is assumed relatively constant, equation (9) predicts that $\Delta^{18}O_L$ will vary as a function of both $\Delta^{18}O_e$ and $E$. In order to test for an influence of $E$ on $\Delta^{18}O_L$, it is necessary to
first account for variation in $\Delta ^{18}O_L$ caused by $\Delta ^{18}O_c$ (Flanagan et al., 1994). To this end, the relative deviation of $\Delta ^{18}O_L$ from $\Delta ^{18}O_c$ ($1 - \Delta ^{18}O_L/\Delta ^{18}O_c$) can be examined, in which case equation (9) can be written as

$$1 - \frac{\Delta ^{18}O_L}{\Delta ^{18}O_c} = 1 - \frac{(1 - e^{-\varphi})}{\varphi}.$$

Equation (10) predicts that $1 - \Delta ^{18}O_L/\Delta ^{18}O_c$ should increase as $E$ increases.

The transfer of the leaf water $^{18}O$ signal to plant organic material can be described by the following equation (Barbour and Farquhar, 2000):

$$\Delta ^{18}O_p = \Delta ^{18}O_L \left(1 - p_{ex}p_x\right) + \epsilon_{wc} + \epsilon_{cp},$$

where $\Delta ^{18}O_p$ is $^{18}O$ enrichment of plant dry matter, $p_{ex}$ is the proportion of O atoms that exchange with local water during synthesis of cellulose, a primary constituent of plant dry matter, $p_x$ is the proportion of unenriched water at the site of tissue synthesis, $\epsilon_{wc}$ is the fractionation between organic oxygen and medium water, and $\epsilon_{cp}$ is the difference in $\Delta ^{18}O$ between tissue dry matter and the cellulose component. For tree stems, $p_{ex}p_x$ has been found to be relatively constant at about 0.4 (Roden et al., 2000; Cernusak et al., 2005). The $\epsilon_{wc}$ is relatively constant at about 27‰ (Barbour, 2007), and for stem dry matter, $\epsilon_{cp}$ appears to be relatively constant at about −5‰ (Borella et al., 1999; Barbour et al., 2001; Cernusak et al., 2005). If these assumptions are valid, variation in $\Delta ^{18}O_p$ should primarily reflect variation in $\Delta ^{18}O_L$. Thus, combining equations (10) and (11) provides a means of testing for an influence of $E$ on $\Delta ^{18}O_p$, assuming that $\Delta ^{18}O_p$ provides a time-integrated record of $\Delta ^{18}O_L$ (Barbour et al., 2004):

$$1 - \frac{\left(\Delta ^{18}O_p - \epsilon_{wc} - \epsilon_{cp}\right)}{1 - p_{ex}p_x} = 1 - \frac{(1 - e^{-\varphi})}{\varphi}.$$

RESULTS

Growth, photosynthesis, and elemental concentrations

Daytime meteorological conditions over the course of the experiment are shown in Table II. Dates of initiation of transpiration measurements and harvest for each species are shown in Table III. Table III also shows the initial and final dry masses, in addition to root/shoot ratios.
Variation in relative growth rate, $r$, among species is shown in Figure 1A; variation in the components of $r$, $A$ and $1/\rho$, is shown in Figures 1B and 1C, respectively. The $r$ varied significantly among functional groups ($P<0.0001$), and among species within functional groups ($P<0.0001$). Gymnosperm trees had the lowest mean value of $r$, whereas angiosperm lianas had the highest mean value; angiosperm trees had a mean value of $r$ intermediate between that of gymnosperm trees and angiosperm lianas (Figure 1A). In contrast, there was less variation among species and functional groups in instantaneous photosynthesis rates expressed on a leaf area basis (Figure 1B), although the species $P.\ caribaea$ and $S.\ hypargyreum$ were notable for having relatively high values of $A$. Variation in $r$ tended to be more closely associated with variation in $1/\rho$ than with variation in $A$. Gymnosperm trees had the lowest mean value of $1/\rho$, whereas angiosperm trees had an intermediate mean value, and angiosperm lianas had the highest mean value (Figure 1C). The liana species $S.\ hypargyreum$ possessed swollen, tuberous roots, which caused it to have a root/shoot ratio much higher than any other species in the study (Table III), and to have a reduced $1/\rho$ relative to the other two liana species (Figure 1C).

Variation in instantaneous photosynthesis, when expressed on a leaf mass basis, was a good predictor of variation in $r$ (Figure 2). The former was measured over several minutes, whereas the latter was measured over several months. Mass-based photosynthesis, $A_m$, is the product of $A$ (mol m$^{-2}$ s$^{-1}$) and specific leaf area, $SLA$ (m$^2$ kg$^{-1}$). The correlation between $A_m$ and $r$ was almost entirely driven by variation in $SLA$, because $A$ on a leaf area basis was not significantly correlated with $r$ ($P=0.14$, $n=94$).

The C and N concentrations of leaves, stems, roots, and whole plants for each species are given in Supplemental Table SI. For whole-plant C concentration, there was significant variation, both among functional groups ($P<0.0001$), and among species within functional groups ($P<0.0001$), as shown in Figure 3A. Gymnosperm trees had a mean C concentration of 49.6%, significantly higher than angiosperm trees and lianas. Angiosperm trees and lianas did not differ from each other in whole-plant C concentration, and had mean values of 45.4% and 44.9%, respectively. For whole plant N concentration, there was also significant variation among functional groups ($P<0.0001$) and among species within functional groups ($P<0.0001$), as shown in Figure 3B. Angiosperm lianas had the highest mean whole-plant N concentration at 1.22%, followed by angiosperm trees at 1.01%, then by gymnosperm trees at 0.82%. Accordingly, there was significant variation among functional groups ($P<0.0001$) and among
species within functional groups ($P<0.0001$) in whole-plant C/N mass ratio (Figure 3C). Gymnosperm trees had the highest mean whole-plant C/N at 64.7 g g$^{-1}$, followed by angiosperm trees at 49.6 g g$^{-1}$, then by angiosperm lianas at 39.0 g g$^{-1}$.

Concentrations of P, Ca, and K, and the N/P mass ratio in leaf dry matter for each species are shown in Table IV. There was significant variation among functional groups ($P<0.0001$) and among species within functional groups ($P<0.0001$) for all elements and for N/P. Angiosperm lianas tended to have higher mean concentrations of P, Ca, and K in their leaf dry matter than angiosperm and gymnosperm trees. The mean leaf N/P was higher in angiosperm trees than in gymnosperm trees or angiosperm lianas; mean values were 9.3, 4.9, and 4.7 g g$^{-1}$, respectively.

When expressed on a leaf area basis, the leaf P concentration was significantly correlated with mean transpiration rate across all individuals ($R^2=0.24$, $P<0.0001$, $n=94$). The equation relating the two was $P_{\text{area}}=0.096MTR+2.75$, where $P_{\text{area}}$ is in mmol m$^{-2}$, and $MTR$ is in mol m$^{-2}$ day$^{-1}$.

**Nitrogen use efficiency**

Equation (2) presents a means for analyzing variation among functional groups and species in whole-plant N use efficiency, $NUE$ (mol C mol$^{-1}$ N s$^{-1}$). We calculated $NUE$ as the product of $r$ and $m_c/m_n$, the whole-plant C to N molar ratio; thus, a relatively high C/N has the effect of increasing $NUE$. Figure 4A shows variation among species in $NUE$. There was significant variation among functional groups ($P<0.0001$) and among species within functional groups ($P<0.0001$). However, unlike results for $r$, angiosperm trees and lianas did not differ from each other with respect to $NUE$ ($P=0.84$). In contrast, $NUE$ of gymnosperm trees was lower than that of both angiosperm trees ($P<0.0001$) and angiosperm lianas ($P<0.0001$). Figures 4B and 4C show variation among species in the $NUE$ components, $A_n$ and $n_l$. Variation in $NUE$ among species tended to reflect variation in $A_n$, the photosynthetic N use efficiency (Figure 4B). The $A_n$ was also higher in angiosperm trees and lianas than in gymnosperm trees (Figure 4B). This variation in $A_n$ was offset to a lesser extent by variation in $n_l$, the proportion of plant N allocated to leaves (Figure 4C). Gymnosperm trees had highest mean $n_l$, at 0.69, followed by angiosperm trees at 0.59, then by angiosperm lianas at 0.50. Thus, a higher allocation of N to leaves in gymnosperm trees compensated to some extent for their much lower $A_n$. However, the $A_n$ was still the dominant control over $NUE$ (Figure 5).
**Transpiration efficiency**

Mean values for each species for $TE_c$, the whole-plant transpiration efficiency of C gain, are shown in Table V. Also shown in Table V are the growth-weighted estimates of the daytime vapor pressure deficit, $D_g$, by species. There was significant variation, both among functional groups ($P<0.0001$), and among species within functional groups ($P<0.0001$), in both $TE_c$ and $D_g$. However, across the full data set, $TE_c$ and $D_g$ were not significantly correlated ($P=0.11, n=94$), suggesting that $D_g$ was not a primary control over $TE_c$. Taking the product of $D_g$ and $TE_c$ allows analysis of variation in $TE_c$ independently of variation in $D_g$, as articulated in equation (5). The $D_g·TE_c$ also varied significantly among functional groups ($P<0.0001$) and among species within functional groups ($P<0.0001$). Angiosperm trees had the highest mean $D_g·TE_c$ at 1.58 Pa mol C mol$^{-1}$ H$_2$O, followed by angiosperm lianas at 1.30 Pa mol C mol$^{-1}$ H$_2$O, then by gymnosperm trees at 1.11 Pa mol C mol$^{-1}$ H$_2$O. Among all species, there was a 3.7-fold variation in $D_g·TE_c$ (i.e., the largest species mean was 3.7 times the smallest species mean).

Table V summarizes for each species the components of $D_g·TE_c$ that we quantified: $\phi_v$, the ratio of leaf-to-air vapor pressure difference to air vapor pressure deficit; $\phi_w$, the ratio of unproductive to productive water loss; and $c_i/c_a$, the ratio of intercellular to ambient CO$_2$ partial pressures during photosynthesis. Although there was a 1.8-fold variation among species in $\phi_v$, this parameter did not appear to be a primary control over $D_g·TE_c$: the term $1/\phi_v$ explained only 13% of variation in $D_g·TE_c$ ($R^2=0.13, P=0.0004, n=94$); moreover, the slope of the relationship between $D_g·TE_c$ and $1/\phi_v$ was negative, opposite to that predicted by equation (5). The parameter $\phi_w$ similarly did not appear to exert a strong control over $D_g·TE_c$: although $D_g·TE_c$ was positively correlated with $1/(1+\phi_w)$ ($R^2=0.18, P<0.0001, n=92$), there was only a 1.1-fold variation in this term among species, suggesting that it could only account for a variation in $D_g·TE_c$ of around 10%. In contrast, the $c_i/c_a$ appeared to be the primary control over $D_g·TE_c$. Among species, there was a 2.3-fold variation in instantaneous measurements of $1-c_i/c_a$, and $c_i/c_a$ explained 46% of variation in $D_g·TE_c$. Regression coefficients are given in Table VI. Furthermore, instantaneous measurements of $1-c_i/c_a$ explained 64% of variation in the composite term $v_g·TE_c(1+\phi_w)$ ($R^2=64, P<0.0001, n=94$). Taking this product means that only the variables $\phi_v$, $c_a$, and $c_i/c_a$ remain on the right side of equation (5).
Variation in instantaneous measurements of \( c_i/c_a \) was largely driven by variation in stomatal conductance, \( g_s \), rather than by variation in photosynthesis, \( A \). If \( g_s \) controls variation in \( c_i/c_a \), then \( c_i/c_a \) should decrease as \( 1/g_s \) increases. The \( 1/g_s \) is equivalent to the stomatal resistance. In contrast, if \( A \) controls \( c_i/c_a \), then \( c_i/c_a \) should decrease as \( A \) increases. Figure 6A shows that instantaneous \( c_i/c_a \) decreased as a linear function of \( 1/g_s \). In contrast, Figure 6B shows that there was a weak tendency for \( c_i/c_a \) to increase as \( A \) increased, opposite to the trend that would be expected if \( A \) were controlling \( c_i/c_a \).

We used measurements of leaf temperature, taken with a hand-held infrared thermometer, to calculate values of \( \phi_v \) for the species harvested on the second and third harvest dates (Table III). We then compared these instantaneous measurements of \( \phi_v \) with our time-integrated estimates for each plant based on leaf energy balance predictions and meteorological data. The time-integrated estimates of \( \phi_v \) compared favorably with the instantaneous measurements of \( \phi_v \) \((R^2=0.42, P<0.0001, n=55)\), thus providing some validation of the former.

**Stable isotope composition**

The C isotope composition of leaves, stems, roots, and whole plants is shown for each species in Table VII. Also shown is the difference in \( \delta^{13}C \) between leaves and the sum of stems plus roots, the heterotrophic component of the plant. Across all individuals, leaf \( \delta^{13}C \) was more negative than stem \( \delta^{13}C \) \((P<0.0001, n=94)\) and root \( \delta^{13}C \) \((P<0.0001, n=94)\), whereas stem \( \delta^{13}C \) was more negative than root \( \delta^{13}C \), but by a much smaller amount \((P=0.0008, n=94)\); mean values for leaf, stem, and root \( \delta^{13}C \) were \(-29.4\), \(-28.1\), and \(-27.8\%)\, respectively.

We converted plant \( \delta^{13}C \) values to \( ^{13}C \) discrimination by assuming \( \delta^{13}C \) of atmospheric CO\(_2\) to be \(-8\%e\). Whole-plant \( \Delta^{13}C \), \( \Delta^{13}C_p \), covered a range from \(18.8\%e\) to \(22.9\%e\) among species, corresponding to \( \delta^{13}C_p \) values ranging from \(-26.3\%e\) to \(-30.2\%e\) (Table VII). There was significant variation in \( \Delta^{13}C_p \) values ranging from \(-26.3\%e\) to \(-30.2\%e\) \((P=0.002)\) and among species within functional groups \((P<0.0001)\). The \( \Delta^{13}C_p \) was lower in angiosperm trees than in gymnosperm trees, whereas angiosperm lianas did not differ significantly from angiosperm or gymnosperm trees. Mean values were \(21.0\%e\), \(21.2\%e\), and \(21.5\%e\) for angiosperm trees, angiosperm lianas, and gymnosperm trees, respectively.
The $\Delta^{13}C_p$ was significantly correlated with instantaneous measurements of $c_i/c_a$ (Figure 7), as predicted by equation (6). We estimated the term $d$ of equation (6) by least-squares regression by assuming fixed values for $a$ and $b$ of 4.4‰ and 29‰, respectively. This resulted in an estimate for $d$ of 3.1‰; the regression equation explained 57% of variation in $\Delta^{13}C_p$. Thus, the predictive power of this relationship was equivalent to that obtained with a standard linear regression, in which both the slope and intercept are free to vary (Figure 7). Using the mean estimate of 3.1‰ for $d$, and values of 4.4‰ and 29‰ for $a$ and $b$, respectively, we calculated a $\Delta^{13}C_p$-based estimate of $c_i/c_a$ for each plant. Mean values of these estimates for each species are given in Table V. There was a 2.4-fold variation among species in the $\Delta^{13}C_p$-based estimates of $1-c_i/c_a$.

Variation in $\Delta^{13}C_p$ was significantly correlated with variation in $D_g \cdot TE_c$ (Figure 8); the former explained 49% of variation in the latter. Regression coefficients and the coefficient of determination for least-squares linear regressions of $TE_c$, $D_g \cdot TE_c$, and $v_g \cdot TE_c$ against $\Delta^{13}C$ of leaves, stems, roots, and whole plants are given in Table VI. In general, whole-plant $\Delta^{13}C$ was a better predictor of variation in $TE_c$ than $\Delta^{13}C$ of leaves, stems, or roots individually. Additionally, weighting of $TE_c$ by $D_g$ or $v_g$ tended to result in modest increases in the proportion of variation explained by the regression models (Table VI).

Correlations between $\Delta^{13}C_p$ and $1/g_s$ and $A$ further supported the conclusion that variation in $c_i/c_a$ was largely driven by variation in $g_s$. The $\Delta^{13}C_p$ decreased as a linear function of $1/g_s$ (Figure 6C). In contrast, the $\Delta^{13}C_p$ showed a weak tendency to increase as a function of $A$ (Figure 6D), opposite to the trend that would be expected if $A$ controlled variation in $c_i/c_a$.

Variation among species in the O isotope composition of stem dry matter is given in Table VII. We calculated the $^{18}$O enrichment above source water of stem dry matter, $\Delta^{18}O_p$, from the mean $\delta^{18}O$ of irrigation water of $-4.3‰$. The observed $\Delta^{18}O_p$ was significantly correlated with the predicted $^{18}$O enrichment of evaporative site water, $\Delta^{18}O_e$, weighted by predicted weekly growth increments (Figure 9). We tested whether the residual variation in $\Delta^{18}O_p$, after accounting for variation in $\Delta^{18}O_e$, was related to transpiration rate by plotting $1-[(\Delta^{18}O_p-\epsilon_{wc}-\epsilon_{cp})/(1-p_{ex}p_s)]/\Delta^{18}O_e$ against the mean transpiration rate, $MTR$. As shown in equation (12), this term should increase with increasing transpiration rate if there is a significant Péclet effect. Our analysis detected a significant relationship between the two parameters.
\(R^2=0.14, P=0.0002, n=94\), supporting the notion of a significant Péclet effect, although there was considerable scatter in the relationship. Using the \(MTR\) and \(E_d/E_d\), we calculated a mean daytime transpiration rate, then used the nonlinear regression routine in SYSTAT to solve for an average value of \(L\), the scaled effective path length, across the full data set. This analysis estimated a mean value of \(L\) for the full data set of 53 mm, with the 95% confidence interval ranging from 43 to 62 mm.

**DISCUSSION**

In this paper, we present a comprehensive comparison of physiological processes in seedlings of conifers, angiosperm trees, and angiosperm lianas under tropical field conditions. The comparison yielded novel insights into physiological differences among these functional groups, when grown in a tropical environment. For example, we observed that liana species, on average, had higher \(1/\rho\) than tree species, and that this trait was associated with faster growth. We also observed that gymnosperm trees had significantly lower whole-plant N use efficiency than both angiosperm trees and angiosperm lianas. In addition, the results provided an integrated account of the physiological controls over growth, whole-plant water and N use efficiency, and stable isotope composition across the full range of species. Relative growth rate, \(r\), was mainly controlled by variation in \(1/\rho\), the amount of assimilative surface area for a given plant biomass (Figure 1). Of the components of \(1/\rho\), the specific leaf area, \(SLA\), played a key role, such that the product of \(SLA\) and instantaneous photosynthesis, \(A\), was a strong predictor of variation in \(r\) (Figure 2). The whole-plant N use efficiency, \(NUE\), was mainly controlled by \(A_n\), the photosynthetic rate for a given amount of leaf N (Figure 5). An increase in the proportional allocation of N to leaves, \(n_l\), in species with low \(A_n\) was observed; however, the increased \(n_l\) compensated to only a relatively modest extent for low \(A_n\) (Figure 4). The primary control over the transpiration efficiency of C uptake, \(TE_c\), was \(c_i/c_a\), the ratio of intercellular to ambient CO\(_2\) partial pressures during photosynthetic gas exchange (Tables V and VI). The \(c_i/c_a\) was also the primary control over whole-plant \(^{13}\text{C}\) discrimination, \(\Delta^{13}\text{C}_p\) (Figure 7), such that variation in \(\Delta^{13}\text{C}_p\) was closely correlated with variation in \(TE_c\) (Table VI and Figure 8). The \(c_i/c_a\), in turn, was largely controlled by stomatal conductance, \(g_s\) (Figure 6). The \(^{18}\text{O}\) enrichment of stem dry matter, \(\Delta^{18}\text{O}_p\), was primarily controlled by the predicted \(^{18}\text{O}\) enrichment of the evaporative sites within leaves, \(\Delta^{18}\text{O}_e\), during photosynthetic gas exchange (Figure 9). Variation in leaf
transpiration rate further explained some of the residual variation in $\Delta^{18}O_p$, not accounted for by variation in $\Delta^{18}O_c$.

**Growth and N use efficiency**

We observed that the term $1/\rho$ was the primary control over variation in $r$, and that $A$ was a relatively conservative parameter among species (Figure 1). These results agree with those presented previously for 24 herbaceous species (Poorter and Remkes, 1990; Poorter et al., 1990), and for many woody species (Cornelissen et al., 1996; Atkin et al., 1998; Wright and Westoby, 2000). For the herbaceous species, $SLA$ was also a key component of $1/\rho$, such that variation in $r$ was not correlated with variation in $A$, but was strongly correlated with variation in $A_m$, the product of $A$ and $SLA$ (Poorter et al., 1990). It should be noted that $A$ was measured near the end of the experiment, and our results thus do not preclude the possibility that variation in $A$ may have modulated $r$ earlier in plant development. Nonetheless, our measurements of $A_m$ explained more than two thirds of total variation in $r$ (Figure 2). Among the species that we grew, the gymnosperm trees generally had lowest $1/\rho$ and $r$, whereas angiosperm lianas had highest $1/\rho$ and $r$, with angiosperm trees intermediate between the two (Figure 1). This pattern suggests that variation in $1/\rho$ could be related to hydraulic efficiency, with the tracheid-bearing gymnosperm species constrained by a lower hydraulic conductance for a given plant mass, and thereby requiring a greater plant mass to support a given amount of assimilative, and thus evaporative, surface area. On the other hand, the angiosperm liana species, having freed themselves from the constraint of structural self-sufficiency, and possessing hydraulically-efficient vessels, might then have required a smaller plant mass to deliver water to a given leaf surface area.

Measurements of whole-plant hydraulic conductance per unit plant mass would be necessary to confirm this hypothesis. However, it would be consistent with differences in hydraulic conductivity observed previously between gymnosperm and angiosperm seedlings (Brodribb et al., 2005).

As shown in equation (1), the term $\phi_c$, the proportion of net C fixation used for respiration, has potential to influence $r$. It was previously observed for 24 herbaceous species that $\phi_c$ ranged from about 0.5 to 0.3, and that $r$ was negatively correlated with $\phi_c$, as predicted by equation (1) (Poorter et al., 1990). Although we did not measure $\phi_c$ in our study, we can speculate that the slower-growing species had higher values, because they generally had higher
whole-plant C concentrations (Figure 3A), which would correlate with higher tissue construction costs (Vertregt and Penning de Vries, 1987; Poorter, 1994). The $r$ was negatively correlated with whole-plant C concentration across the full data set ($R^2=0.35$, $P<0.0001$, $n=94$).

The leaf N/P mass ratios that we observed (Table IV) were generally low for tropical vegetation (Reich and Oleksyn, 2004). However, they are consistent with a previous study conducted under similar conditions, but with variable amounts of rice husk mixed into the experimental soil (Cernusak et al., 2007b). At a similar rice husk/soil mixture as used in the present study, we previously observed a mean leaf N/P ratio of 5.9 for *Ficus insipida* (Cernusak et al., 2007b), whereas the overall mean for all species in the present study was 7.2. The generally low leaf N/P ratios suggest that plant growth in the present study was constrained primarily by N availability, rather than by P availability (Koerselman and Meuleman, 1996; Aerts and Chapin, 2000). This is consistent with the addition of rice husks increasing the C/N ratio of the experimental soil, thereby favoring microbial immobilization of soil N, and thus reducing N availability to plants. This provided a useful experimental basis for comparing N use efficiency among the species in our study, because N was likely the nutrient most limiting plant growth.

We calculated whole-plant N use efficiency, NUE, as the product of $r$ and $m_c/m_n$, the whole-plant molar ratio of C to N. Although the gymnosperm species had higher C/N than the angiosperm species (Figure 4), they were still at a marked disadvantage with the respect to NUE. Such disadvantage resulted primarily from a lower $A_n$ (Figures 4 and 5). This difference between gymnosperm and angiosperm seedlings, whereby the former employed N much less efficiently than the latter for accumulating C, could be decisive in determining competitive outcomes between the two. Although productivity in tropical forests is generally considered P limited, it has also been reported that N availability can constrain tree growth in both montane (Tanner et al., 1998) and lowland (LeBauer and Treseder, 2008) tropical forests. Thus, a higher NUE may contribute to angiosperm dominance in tropical environments. The low $A_n$ in the gymnosperm species compared to the angiosperm species resulted primarily from lower SLA, because A was generally similar between the two groups, and leaf N concentration was lower in gymnosperms than in angiosperms (Supplementary Table SI).
Transpiration efficiency

The species included in the study exhibited a large variation in the transpiration efficiency of C uptake, $TE_c$ (Table V). This is consistent with previous results showing large variation in $TE_c$ among seven tropical tree species (Cernusak et al., 2007a). When the $TE_c$ for each individual plant was normalized according to its growth-weighted mean daytime vapor pressure deficit, $D_g$, the variation among species was still apparent, suggesting that $D_g$ was not a primary control over $TE_c$. Additionally, the relative ranking among species in the present study was consistent with results for three species that were also measured previously (Cernusak et al., 2007a); in the present study *P. pinnatum* had the highest $D_g\cdot TE_c$, *S. macrophylla* an intermediate value, and *T. grandis* the lowest value (3.05, 1.12, and 0.82 Pa mol C mol$^{-1}$ H$_2$O, respectively). Previously, we observed that $TE_c$ for these three species was 3.97, 2.88, and 1.63 mmol C mol$^{-1}$ H$_2$O, respectively (Cernusak et al., 2007a).

In the present study, we were able to confirm that $c_i/c_a$ was the primary control over $D_g\cdot TE_c$. The $\phi_v$ also showed a moderate variation among species, suggesting that it could be an important source of variation in $D_g\cdot TE_c$ (Table V); however, the $\phi_v$ tended to be negatively correlated with $c_i/c_a$, due to a mutual dependence of the two parameters on $g_s$. Thus, it appeared that variation in $\phi_v$ mostly served to dampen what would have been the full effect of variation in $c_i/c_a$ on $D_g\cdot TE_c$. For example, plants with low $g_s$ tended to have low $c_i/c_a$ (Figure 6), which would increase $D_g\cdot TE_c$, as shown in equation (5). All else being equal, the low $g_s$ would also cause leaf temperature to increase, thereby increasing $\phi_v$, which would then cause a counteracting decrease in $D_g\cdot TE_c$. However, it is clear from the large variation in $D_g\cdot TE_c$ and its correlation with $c_i/c_a$ (Table VI) that variation in $\phi_v$ only dampened, and did not completely cancel, the effect of $c_i/c_a$ on $D_g\cdot TE_c$. Of the other terms in equation (5), we found that $\phi_w$, the unproductive water loss as a proportion of that associated with photosynthesis, played only a minor role in modulating $D_g\cdot TE_c$, in agreement with previous results (Cernusak et al., 2007b). Finally we consider variation in $1-\phi_c$: although this term is an important functional trait, and may play an important role in modulating $r$, it likely plays a lesser role in controlling $D_g\cdot TE_c$ than $c_i/c_a$. For example if $\phi_c$ varied among species from 0.3 to 0.5 (Poorter et al., 1990), the term $1-\phi_c$ would vary from 0.5 to 0.7, whereas we observed variation in $1-c_i/c_a$ from 0.12 to 0.27 (Table V). Thus, the former would be associated with a 1.4-fold variation in $D_g\cdot TE_c$, and the latter with a 2.3-fold variation in $D_g\cdot TE_c$. 

There was significant variation in $D_gTE_c$ among the plant functional groups that we studied, such that angiosperm trees had highest $D_gTE_c$, on average, and gymnosperm trees lowest, with angiosperm lianas intermediate between the two. Angiosperm trees also had an advantage over gymnosperm trees if water-use efficiency was analyzed as $TE_c$, $v_gTE_c$, $c_i/c_a$, or $\Delta^{13}C_p$. Thus in addition to having an advantage over gymnosperm seedlings in N use efficiency, angiosperm seedlings may also have a competitive advantage in terms of water-use efficiency, when grown in tropical environments. However, it should be emphasized that our experiment was carried out under well-watered conditions, and thus may not necessarily be indicative of trends when water availability is limiting to plant growth.

Of the gymnosperm species that we grew, only one, *Podocarpus guatemalensis*, occurs naturally in tropical forests of Panama. Although generally associated with highland forests, this species also occurs on low-lying islands off both the Pacific and Atlantic coasts. Unfortunately, only one individual of *P. guatemalensis* survived in our experiment, and we therefore excluded it from all species-level analyses. However, the lone surviving individual was interesting in that it had the highest $D_gTE_c$ and lowest $c_i/c_a$ of any plant in the study (Figures 7 and 8). The $c_i/c_a$ of 0.63 that we observed for this individual of *P. guatemalensis* is similar to a $c_i/c_a$ of about 0.60 observed previously for well-watered *Podocarpus lawrencii* (Brodribb, 1996). Further research is necessary to determine whether high water-use efficiency is a common trait within the genus *Podocarpus*, and whether this trait contributes to the ability of *Podocarpus* to persist in otherwise angiosperm-dominated tropical forests.

**Stable isotope composition**

Whole-plant $^{13}C$ discrimination, $\Delta^{13}C_p$, showed a strong correlation with instantaneous measurements of $c_i/c_a$ (Figure 7), suggesting that in general $\Delta^{13}C_p$ was a faithful recorder of $c_i/c_a$, as predicted by equation (6). The mean value for $d$ that we estimated for the full data set was 3.1‰, reasonably similar to a value of 4.0‰, recently estimated for *Ficus insipida* (Cernusak et al., 2007b). The $\Delta^{13}C_p$ was also a reasonably good predictor of variation in $TE_c$, $D_gTE_c$, and $v_gTE_c$ (Table VI). We previously observed that the relationship between $\Delta^{13}C_p$ and $TE_c$ broke down at the species level, appearing to reflect species-specific offsets in the relationship between the two parameters (Cernusak et al., 2007a). Whereas there was some evidence of similar behavior in the present study, as can be seen in Figure 8, the species-level relationship between
$\Delta^{13}C_p$ and $D_{g}TE_{c}$ was generally much stronger in the present study. For example, in a least-squares linear regression between $\Delta^{13}C_p$ and $D_{g}TE_{c}$ using species means, the former explained 57% of variation in the latter ($R^2=0.57$, $P=0.002$, $n=14$); if $P. guatemalensis$ was included in the regression, the $\Delta^{13}C_p$ explained 77% of variation in $D_{g}TE_{c}$ ($R^2=0.77$, $P<0.0001$, $n=15$). The main difference between the earlier study (Cernusak et al., 2007a) and the present study was likely the range of variation in $\Delta^{13}C_p$ exhibited by the particular species that comprised the experiments. In the earlier study, mean values for $\Delta^{13}C_p$ at the species level ranged from only 20.3 to 21.7‰, whereas in the present study, species means ranged from 18.8 to 22.9‰; including the individual of $P. guatemalensis$ would further extend the lower range to 16.9‰.

Although our results show a generally strong correlation between $\Delta^{13}C_p$ and $D_{g}TE_{c}$, we suggest that it is best to err on the side of caution when interpreting variation among species in the former as indicative of variation among species in the latter. As shown in equation (7), there are many terms with potential to influence the relationship between $\Delta^{13}C_p$ and $D_{g}TE_{c}$, not least of which is variation in $d$, which could be associated with variation among species in mesophyll conductance to CO$_2$ (Lloyd et al., 1992; Warren and Adams, 2006; Seibt et al., 2008). Moreover, as was previously the case (Cernusak et al., 2007a), we observed significant variation among species in the difference between $\delta^{13}C$ of leaves and that of stems and roots (Table VII).

The mechanistic basis for such variation among species in the $\delta^{13}C$ difference between leaves and heterotrophic tissues is not well understood (Hobbie and Werner, 2004; Badeck et al., 2005).

The $^{18}O$ enrichment of stem dry matter, $\Delta^{18}O_p$, varied significantly among species, and much of the observed variation in $\Delta^{18}O_p$ could be explained by variation in $\Delta^{18}O_e$, the predicted $^{18}O$ enrichment of evaporative sites within leaves (Figure 9). Because plants were grown over different time periods throughout the year, and due to predicted differences in leaf temperature, there was a reasonable variation among species in predicted $\Delta^{18}O_e$. Species means for growth-weighted $\Delta^{18}O_e$ ranged from 6.9 to 13.0‰ (14.6‰ for $P. guatemalensis$), and explained 73% of variation in the observed species means for $\Delta^{18}O_p$ ($R^2=0.73$, $P=0.0001$, $n=14$), or 75% if $P. guatemalensis$ was included ($R^2=0.75$, $P<0.0001$, $n=15$). These correlations suggest that the assumptions described in the theory section for the $\Delta^{18}O_p$ model are reasonable. Additionally, we observed that the term $1-[(\Delta^{18}O_p-\epsilon_{wc}-\epsilon_{cp})/(1-p_{ex}p_{c})]/\Delta^{18}O_e$ was significantly related to the mean daytime transpiration rate across the full data set, providing evidence for a Péclet effect, as
articulated in equation (12). This result is consistent with an experiment involving three temperate tree species (Barbour et al., 2004). In the present study, we estimated a mean scaled effective path length, \( L \), for the full data set of 53 mm, similar to a value of 54 mm estimated previously for *Eucalyptus globulus* (Cernusak et al., 2005). Analysis of \( \Delta^{18}O_p \) in stem dry matter likely provides an advantage over analysis of leaf dry matter for data sets such as ours, which comprise diverse sets of species, because \( \varepsilon_{cp} \) for stem dry matter tends to be less variable within and among species than \( \varepsilon_{cp} \) for leaf dry matter (Borella et al., 1999; Barbour et al., 2001; Cernusak et al., 2004; Cernusak et al., 2005).

Given a sound theoretical understanding of sources of variation in \( \delta^{13}C \) and \( \delta^{18}O \) in plant dry matter, it should be possible to use such isotopic data to constrain physiological models of tropical forest trees. Data from the present experiment support the suggestion that measurements of \( \delta^{13}C \) can be used to make time-integrated estimates of \( c_i/c_a \) at the tree or stand scale. The photosynthetic rate, \( A \), can then be predicted from \( c_i \), assuming \( c_a \) is known or can be predicted. Finally, \( g_s \) can be calculated from \( A, c_i, \) and \( c_a \). An example of this modeling approach was recently provided (Buckley, 2008), along with a discussion of its advantages and disadvantages.

In the case of \( \delta^{18}O \), it should be possible to use this signal to reconstruct the ratio of ambient to intercellular vapor pressures, \( e_a/e_i \), during photosynthesis (Farquhar et al., 1989b; Sternberg et al., 1989). The strong relationship that we observed between stem dry matter \( \Delta^{18}O_p \) and the predicted \( \Delta^{18}O_e \) (Figure 9) supports this idea. However, our analysis also confirmed that the relationship between \( \Delta^{18}O_p \) and \( \Delta^{18}O_e \) was further modified by transpiration rate, \( E \), suggesting that it may be necessary to obtain information independently about \( E \) in order to calculate \( e_a/e_i \) from \( \Delta^{18}O_p \). Such information might be obtained from sap flux measurements or eddy covariance data, for example. Assuming \( e_a \) is known or can be predicted, an estimate of \( e_i \) based on \( \Delta^{18}O_p \) might then be particularly valuable, as it was recently suggested that the leaf-to-air vapor pressure difference, \( e_i-e_a \), will likely be an important control over productivity in tropical forest trees in the face of changing climate (Lloyd and Farquhar, 2008).

**CONCLUSION**

We observed that \( 1/\rho \) was an important control over relative growth rate, \( r \), in a diverse group of seedlings grown under tropical field conditions, including gymnosperm trees.
angiosperm trees, and angiosperm lianas. The gymnosperm trees generally had lower $1/\rho$ and $r$ than the angiosperm species, and this may have reflected differences in the hydraulic efficiency of plant biomass among functional groups. Additionally, we observed that $A_n$, the photosynthetic N use efficiency, was the primary control over whole-plant N use efficiency, and that the gymnosperm species appeared to be at a significant disadvantage with respect to this trait compared to angiosperm species. Variation in whole-plant water use efficiency among species was primarily controlled by $c_i/c_a$, which in turn varied as a function of stomatal conductance. Whole-plant $^{13}$C discrimination was also controlled by $c_i/c_a$, and thus correlated with whole-plant water use efficiency. The $^{18}$O enrichment of stem dry matter of the experimental plants varied primarily as a function of the predicted $^{18}$O enrichment of evaporative site water within leaves, and secondarily as a function of the mean daytime transpiration rate. Results provided quantitative information about the mechanisms controlling fluxes of C and water between forest trees and the atmosphere, and the coupling of these processes to plant N status. Moreover, our data set enabled rigorous testing of the theoretical basis for variation in $^{13}$C and $^{18}$O of plant dry matter; measurements of these stable isotope ratios could prove useful for parameterizing forest ecosystem process models.

MATERIALS AND METHODS

Study site and plant material

The study was carried out at the Santa Cruz Experimental Field Facility, a part of the Smithsonian Tropical Research Institute, located in Gamboa, Republic of Panama ($9^\circ 07'$ N, $79^\circ 42'$ W), at an altitude of approximately 28 m above sea level. Average meteorological conditions at the study site during the experiment are shown in Table II. These values were calculated from data collected on site every 15 minutes by an automated weather station (Winter et al., 2001; Winter et al., 2005). Seedlings of *C. lusitanica*, *P. caribaea*, and *T. occidentalis* were obtained from a commercial nursery in Chiriqui Province, Republic of Panama. All other species were grown from seed collected in the Panama Canal Watershed, or obtained as seedlings from PRORENA, a native species reforestation initiative operated through the Center for Tropical Forest Science at the Smithsonian Tropical Research Institute. Familial associations for each species are shown in Table III. The species *C. lusitanica* and *P. caribaea* are conifers, with native distributions extending from Mexico to Nicaragua. *T. occidentalis* is a conifer native
to north-eastern North America, and *T. grandis* is a timber species native to south and southeast Asia. All other species included in the study occur naturally in Panama.

The initial dry mass at the commencement of transpiration measurements for each species is shown in Table III; these dry masses were estimated by harvesting three to five individuals judged to be similar in size to the seedlings retained for the experiment. Seedlings were transplanted individually into 38 L plastic pots (Rubbermaid Round Brute, Consolidated, Twinsburg, OH, USA). Each pot contained 25 kg of dry soil mixture, which comprised 60% by volume dark, air-dried top soil, and 40% by volume air-dried rice husks. The rice husks were added to improve soil structure and drainage. The pot water content was brought to field capacity by addition of 8 kg water. The soil surface was covered with 2 kg gravel to minimize soil evaporation, and the outer walls of each pot were lined with reflective insulation to minimize heating by sunlight. A metal trellis was added to each pot containing a liana seedling. The pots were situated under a rain shelter with a glass roof, such that there was essentially no interception of rain by the pots in the otherwise open-air conditions. The initiation of measurements varied among species, due to temporal variation in availability of seed and seedlings. Harvest dates also varied, depending on the date of initiation of measurements and growth rates; there were three harvests in total. The start and end dates of transpiration measurements for each species are given in Table III.

**Growth and transpiration efficiency measurements**

Pots were weighed at a minimum frequency of once per week to the nearest 5 g with a 64 kg capacity balance (Sartorius QS64B, Thomas, Swedesboro, NJ, USA). After the mass was recorded, water was added to each pot to restore it to its mass at field capacity. As plant water use increased with increasing plant size, the pots were weighed and watered more frequently. We endeavored to maintain pot water content above 5 kg at all times, such that the range of soil water contents experienced by the plants ranged approximately from field capacity to 60% of field capacity. Control pots without plants were deployed among the pots with plants in a ratio of one control pot to each six planted pots. The control pots were weighed each week to estimate soil evaporation. Cumulative plant water use was calculated as the sum of pot water loss over the course of the experiment minus the average water loss of control pots for the same time period. Shortly before plant harvest, the pots were weighed at dawn and dusk for two days, in
order to calculate nighttime transpiration separately from daytime transpiration. Immediately following plant harvest, total plant leaf area was measured with a leaf area meter (LI-3100, Li-Cor Inc, Lincoln, NE, USA). Leaves, stems, and roots were separated at harvest and oven-dried to a constant mass at 70°C; they were then weighed to the nearest 0.02 g.

The mean relative growth rate, \( r \), of each plant was calculated as 
\[
  r = \frac{\ln(m_{c2}) - \ln(m_{c1})}{t},
\]
where \( \ln(m_{c2}) \) and \( \ln(m_{c1}) \) are natural logarithms of the C mass at the end and beginning of the experiment, respectively, and \( t \) is the duration of the experiment (Blackman, 1919). The mean transpiration rate, \( MTR \), over the course of the experiment was calculated as the cumulative water transpired divided by the leaf area duration (Sheshshayee et al., 2005):
\[
  MTR = \frac{E_t}{(LA_1 + LA_2)0.5t},
\]
where \( E_t \) is cumulative water transpired, and \( LA_1 \) and \( LA_2 \) are the leaf area at the beginning and end of the experiment, respectively. The transpiration efficiency of C gain, \( TE_c \), was calculated as 
\[
  TE_c = \frac{(m_{c2} - m_{c1} + l_c)}{E_t},
\]
where \( l_c \) is the C mass of leaf litter abscised during the experiment.

In order to compare the \( D \) experienced by species that were grown over different time periods (Table III), we calculated a growth-weighted \( D \) for each individual plant. Dry matter increments were predicted at weekly time steps for each plant using relative growth rates calculated over the full experiment. Thus, the dry matter increment for week 1, \( w_1 \), was calculated as 
\[
  w_1 = m_1 - m_0,
\]
where \( m_0 \) was initial plant dry mass, and \( m_1 \) was calculated as 
\[
  m_1 = m_0 e^{rt},
\]
with \( t = 7 \) days; the \( r \) was calculated as described above. The dry matter increment in week 2, \( w_2 \), was then calculated as 
\[
  w_2 = m_2 - m_1,
\]
where \( m_2 \) was calculated as 
\[
  m_2 = m_1 e^{rt},
\]
with \( t \) again set at 7 days, and so on. For each week during the experimental period, we also calculated an average daytime \( D \) from the meteorological data collected at 15-minute intervals. Growth-weighted \( D \), \( D_g \), was then calculated as
\[
  D_g = \frac{\sum_{i=1}^{n} D_i w_i}{\sum_{i=1}^{n} w_i},
\]
where \( D_i \) is the average daytime \( D \) for week \( i \) (kPa), and \( w_i \) is the predicted dry matter increment for week \( i \) (g).

In addition to calculating a growth-weighted \( D \) for each species, we also predicted a growth-weighted \( v \). Average weekly \( v \) for each plant was predicted using a leaf energy balance model developed by D.G.G. dePury and G.D. Farquhar (unpublished data), and described by
Barbour et al. (2000a). The model was parameterized with weekly average daytime values for air temperature, relative humidity, irradiance, and wind speed taken from the data collected by the automated weather station. Stomatal conductance for each plant, measured as described below, was further used to parameterize the model. The model predicted average weekly daytime leaf temperature for each plant. The intercellular water vapor pressure, $e_i$, was then calculated as the saturation vapor pressure at leaf temperature, and this value was used to calculate an average weekly value of $v$ for each plant. The growth-weighted $v$, $v_g$, was then calculated as in equation (13), but replacing $D_i$ with $v_i$, the average daytime $v$ for week $i$.

In a similar fashion, we predicted a growth-weighted $\Delta^{18}O_e$ for each plant. For each weekly time step, the predicted value of $e_a/e_i$ was used with equation (8) to calculate average weekly daytime $\Delta^{18}O_e$. The $\Delta^{18}O_v$ was assumed equal to $-\varepsilon^+$, calculated from air temperature (Bottinga and Craig, 1969). Growth-weighted $\Delta^{18}O_e$, $\Delta^{18}O_{eg}$, was then calculated for each plant as in equation (13), but replacing $D_i$ with $\Delta^{18}O_{ei}$, the average daytime $\Delta^{18}O_e$ for week $i$.

**Leaf gas exchange and leaf temperature measurements**

We measured leaf gas exchange on three to five leaves per plant in the week preceding plant harvest with a Li-6400 portable photosynthesis system (Li-Cor Inc). Leaves were illuminated with an artificial light source (6400-02B LED, Li-Cor Inc, Lincoln, NE, USA) at a photon flux density of 1200 µmol m$^{-2}$ s$^{-1}$. Measurements were made during both the morning and afternoon for each plant, and the mean of the two sets of measurements was taken for each individual. The mean leaf temperature during measurements was $32.7 \pm 1.5^\circ C$ (mean ± 1 SD), and the mean $v$ was $1.55 \pm 0.46$ kPa (mean ± 1 SD).

Several days prior to the harvests that took place on 11 March 2006 and 10 May 2006 (Table III), we made measurements of leaf temperature with a hand-held infrared thermometer (Raytek MT Minitemp, Forestry Suppliers Inc, USA). Measurements were repeated two to three times on three to five leaves per plant under clear-sky conditions near midday. Values for each plant were averaged, and $v$ was calculated from measurements of relative humidity and air temperature, assuming $e_i$ was at saturation at the average leaf temperature for each plant. Leaf temperature was not measured for the plants harvested on 13 December 2005 (Table III).
**Stable isotope and elemental analyses**

Leaf, stem, and root dry matter were ground to a fine, homogeneous powder for analysis of isotopic and elemental composition. The $\delta^{13}C$, total C, and total N concentrations were determined on sub-samples of approximately 3 mg, combusted in an elemental analyzer (ECS 4010, Costech Analytical Technologies, Valencia, CA, USA) coupled to a continuous flow isotope ratio mass spectrometer (Delta XP, Finnigan MAT, Bremen, Germany). The $\delta^{18}O$ of stem dry matter was determined on sub-samples of approximately 1 mg (Delta XP, Finnigan MAT), following pyrolysis in a high temperature furnace (Thermoquest TC/EA, Finnigan MAT). 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 were expressed in delta notation with respect to the standards of PeeDee Belemnite and Vienna Standard Mean Ocean Water, respectively. The $^{13}C$ discrimination of plant dry matter ($\Delta^{13}C_p$) was calculated as $\Delta^{13}C_p = (\delta^{13}C_a - \delta^{13}C_p)/(1 + \delta^{13}C_p)$, where $\delta^{13}C_a$ is the $\delta^{13}C$ of CO$_2$ in air and $\delta^{13}C_p$ is that of plant dry matter. We assumed a $\delta^{13}C_a$ of -8‰. The oxygen isotope enrichment of stem dry matter ($\Delta^{18}O_p$) was calculated as $\Delta^{18}O_p = (\delta^{18}O_p - \delta^{18}O_s)/(1 + \delta^{18}O_s)$, where $\delta^{18}O_p$ is $\delta^{18}O$ of stem dry matter, and $\delta^{18}O_s$ is that of irrigation (source) water. Irrigation water was drawn from two 800 L tanks, sealed to prevent evaporation, which were periodically refilled with tap water, to buffer against short term variation in $\delta^{18}O_s$. The tank water had a mean $\delta^{18}O$ of $-4.3 \pm 0.5‰$ (mean $\pm$ 1 SD, $n=6$); we therefore calculated $\Delta^{18}O_p$ assuming a $\delta^{18}O_s$ of $-4.3‰$.

Leaf dry matter was further analyzed for P, K, and Ca concentrations by acid digestion and detection on an inductively-coupled plasma optical-emission spectrometer (Perkin Elmer Inc, Wellesley, MA, USA). Leaf samples were prepared by digesting approximately 200 mg sample material under pressure in PTFE vessels with 2 mL of concentrated nitric acid.

**Statistical analyses**

We analyzed relationships between continuous variables using least-squares linear regression. Variation among species and among functional groups (gymnosperm trees, angiosperm trees, and angiosperm lianas) was assessed with a nested design in the general linear model routine of SYSTAT 11 (SYSTAT Software Inc, Richmond, CA, USA); functional group and species nested within functional group were taken as independent factors. For these analyses, the number of observations was 93, the degrees of freedom for functional group was 2.
the degrees of freedom for species nested within functional group was 11, and the degrees of freedom error was 79. Pair-wise comparisons among species or functional groups were then carried out according to Tukey’s method. Among the study species, there was one, *Podocarpus guatemalensis*, for which only one individual survived. All other species comprised between five and eight individuals, as shown in Table III. Because there was only one individual of *P. guatemalensis*, we excluded this species from analyses aimed at assessing variation among functional groups and species. However, we included the individual in linear regression analyses of continuous variables. We considered it important to report data for this individual, as it represents the only gymnosperm species in the study native to the tropical forests of Panama.

**Supplemental data**

The following materials are available in the online version of this article.

Supplemental Table SI. The C and N concentrations of experimental plants.

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FIGURE LEGENDS

Figure 1. Variation among species in mean relative growth rate (A), net photosynthesis, expressed on a leaf area basis (B), and leaf area per unit plant C mass, \( 1/\rho \) (C). Error bars represent one standard error. Sample sizes for each species are given in Table III.

Figure 2. Mean relative growth rate plotted against instantaneous measurements of photosynthesis expressed on a leaf mass basis. Open symbols with internal cross hairs refer to gymnosperm tree species; completely open symbols refer to angiosperm liana species; closed symbols and closed symbols with internal cross hairs refer to angiosperm tree species.

Figure 3. Variation among species in the C concentration of dry matter on a whole-plant basis (A), the N concentration of dry matter on a whole-plant basis (B), and the C/N mass ratio of dry matter on a whole-plant basis (C). Error bars represent one standard error. Sample sizes for each species are given in Table III.

Figure 4. Variation among species in whole-plant N use efficiency (A), photosynthetic N use efficiency (B), and \( n_l \), the leaf N content as a proportion of whole-plant N content (C). Error bars represent one standard error. Sample sizes for each species are given in Table III.

Figure 5. Whole-plant N use efficiency plotted against photosynthetic N use efficiency. Whole-plant N use efficiency was calculated from mean relative growth rate, measured over several months, whereas photosynthetic N use efficiency was calculated from instantaneous photosynthesis measurements, taken over several minutes. Different symbols refer to different species, as detailed in Figure 2.

Figure 6. The top two panels show instantaneous measurements of \( c_i/c_a \) plotted against stomatal resistance (A) and photosynthesis (B); the bottom two panels show whole-plant \(^{13}\)C discrimination plotted against stomatal resistance (C) and photosynthesis (D). Stomatal resistance is the inverse of stomatal conductance. Different symbols refer to different species, as described for Figure 2.

Figure 7. Whole-plant \(^{13}\)C discrimination plotted against the ratio of intercellular to ambient CO\(_2\) partial pressures determined from instantaneous gas exchange measurements. Different symbols refer to different species, as defined in Figure 2.

Figure 8. The product of transpiration efficiency of C gain (\( TE_c \)) and daytime vapor-pressure deficit of ambient air (\( D_g \)) plotted against whole-plant \(^{13}\)C discrimination. Different symbols refer to different species, as defined in Figure 2. Daytime air vapor-pressure deficit was weighted according to the predicted weekly growth increment for each individual plant.

Figure 9. The \(^{18}\)O enrichment of stem dry matter relative to irrigation water plotted against the predicted \(^{18}\)O enrichment of water at the evaporative sites in leaves. The predicted \( \Delta^{18}\)O\(_e\) was weighted according to the predicted weekly growth increment for each individual plant. Different symbols refer to different species, as defined in Figure 2.
### Table I. Abbreviations and symbols used in the text.

| Symbol | Description |
|--------|-------------|
| A      | Area-based photosynthesis rate (µmol CO_2 m^{-2} s^{-1}) |
| A_m   | Mass-based photosynthesis rate (nmol CO_2 g^{-1} s^{-1}) |
| A_n   | Photosynthetic N use efficiency (µmol CO_2 mol^{-1} N s^{-1}) |
| a     | Discrimination against ^13C during diffusion through stomata |
| b     | Discrimination against ^13C during carboxylation by Rubisco |
| C     | Molar concentration of water (mol m\(^{-3}\)) |
| c_a   | Partial pressure of CO_2 in ambient air (Pa) |
| c_i   | Partial pressure of CO_2 in leaf intercellular air spaces (Pa) |
| D     | Vapor pressure deficit of ambient air (kPa) |
| D_g   | Growth-weighted vapor pressure deficit of ambient air (kPa) |
| D_i   | Average daytime vapor pressure deficit of ambient air during week \(i\) (kPa) |
| D_{18} | Diffusivity of H_2^{18}O in water (m\(^{2}\) s^{-1}) |
| d     | Discrimination against ^13C during C_1 photosynthesis not associated with \(a\) or \(b\) |
| E     | Transpiration rate (mmol m\(^{-2}\) s\(^{-1}\)) |
| E_d   | Daytime transpiration rate (mmol m\(^{-2}\) s\(^{-1}\)) |
| E_c   | Cumulative transpiration over the course of the experiment (mol) |
| E_n   | Nighttime transpiration rate (mmol m\(^{-2}\) s\(^{-1}\)) |
| e_a   | Vapor pressure of ambient air (kPa) |
| e_i   | Vapor pressure in leaf intercellular air spaces (kPa) |
| g_s   | Stomatal conductance (mol m\(^{-2}\) s\(^{-1}\)) |
| L     | Scaled effective path length relating to ^18O advection and diffusion (m) |
| LAR   | Leaf area ratio (m\(^{2}\) kg\(^{-1}\)) |
| LA_1  | Leaf area at the initiation of the experiment (m\(^{2}\)) |
| LA_2  | Leaf area at the conclusion of the experiment (m\(^{2}\)) |
| l     | Light period as a fraction of 24 hours |
| l_c   | Mass of C in leaf litter abscised during the experiment (mol C) |
| m_c   | Plant C mass (mol C) |
| m_c_1 | Plant C mass at the initiation of the experiment (mol C) |
| m_c_2 | Plant C mass at the conclusion of the experiment (mol C) |
| m_n   | Plant N mass (mol N) |
| MTR   | Mean transpiration rate over the course of the experiment (mol m\(^{-2}\) day\(^{-1}\)) |
| NAR   | Net assimilation rate (g dry matter m\(^{-2}\) s\(^{-1}\)) |
| NUE   | Whole-plant N use efficiency (mol C mol^{-1} N day^{-1}) |
| n_l   | Leaf N as a proportion of whole-plant N |
| P_{area} | Leaf P per unit area (mmol m\(^{-2}\)) |
| p_{ex} | Proportion of O atoms exchanging with medium water during cellulose synthesis |
| p_x   | Proportion of water in developing cells not subject to evaporative ^18O enrichment |
| R     | The ^18O/^16O ratio of any water or dry matter component of interest |
| R_{a} | The ^13C/^12C ratio of CO_2 in ambient air |
| R_{p} | The ^13C/^12C ratio of plant C |
| R_{s} | The ^18O/^16O ratio of irrigation (source) water |
| r     | Relative growth rate (mol C mol^{-1} C day^{-1}) |
| SLA   | Specific leaf area (m\(^{2}\) kg\(^{-1}\)) |
| TE_c  | Whole-plant transpiration efficiency of C gain (mmol C mol^{-1} H_2O) |
| v     | Leaf-to-air vapor pressure difference (kPa) |
| v_g   | Growth-weighted leaf-to-air vapor pressure difference (kPa) |
| v_{i} | Average daytime leaf-to-air vapor pressure difference for week \(i\) (kPa) |
| w_{i} | Predicted dry matter increment for week \(i\) (g) |
| \Delta_{13}C | Discrimination against ^13C |
| Symbol | Definition |
|--------|------------|
| $\Delta^{13}C_p$ | Discrimination against $^{13}C$ in dry matter of the whole plant relative to CO$_2$ in air |
| $\Delta^{18}O_e$ | The $^{18}O$ enrichment of evaporative sites within leaves compared to source water |
| $\Delta^{18}O_{eg}$ | Growth-weighted prediction of $\Delta^{18}O_e$ over the course of the experiment |
| $\Delta^{18}O_{ei}$ | Predicted average daytime $\Delta^{18}O_e$ for week $i$ |
| $\Delta^{18}O_L$ | The $^{18}O$ enrichment of average lamina leaf water compared to source water |
| $\Delta^{18}O_p$ | The $^{18}O$ enrichment of stem dry matter compared to source water |
| $\Delta^{18}O_v$ | The $^{18}O$ enrichment of atmospheric water vapor compared to source water |
| $\delta^{13}C$ | The $^{13}C/^{12}C$ ratio relative to the PeeDee Belmnite international standard |
| $\delta^{13}C_a$ | The $\delta^{13}C$ of CO$_2$ in ambient air |
| $\delta^{13}C_p$ | The $\delta^{13}C$ of plant C |
| $\delta^{18}O$ | The $^{18}O/^{16}O$ ratio relative to Vienna Standard Mean Ocean Water |
| $\delta^{18}O_P$ | The $\delta^{18}O$ of stem dry matter |
| $\delta^{18}O_s$ | The $\delta^{18}O$ of irrigation (source) water |
| $\varepsilon_{cp}$ | The $\delta^{18}O$ difference between plant dry matter and cellulose extracted from it |
| $\varepsilon_k$ | Kinetic H$_2^{18}O$ fractionation for diffusion through stomata and leaf boundary layer |
| $\varepsilon_{wc}$ | The $^{18}O$ enrichment of cellulose compared to the water in which it formed |
| $\varepsilon^*$ | Equilibrium H$_2^{18}O$ fractionation during the phase change from liquid to gas |
| $\phi_c$ | Proportion of net photosynthesis subsequently used for respiration |
| $\phi_v$ | Scaling factor to convert $D$ to $v$ ($=v/D$) |
| $\phi_p$ | Unproductive water loss as proportion of that associated with photosynthesis |
| $\phi$ | Péclet number |
| $\rho$ | Plant C mass per unit leaf area (mol C m$^{-2}$) |
Table II. Average daytime meteorological conditions at the study site over the course of the experiment. Values are monthly means of measurements taken every 15 min between the hours of 0700 and 1730 local time. We focused on daytime hours to characterize conditions during photosynthetic gas exchange.

|                | 2005         |              | 2006         |              |
|----------------|--------------|--------------|--------------|--------------|
|                | Jun  | Jul  | Aug  | Sep  | Oct  | Nov  | Dec  | Jan  | Feb  | Mar  | Apr  | May  |
| Air temperature (°C) | 28.0 | 26.7 | 28.6 | 29.6 | 30.0 | 27.7 | 30.7 | 28.1 | 27.9 | 29.1 | 28.7 | 28.2 |
| Relative humidity (%) | 81.3 | 82.9 | 82.6 | 83.3 | 79.3 | 84.2 | 75.0 | 72.5 | 68.8 | 68.3 | 74.1 | 80.1 |
| Vapor pressure deficit (kPa) | 0.73 | 0.62 | 0.71 | 0.70 | 0.89 | 0.60 | 1.12 | 1.06 | 1.18 | 1.31 | 1.03 | 0.78 |
| Wind speed (m s⁻¹) | 0.33 | 0.26 | 0.31 | 0.33 | 0.45 | 0.29 | 0.50 | 0.61 | 0.87 | 0.82 | 0.75 | 0.38 |
| Photon flux density (µmol m⁻² s⁻¹) | 685  | 655  | 649  | 628  | 750  | 602  | 743  | 808  | 859  | 925  | 854  | 685  |
Table III. Experimental time period, initial and final plant dry mass, and root to shoot ratio for each species in the study. Values for final dry mass and root to shoot ratio are given as the mean, with the standard deviation in parentheses. A standard deviation is not given for *P. guatemalensis* because only one plant survived for this species.

| Species                               | Family         | Start date  | End date   | Number of plants | Initial dry mass (g) | Final dry mass (g) | Root to shoot ratio (g g\(^{-1}\)) |
|---------------------------------------|----------------|-------------|------------|------------------|----------------------|-------------------|-----------------------------------|
| Gymnosperm tree species               |                |             |            |                  |                      |                   |                                   |
| *Cupressus lusitanica* Mill.          | Cupressaceae   | 23 May 2005 | 13 Dec 2005| 8                | 5.8                  | 60.2 (19.0)       | 0.36 (0.06)                       |
| *Pinus caribaea* Morelet              | Pinaceae       | 23 May 2005 | 13 Dec 2005| 8                | 5.6                  | 64.6 (30.9)       | 0.27 (0.09)                       |
| *Podocarpus guatemalensis* Standl.    | Podocarpaceae  | 23 May 2005 | 11 May 2006| 1                | 0.8                  | 107.3 (NA)        | 0.16 (NA)                         |
| *Thuja occidentalis* L.               | Cupressaceae   | 26 Apr 2004 | 13 Dec 2005| 8                | 4.7                  | 41.4 (16.5)       | 0.47 (0.08)                       |
| Angiosperm tree species               |                |             |            |                  |                      |                   |                                   |
| *Calophyllum longifolium* Willd.      | Clusiaceae     | 11 Jul 2005 | 10 Mar 2006| 6                | 3.1                  | 62.6 (27.0)       | 0.39 (0.08)                       |
| *Clusia praetensis* Seem.             | Clusiaceae     | 22 Aug 2005 | 11 May 2006| 6                | 0.2                  | 112.2 (62.3)      | 0.76 (0.26)                       |
| *Heronicia alchormoides* Allemão      | Euphorbiaceae  | 20 Jun 2005 | 13 Dec 2005| 6                | 0.2                  | 36.6 (30.2)       | 1.10 (0.13)                       |
| *Luehea seemannii* Triana & Planch.   | Tiliaceae      | 29 Aug 2005 | 10 Mar 2006| 7                | 0.1                  | 39.8 (7.3)        | 0.61 (0.10)                       |
| *Platyomiscium pinnatum* (Jacq.) Dugand | Fabaceae     | 7 Nov 2005  | 10 Mar 2006| 2                | 0.6                  | 53.9 (16.6)       | 0.58 (0.20)                       |
| *Platyomiscium pinnatum* (Jacq.) Dugand | Fabaceae     | 7 Nov 2005  | 11 May 2006| 3                | 0.6                  | 21.6 (13.3)       | 0.36 (0.14)                       |
| *Swietenia macrophylla* King          | Meliaceae      | 7 Nov 2005  | 11 May 2006| 7                | 0.8                  | 24.3 (11.8)       | 0.34 (0.10)                       |
| *Tabebuia rosea* (Bertol.) A. DC.     | Bignoniaceae   | 20 Jun 2005 | 13 Dec 2005| 6                | 0.6                  | 64.8 (11.0)       | 0.92 (0.12)                       |
| *Tectona grandis* Linn.               | Verbenaceae    | 17 May 2004 | 11 May 2006| 7                | 0.1                  | 45.5 (11.3)       | 0.93 (0.17)                       |
| Angiosperm liana species              |                |             |            |                  |                      |                   |                                   |
| *Gouania lupuloides* (L.) Urb.        | Rhamnaceae     | 29 Aug 2005 | 10 Mar 2006| 6                | 0.01                 | 31.5 (15.6)       | 0.47 (0.20)                       |
| *Mikania leiostachya* Benth.          | Asteraceae     | 29 Aug 2005 | 10 Mar 2006| 3                | 0.1                  | 21.2 (4.3)        | 0.37 (0.27)                       |
| *Mikania leiostachya* Benth.          | Asteraceae     | 14 Nov 2005 | 11 May 2006| 3                | 0.3                  | 23.2 (4.8)        | 0.25 (0.06)                       |
| *Stigmaphyllon hypargyreum* Triana & Planch. | Malphigiaceae | 29 Aug 2005 | 10 Mar 2006| 7                | 0.1                  | 50.3 (20.3)       | 1.99 (0.21)                       |
Table IV. Leaf P, Ca, and K concentrations, and N/P ratios of experimental plants. Values are given as the mean for each species, with the standard deviation in parentheses. No standard deviation is given for *P. guatemalensis*, because only one individual of this species survived. Sample sizes for the other species ranged from five to eight individuals, as shown in Table III.

| Species                  | P (g kg⁻¹) | Ca (g kg⁻¹) | K (g kg⁻¹) | N/P (g g⁻¹) |
|--------------------------|------------|-------------|------------|-------------|
| **Gymnosperm tree species** |            |             |            |             |
| *Cupressus lusitanica*   | 3.10 (0.76)| 8.3 (0.9)   | 20.9 (2.8) | 3.6 (0.8)   |
| *Pinus caribaea*         | 1.43 (0.47)| 3.6 (1.4)   | 9.6 (1.6)  | 7.6 (1.4)   |
| *Podocarpus guatemalensis* | 2.48 (NA)| 8.1 (NA)   | 16.9 (NA)  | 5.9 (NA)   |
| *Thuja occidentalis*     | 4.24 (0.86)| 10.5 (2.2) | 16.8 (1.5) | 3.4 (0.8) |
| **Angiosperm tree species** |            |             |            |             |
| *Calophyllum longifolium*| 0.94 (0.23)| 7.7 (0.8)   | 8.4 (1.4)  | 12.4 (1.5)  |
| *Clusia pratensis*       | 1.38 (0.47)| 13.6 (1.1)  | 14.4 (5.2) | 9.7 (2.1)  |
| *Hyeronima alchorneoides*| 2.22 (0.59)| 12.5 (3.9)  | 24.0 (4.3) | 6.8 (1.5)  |
| *Luehea seemannii*       | 3.59 (0.81)| 18.0 (2.1)  | 14.2 (1.6) | 5.0 (1.4)  |
| *Platymiscium pinnatum*  | 1.68 (0.23)| 10.2 (2.1)  | 16.7 (4.3) | 18.2 (5.7) |
| *Swietenia macrophylla*  | 1.33 (0.35)| 13.4 (1.5)  | 20.3 (3.0) | 12.0 (2.8) |
| *Tabebuia rosea*         | 1.43 (0.04)| 14.4 (4.3)  | 15.1 (4.4) | 11.1 (2.2) |
| *Tectona grandis*        | 5.96 (0.42)| 8.6 (1.0)   | 13.3 (2.1) | 2.0 (0.2)  |
| **Angiosperm liana species** |            |             |            |             |
| *Gouania lupuloides*     | 5.33 (1.15)| 14.4 (2.1)  | 24.5 (2.4) | 4.4 (0.9)  |
| *Mikania leiotachya*     | 2.66 (0.30)| 13.2 (1.4)  | 29.5 (3.5) | 6.6 (1.2)  |
| *Stigmaphyllum hypargyreum* | 6.23 (1.09)| 23.4 (3.3)  | 24.6 (4.0) | 3.5 (0.5)  |
Table V. Transpiration efficiency and related parameters for each species. Symbol definitions are as follows: transpiration efficiency of C uptake (TE\textsubscript{c}); growth-weighted daytime vapor pressure deficit (D\textsubscript{g}) and leaf-to-air vapor pressure difference (v\textsubscript{g}); the ratio of nighttime to daytime transpiration (E\textsubscript{n}/E\textsubscript{d}); and the ratio of intercellular to ambient CO\textsubscript{2} partial pressures (c\textsubscript{i}/c\textsubscript{a}). The c\textsubscript{i}/c\textsubscript{a} is given as the value measured with a portable photosynthesis system (instantaneous), or as the value estimated from whole-plant $\Delta^{13}$C discrimination ($\Delta^{13}$C\textsubscript{P}-based). Values are given as the mean for each species, with the standard deviation in parentheses. No standard deviation is given for P. guatemalensis, because only one individual of this species survived. Sample sizes for the other species ranged from five to eight individuals, as shown in Table III.

| Species                       | TE\textsubscript{c} (mmol C mol\textsuperscript{-1} H\textsubscript{2}O) | D\textsubscript{g} (kPa) | v\textsubscript{g} (kPa) | $\phi$\textsubscript{v} (=v\textsubscript{g}/D\textsubscript{g}) | $\phi$\textsubscript{w} (=E\textsubscript{n}/E\textsubscript{d}) | Instantaneous c\textsubscript{i}/c\textsubscript{a} | $\Delta^{13}$C\textsubscript{P}-based c\textsubscript{i}/c\textsubscript{a} |
|-------------------------------|-------------------------|-------------------------|-------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Gymnosperm tree species**    |                         |                         |                         |                             |                             |                             |                             |
| *Cupressus lusitanica*         | 1.29 (0.20)             | 0.76 (0.01)             | 0.90 (0.07)             | 1.17 (0.08)                 | 0.11 (0.02)                 | 0.78 (0.04)                 | 0.80 (0.02)                 |
| *Pinus caribaea*               | 1.20 (0.19)             | 0.76 (0.01)             | 0.72 (0.05)             | 0.95 (0.07)                 | 0.04 (0.02)                 | 0.86 (0.03)                 | 0.88 (0.02)                 |
| *Podocarpus guatemalensis*     | 3.48 (NA)               | 1.04 (NA)               | 1.71 (NA)               | 1.64 (NA)                  | 0.04 (NA)                  | 0.63 (NA)                  | 0.63 (NA)                  |
| *Thuja occidentalis*           | 1.85 (0.19)             | 0.76 (0.01)             | 0.92 (0.03)             | 1.21 (0.05)                | 0.03 (0.03)                | 0.80 (0.02)                | 0.78 (0.01)                |
| **Angiosperm tree species**    |                         |                         |                         |                             |                             |                             |                             |
| *Calophyllum longifolium*      | 1.83 (0.24)             | 1.05 (0.02)             | 1.53 (0.12)             | 1.46 (0.10)                | 0.04 (0.04)                | 0.76 (0.04)                | 0.78 (0.05)                |
| *Clusia pratensis*             | 2.16 (0.22)             | 1.02 (0.01)             | 1.42 (0.06)             | 1.39 (0.06)                | 0.01 (0.01)                | 0.76 (0.03)                | 0.73 (0.01)                |
| *Hyeronima alchorneoides*      | 1.50 (0.20)             | 0.83 (0.01)             | 1.37 (0.04)             | 1.65 (0.05)                | 0.08 (0.01)                | 0.80 (0.02)                | 0.85 (0.01)                |
| *Luehea seemannii*             | 0.98 (0.15)             | 1.20 (0.00)             | 1.43 (0.20)             | 1.19 (0.16)                | 0.05 (0.01)                | 0.80 (0.03)                | 0.81 (0.02)                |
| *Platyrisicum pinnatum*        | 2.76 (0.62)             | 1.12 (0.10)             | 1.53 (0.28)             | 1.39 (0.36)                | 0.02 (0.01)                | 0.73 (0.09)                | 0.71 (0.06)                |
| *Swietenia macrophylla*        | 1.07 (0.22)             | 1.05 (0.01)             | 1.21 (0.03)             | 1.15 (0.03)                | 0.07 (0.01)                | 0.86 (0.02)                | 0.88 (0.02)                |
| *Tabebuia rosea*               | 1.92 (0.24)             | 0.82 (0.00)             | 1.37 (0.18)             | 1.67 (0.21)                | 0.11 (0.02)                | 0.75 (0.06)                | 0.79 (0.03)                |
| *Tectona grandis*              | 0.84 (0.07)             | 0.98 (0.01)             | 1.20 (0.08)             | 1.23 (0.09)                | 0.08 (0.01)                | 0.88 (0.03)                | 0.81 (0.03)                |
| **Angiosperm liana species**   |                         |                         |                         |                             |                             |                             |                             |
| *Gouania lupuloides*           | 0.96 (0.34)             | 1.25 (0.01)             | 1.37 (0.08)             | 1.10 (0.06)                | 0.04 (0.01)                | 0.84 (0.03)                | 0.80 (0.02)                |
| *Mikania leiostachya*          | 0.89 (0.23)             | 1.11 (0.10)             | 1.39 (0.20)             | 1.25 (0.12)                | 0.07 (0.02)                | 0.79 (0.05)                | 0.80 (0.06)                |
| *Stigmaphyllon hypargyreum*    | 1.35 (0.14)             | 1.21 (0.01)             | 1.18 (0.06)             | 0.97 (0.05)                | 0.03 (0.01)                | 0.82 (0.03)                | 0.82 (0.02)                |
Table VI. The proportion of variation in transpiration efficiency explained by $^{13}$C discrimination and instantaneous $c_i/c_a$. Linear regression equations were fitted with the following parameters alternatively used as dependent variables: transpiration efficiency of C uptake ($TE_c$); the product of $TE_c$ and growth-weighted vapor pressure deficit ($D_g·TE_c$); and the product of $TE_c$ and growth-weighted leaf-to-air vapor pressure difference ($v_g·TE_c$). Independent variables were whole-plant $^{13}$C discrimination ($\Delta^{13}C$); $^{13}$C of leaves, stems, or roots individually; and instantaneous $c_i/c_a$. For each analysis, $n$=94. All regression coefficients were significant at $P<0.0001$.

| Dependent variable | Intercept | Whole-plant $\Delta^{13}C$ (‰) | Leaf $\Delta^{13}C$ (‰) | Stem $\Delta^{13}C$ (‰) | Root $\Delta^{13}C$ (‰) | Instantaneous $c_i/c_a$ | Model $R^2$ |
|--------------------|-----------|----------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|-----------|
| $TE_c$             | 7.76      | -0.298                           |                          |                          |                          |                          | 0.45      |
| $D_g·TE_c$         | 8.40      | -0.330                           |                          |                          |                          |                          | 0.49      |
| $v_g·TE_c$         | 14.4      | -0.594                           |                          |                          |                          |                          | 0.53      |
| $TE_c$             | 7.04      | -0.253                           |                          |                          |                          |                          | 0.38      |
| $D_g·TE_c$         | 8.03      | -0.300                           |                          |                          |                          |                          | 0.48      |
| $v_g·TE_c$         | 12.9      | -0.501                           |                          |                          |                          |                          | 0.45      |
| $TE_c$             | 6.87      | -0.262                           |                          |                          |                          |                          | 0.40      |
| $D_g·TE_c$         | 7.42      | -0.290                           |                          |                          |                          |                          | 0.45      |
| $v_g·TE_c$         | 12.8      | -0.528                           |                          |                          |                          |                          | 0.50      |
| $TE_c$             | 6.66      | -0.255                           |                          |                          |                          |                          | 0.36      |
| $D_g·TE_c$         | 6.53      | -0.251                           |                          |                          |                          |                          | 0.32      |
| $v_g·TE_c$         | 11.8      | -0.487                           |                          |                          |                          |                          | 0.40      |
| $TE_c$             | 7.30      | -7.27                            |                          |                          |                          |                          | 0.51      |
| $D_g·TE_c$         | 7.21      | -7.20                            |                          |                          |                          |                          | 0.46      |
| $v_g·TE_c$         | 13.6      | -14.6                            |                          |                          |                          |                          | 0.62      |
Table VII. The C and O isotope composition of experimental plants. Whole-plant δ\textsuperscript{13}C values were calculated by weighting the δ\textsuperscript{13}C for each tissue by the fraction of C in that tissue relative to the whole plant. The δ\textsuperscript{13}C of stems plus roots was calculated similarly to show the difference between the δ\textsuperscript{13}C of leaves and heterotrophic tissues. Values are given as the mean for each species, with the standard deviation in parentheses. No standard deviation is given for *P. guatemalensis*, because only one individual of this species survived. Sample sizes for the other species ranged from five to eight individuals, as shown in Table III.

| Species                        | Carbon-isotope ratio (δ\textsuperscript{13}C, ‰) | Oxygen-isotope ratio (δ\textsuperscript{18}O, ‰) |
|-------------------------------|-----------------------------------------------|-----------------------------------------------|
|                               | Leaves Stems Roots Whole plant Leaves – (stems + roots) | Stems                                          |
| Gymnosperm tree species       |                                               |                                               |
| *Cupressus lusitanica*        | -29.7 (0.3) -27.8 (0.4) -26.8 (1.0) -28.5 (0.5) -2.4 (0.5) | 20.1 (0.4)                                     |
| *Pinus caribaea*              | -30.7 (0.6) -30.0 (0.6) -29.4 (0.4) -30.2 (0.5) -0.9 (0.2) | 20.0 (0.3)                                     |
| *Podocarpus guatemalensis*    | -25.4 (NA) -23.3 (NA) -23.5 (NA) -24.5 (NA) -2.0 (NA) | 24.7 (NA)                                     |
| *Thuja occidentalis*          | -28.9 (0.2) -27.5 (0.3) -27.2 (0.6) -28.0 (0.3) -1.6 (0.5) | 20.7 (0.4)                                     |
| Angiosperm tree species       |                                               |                                               |
| *Calophyllum longifolium*     | -28.4 (1.3) -27.2 (0.8) -28.1 (1.2) -28.0 (1.1) -0.7 (0.5) | 23.8 (0.6)                                     |
| *Clusia pratensis*            | -27.1 (0.3) -26.4 (0.4) -26.9 (0.3) -26.9 (0.3) -0.4 (0.1) | 23.0 (0.3)                                     |
| *Hyeronima alchorneoides*     | -31.0 (0.1) -29.8 (0.3) -28.7 (0.4) -29.6 (0.3) -2.0 (0.3) | 20.6 (0.3)                                     |
| *Luehea seemannii*            | -29.3 (0.4) -28.1 (0.8) -28.2 (0.4) -28.6 (0.4) -1.1 (0.3) | 22.8 (0.2)                                     |
| *Platymiscium pinnatum*       | -27.3 (1.1) -25.7 (1.6) -25.4 (2.0) -26.3 (1.3) -1.7 (0.8) | 24.8 (1.3)                                     |
| *Swietenia macrophylla*       | -31.6 (0.4) -29.3 (0.3) -29.1 (0.4) -30.3 (0.4) -2.4 (0.1) | 23.9 (0.5)                                     |
| *Tabebuia rosea*              | -29.6 (0.8) -28.0 (0.6) -27.5 (0.8) -28.1 (0.7) -2.0 (0.5) | 23.2 (0.3)                                     |
| *Tectona grandis*             | -29.5 (0.5) -28.0 (0.5) -27.9 (0.6) -28.6 (0.6) -1.5 (0.2) | 22.9 (0.3)                                     |
| Angiosperm liana species      |                                               |                                               |
| *Gouania lupuloides*          | -29.2 (0.5) -28.0 (0.5) -28.3 (0.3) -28.4 (0.4) -1.1 (0.5) | 24.8 (0.4)                                     |
| *Mikania leiostachya*         | -29.3 (1.3) -28.0 (1.1) -27.0 (1.3) -28.3 (1.3) -1.7 (0.2) | 23.8 (1.0)                                     |
| *Stigmaphyllum hypargyreum*   | -29.5 (0.7) -29.2 (0.4) -28.7 (0.5) -28.9 (0.5) -0.7 (0.3) | 22.4 (1.1)                                     |
Figure 1. Variation among species in mean relative growth rate (A), net photosynthesis, expressed on a leaf area basis (B), and leaf area per unit plant C mass, 1/ρ (C). Error bars represent one standard error. Sample sizes for each species are given in Table III.
Figure 2. Mean relative growth rate plotted against instantaneous measurements of photosynthesis expressed on a leaf mass basis. Open symbols with internal cross hairs refer to gymnosperm tree species; completely open symbols refer to angiosperm liana species; closed symbols and closed symbols with internal cross hairs refer to angiosperm tree species.
Figure 3. Variation among species in the C concentration of dry matter on a whole-plant basis (A), the N concentration of dry matter on a whole-plant basis (B), and the C/N mass ratio of dry matter on a whole-plant basis (C). Error bars represent one standard error. Sample sizes for each species are given in Table III.
Figure 4. Variation among species in whole-plant N use efficiency (A), photosynthetic N use efficiency (B), and $n_l$, the leaf N content as a proportion of whole-plant N content (C). Error bars represent one standard error. Sample sizes for each species are given in Table III.
Figure 5. Whole-plant N use efficiency plotted against photosynthetic N use efficiency. Whole-plant N use efficiency was calculated from mean relative growth rate, measured over several months, whereas photosynthetic N use efficiency was calculated from instantaneous photosynthesis measurements, taken over several minutes. Different symbols refer to different species, as detailed in Figure 2.
Figure 6. The top two panels show instantaneous measurements of $c_i/c_a$ plotted against stomatal resistance (A) and photosynthesis (B); the bottom two panels show whole-plant $^{13}$C discrimination plotted against stomatal resistance (C) and photosynthesis (D). Stomatal resistance is the inverse of stomatal conductance. Different symbols refer to different species, as described for Figure 2.
Figure 7. Whole-plant $^{13}$C discrimination plotted against the ratio of intercellular to ambient CO$_2$ partial pressures determined from instantaneous gas exchange measurements. Different symbols refer to different species, as defined in Figure 2.
**Figure 8.** The product of transpiration efficiency of C gain \((TE_c)\) and daytime vapor-pressure deficit of ambient air \((D_g)\) plotted against whole-plant \(^{13}\text{C}\) discrimination. Different symbols refer to different species, as defined in Figure 2. Daytime air vapor-pressure deficit was weighted according to the predicted weekly growth increment for each individual plant.
Figure 9. The $^{18}$O enrichment of stem dry matter relative to irrigation water plotted against the predicted $^{18}$O enrichment of water at the evaporative sites in leaves. The predicted $\Delta^{18}$O$_e$ was weighted according to the predicted weekly growth increment for each individual plant. Different symbols refer to different species, as defined in Figure 2.