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

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Summary

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

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

• Significant variation was observed in TE_C among the C_3 tree species. Values ranged from 1.6 mmol C mol^{-1} H_2O for teak (*Tectona grandis*) to 4.0 mmol C mol^{-1} H_2O for a legume, *Platymiscium pinnatum*.

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

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

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Introduction

The term water-use efficiency describes a plant's photosynthetic production rate relative to the rate at which it transpires water to the atmosphere. It is a measure of plant performance that has long been of interest to agronomists, foresters and ecologists (Bacon, 2004). In cropping systems, improving water-use efficiency presents a means of increasing crop production in the face of finite water supplies (Richards *et al.*, 2002). In forestry systems, water-use efficiency is a critical link between wood production and water management. In global-change research, water-use efficiency links the carbon and water cycles of terrestrial vegetation, and is expected to increase in a future, high-CO_2 world (Guehl *et al.*, 1994; Farquhar, 1997; Winter *et al.*, 2001a).

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

the C_3 group, which comprises the overwhelming majority of tropical tree species.

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

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

Theory

Four definitions of water-use efficiency are applied in this paper: photosynthetic water-use efficiency (PWUE), transpiration efficiency of C gain (TE_C), transpiration efficiency of N acquisition (TE_N), and transpiration ratio (TR). The PWUE describes the relative rates of exchange of CO_2 and water vapour between photosynthesizing leaves and the surrounding atmosphere (mmol CO_2 mol^{-1} H_2O). The TE_C describes water-use efficiency at the whole-plant level as the rate of C accumulation in dry matter relative to cumulative water loss (mmol C mol^{-1} H_2O). Determinations of TE_C usually integrate over several weeks to months, and are based on gravimetric measurements of dry matter accumulation and plant water loss, in combination with elemental analyses of the C mass fraction of plant dry matter. The TE_N is determined in the same way, but using plant N content rather than plant C content. The TR is a measurement of water-use efficiency that has been employed since early research on the topic (Briggs & Shantz, 1914; Shantz & Piemeisel, 1927), and expresses the cumulative amount of water transpired by a plant during the production of a given amount of dry matter. Mathematically, it is the reciprocal of TE_C, but taking dry matter accumulation as the measure of plant production, rather than C accumulation. Typical units for TR are g H_2O g^{-1} DM.

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

$$\begin{aligned} \text{PWUE} = A/E &= [g_c(p_a - p_i)]/[g_w(w_i - w_a)] \\ &= (p_a - p_i)/1.6v \end{aligned} \qquad \text{Eqn 1}$$

where A is net photosynthesis (μmol CO_2 m^{-2} s^{-1}); E is transpiration (mmol H_2O m^{-2} s^{-1}); g_c and g_w are stomatal conductances to CO_2 and water vapour, respectively (mol m^{-2} s^{-1}); p_a and p_i are atmospheric and intercellular CO_2 partial pressures (μbar); w_a and w_i are atmospheric and intercellular water vapour pressures (mbar), 1.6 is the ratio of diffusivities for water vapour and CO_2 in air, and v is the leaf to air vapour pressure difference, equal to $(w_i - w_a)$.

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

$$\begin{aligned} TE_C &= [(1 - \phi_c)(p_a - p_i)]/[1.6v(1 + \phi_w)] \\ &= [p_a(1 - \phi_c)(1 - p_i/p_a)]/[1.6v(1 + \phi_w)] \end{aligned} \qquad \text{Eqn 2}$$

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

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

$$\Delta = a - d + (b - a)p_i/p_a \qquad \text{Eqn 3}$$

where a is the $^{13}C/^{12}C$ fractionation caused by gaseous diffusion through stomata (4.4‰), and b is the effective fractionation caused by the major carboxylating enzymes in C_3 plants (approx. 27‰). The term d summarizes collectively the fractionations caused by dissolution of CO_2 and liquid-phase diffusion, photorespiration and 'dark' respiration (Farquhar *et al.*, 1989a). The effects on the overall Δ of fractionations associated with these processes are thought to be small, but significant (Brugnoli & Farquhar, 2000; Ghashghaie *et al.*, 2003). Δ is defined with respect to atmospheric CO_2 as $\Delta = R_a/R_p - 1$, where R_a is $^{13}C/^{12}C$ of atmospheric CO_2 and R_p is $^{13}C/^{12}C$ of plant material (Farquhar & Richards, 1984).

Equations 2 and 3 suggest that both TE_C and Δ share a mutual dependence on p_i/p_a. Combining these two equations yields:

$$TE_C = [p_a(1-\phi_c)(b-d-\Delta)]/[1.6v(1+\phi_w)(b-a)] \quad \text{Eqn 4}$$

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

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

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

where 3/2 is the molar weight ratio of water vapour and C, and m_C is the mass fraction of C in plant dry matter. Note that TR would then be multiplied by the scaling factor 1000 to give units of g H_2O g^{-1} DM.

Materials and Methods

Plant growth and water use

The experiment took place at the Smithsonian Tropical Research Institute's Santa Cruz Experimental Field Facility in Gamboa, Republic of Panama. The species grown for the experiment and their taxonomic families are given in Table 1. Most are tree species used in reforestation projects in Panama. The experiment started at the end of April 2004 and lasted until mid-September 2004. Seedlings were grown either from seed collected in the Panama Canal watershed, or from seedlings obtained from the native species reforestation project (PRORENA) based at the Smithsonian Tropical Research Institute. Initial seedling dry weights ranged from 0.1 to 0.6 g. Seedlings were grown in 38-l plastic containers (Rubbermaid Round Brute, Consolidated, Twinsburg, OH, USA). The pots had a height of 44 cm, upper diameter of 36.5 cm, and lower diameter of 32 cm. They contained 1.8 kg charcoal at the base to improve drainage. Each pot contained 35 kg soil, which comprised a mixture of 60% (v/v) dark, air-dried topsoil and 40% leaf litter. All pots were filled with the same topsoil/leaf litter mixture, carefully homogenized to ensure a uniform rooting environment for all plants. Pots were not fertilized during the experiment. The soil surface was covered with 2 kg gravel to minimize evaporation. The water content of the soil was brought to field capacity by adding 12 kg of water to each pot. The side walls of the pots were covered with reflective insulation to prevent excessive heating in direct sunlight. The pots were situated under a transparent rain shelter with a glass roof, which typically reduced the incoming photon flux density by approx. 20%. In addition to the pots containing plants, eight control pots without plants were also deployed; these were used to estimate evaporation from the soil surface independently of plant transpiration.

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

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

Species	Family	Start date	End date	No. plants	Final DW (g)	Final/initial DW	Mean VPD (kPa)
Dalbergia retusa Hemsl.	Fabaceae	26 Apr 2004	4 Aug 2004	7	63.8 (8.1)	709	0.62
Ficus insipida Willd.	Moraceae	26 Apr 2004	20 Aug 2004	6	43.6 (2.5)	436	0.59
Pachira quinata (Jacq.) W.S. Alverson	Bombacaceae	26 Apr 2004	5 Jul 2004	8	53.1 (3.2)	295	0.64
Platymiscium pinnatum (Jacq.) Dugand	Fabaceae	26 Apr 2004	9 Aug 2004	8	87.2 (12.8)	224	0.63
Pseudobombax septenatum (Jacq.) Dugand	Bombacaceae	26 Apr 2004	27 Aug 2004	7	68.4 (9.6)	684	0.59
Swietenia macrophylla King	Meliaceae	10 May 2004	10 Sep 2004	6	71.5 (6.8)	477	0.57
Tectona grandis Linn. f.	Verbenaceae	17 May 2004	15 Sep 2004	4	71.3 (12.9)	713	0.60
Saccharum spontaneum L.	Poaceae	26 Apr 2004	14 Jul 2004	7	47.0 (5.8)	86	0.62

All are C_3 tree species with the exception of *S. spontaneum*, a C_4 grass. Values for final dry weight and final/initial dry weight are means; values in parentheses are 1 SE. Mean VPD for each species is the average midday vapour pressure deficit over the 45 d preceding plant harvest, when the majority of plant growth took place.

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

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

Values for September are averages for 1–15 September (which was the final day of the experiment).

determined with a LI-3100 leaf-area meter (Li-Cor, Lincoln, NE, USA). Leaf-area data were not taken for *Saccharum spontaneum*. This species is therefore excluded from analyses involving leaf area.

Meteorological conditions

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

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

Figure 1 shows the measured variation in free water evaporation over the course of the experiment. Although there were slight differences in the monthly averages of this parameter (Table 2), it is clear from Fig. 1 that there was no marked trend during the experiment, such as would occur during the transition from wet season to dry season, for example. Nonetheless, because the different species in the experiment were harvested at different times, it is possible that they were exposed to slightly different evaporative conditions. The final column in Table 2 shows the average midday VPD during the 45 d preceding plant harvest for each species. This is the period during which the majority of plant growth took place. The maximal proportional difference in average midday VPD between species was of the order of 10%.

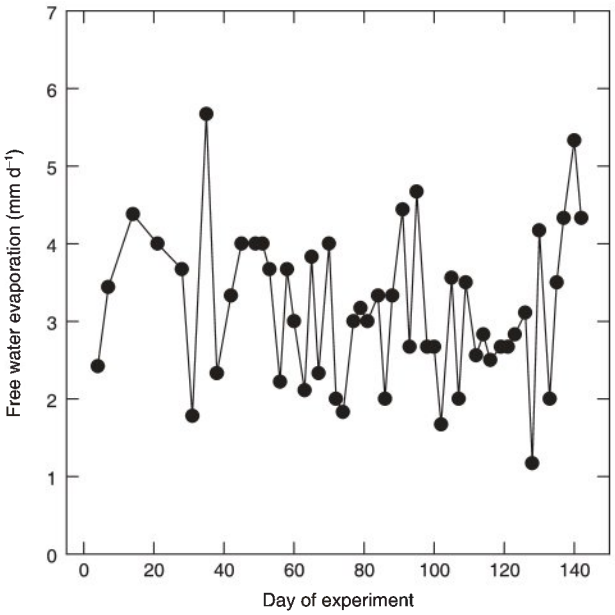

Fig. 1 Variation in mean daily free water evaporation over the course of the experiment. Values are average measurements made with three Etgage evaporimeters located under the rain shelter alongside the experimental plants. Day 1 of the experiment was 26 April 2004; it concluded on 15 September 2004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

Elemental and isotopic analyses

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

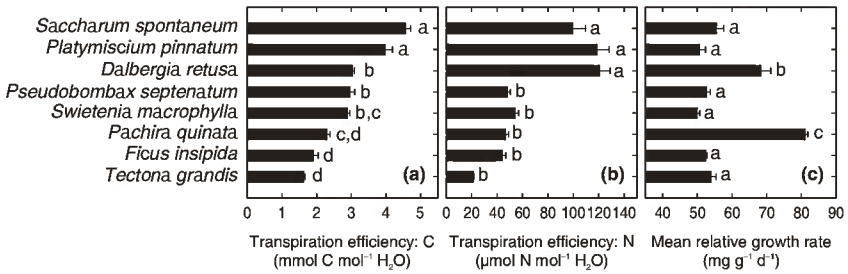

Fig. 2 (a) Transpiration efficiency of carbon gain; (b) transpiration efficiency of nitrogen acquisition; (c) mean relative growth rate for the eight species grown for the experiment. *Saccharum spontaneum* is a C_4 grass; the other species are C_3 trees. *Platymiscium pinnatum* and *Dalbergia retusa* support symbiotic N fixation in root nodules; the other species do not. Mean relative growth rate is expressed on a dry mass basis. Bars followed by different letters are significantly different at $P < 0.05$. Error bars, ±1 SE.

plant dry matter were measured in samples of approx. 10 mg in an elemental analyser (CE Instruments). Isotopic analyses were performed at the Idaho Stable Isotopes Laboratory at the University of Idaho, Moscow, ID, USA. Elemental analyses were performed at the Smithsonian Tropical Research Institute, Republic of Panama. Whole-plant isotopic compositions were calculated by mass balance using the dry mass of each plant organ (leaves, stems and roots) and its elemental and isotopic composition. Carbon-isotope discrimination (Δ) was calculated from measured $\delta^{13}C$ values according to the formula $\Delta = (\delta_a - \delta_p)/(1 + \delta_p)$, where δ_a is the $\delta^{13}C$ of CO_2 in air and δ_p is the $\delta^{13}C$ of plant dry matter. The δ_a was assumed to be −8‰, in accordance with observed daytime δ_a in the vicinity of Panama City (Winter & Holtum, 2002). The isotopic composition of the C_4 grass species *S. spontaneum*, grown alongside the C_3 trees, supported this assumption.

Statistical analyses

Variation among species in water-use efficiency, isotopic composition and elemental composition was assessed by ANOVA. When significant variation was detected, differences among individual species were assessed using Tukey's method for pairwise comparisons. Variation in isotopic and elemental composition among leaves, stems and roots was analysed by paired *t*-tests. A Bonferroni correction was applied to the significance tests to account for the fact that more than one paired *t*-test was made for each dependent variable. Least-squares linear regression was used to analyse relationships between TE_C and potential explanatory variables such as leaf N concentration and isotopic composition. Analysis of covariance (ANCOVA) was used to assess the relationship between TE_C and Δ. Species was taken as the independent variable and Δ as the covariate to test whether the relationship between TE_C and Δ differed among species. All statistical analyses were performed in SYSTAT ver. 9.0 (SPSS, Chicago, IL, USA).

Results

Transpiration efficiency and growth

We observed significant variation among species in TE_C (Fig. 2a). As expected, the species with the highest TE_C was the C_4 grass *S. spontaneum*. Surprisingly, however, mean TE_C for the C_3 tree *Platymiscium pinnatum* was not significantly different from that of *S. spontaneum*. Among the seven C_3 tree species, there was large variation in mean values for TE_C. Species means ranged from 4.0 mmol C mol^{-1} H_2O for *P. pinnatum* to 1.6 mmol C mol^{-1} H_2O for *Tectona grandis*.

We also observed significant variation among species in TE_N (Fig. 2b). The amount of N taken up for a given amount of water transpired to the atmosphere was significantly higher in *S. spontaneum* and in the two leguminous trees, *P. pinnatum* and *Dalbergia retusa*, than in the other five tree species. Among the other five, *T. grandis* had the lowest TE_N. There was a nearly sixfold variation in TE_N among species, with *T. grandis* having a mean value of 21 μmol N mol^{-1} H_2O, and *D. retusa* having a mean value of 121 μmol N mol^{-1} H_2O.

Mean relative growth rate (RGR) varied significantly among species (Fig. 2c). This was largely caused by two species, *D. retusa* and *Pachira quinata*, which had higher RGR than the other six. Of these two, the RGR of *P. quinata* was significantly higher than that of *D. retusa*. Across the C_3 tree species, variation in RGR was significantly correlated with variation in specific leaf area, SLA (m^2 kg^{-1}). The relationship between the two was $RGR = 1.98SLA + 23.1$ ($R^2 = 0.37$, $P < 0.0001$, $n = 46$). The RGR was also correlated with leaf area ratio, LAR (m^2 kg^{-1}), according to the relationship $RGR = 4.23LAR + 31.4$ ($R^2 = 0.27$, $P = 0.0001$, $n = 46$). There was no significant correlation between RGR and TE_C ($P = 0.09$, $n = 53$), or RGR and TE_N ($P = 0.91$, $n = 53$).

Carbon and nitrogen concentrations

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

Whole-plant N concentration also varied among species (Table 3). Values ranged from 2.1% for the legume *D. retusa* to 0.7% for *T. grandis*. Values for leaves ranged from 3.3% for *D. retusa* to 1.0% for *T. grandis*. Across all species, leaves had

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

Species	Carbon concentration (%)				Nitrogen concentration (%)			
	Leaves	Stems	Roots	Whole plant	Leaves	Stems	Roots	Whole plant
Dalbergia retusa	47.6 (0.7)	43.1 (0.2)	44.8 (0.4)	45.2 (0.3) a	3.28 (0.53)	1.29 (0.29)	1.38 (0.30)	2.10 (0.40) a
Ficus insipida	39.7 (0.2)	40.5 (0.3)	43.2 (0.5)	41.0 (0.1) d	1.73 (0.09)	0.64 (0.08)	0.95 (0.05)	1.11 (0.06) c
Pachira quinata	44.1 (0.3)	41.1 (0.5)	42.4 (0.5)	42.6 (0.1) b	1.79 (0.19)	0.59 (0.06)	0.61 (0.07)	1.01 (0.09) c
Platymiscium pinnatum	46.5 (0.7)	43.9 (0.5)	43.9 (0.5)	44.8 (0.3) a	2.69 (0.61)	0.98 (0.35)	1.05 (0.18)	1.57 (0.35) b
Pseudobombax septenatum	43.3 (0.8)	41.6 (0.6)	41.1 (0.4)	42.0 (0.5) b,d	1.41 (0.13)	0.51 (0.06)	0.53 (0.05)	0.80 (0.08) c
Swietenia macrophylla	45.1 (0.4)	43.2 (0.4)	44.2 (0.4)	44.1 (0.2) a,c	1.53 (0.07)	0.46 (0.16)	0.93 (0.11)	0.97 (0.14) c
Tectona grandis	43.6 (0.3)	41.6 (0.4)	42.8 (0.4)	42.9 (0.2) b,c	1.01 (0.06)	0.32 (0.09)	0.44 (0.05)	0.65 (0.02) c
Saccharum spontaneum	41.4 (2.1)	na	40.1 (1.8)	41.1 (1.5) d	1.17 (0.31)	na	0.68 (0.10)	1.05 (0.26) c

Values are means (SD). Whole-plant values were calculated by mass balance in conjunction with the dry weight of each plant organ. Values within a column followed by different letters are statistically different at $P < 0.05$.

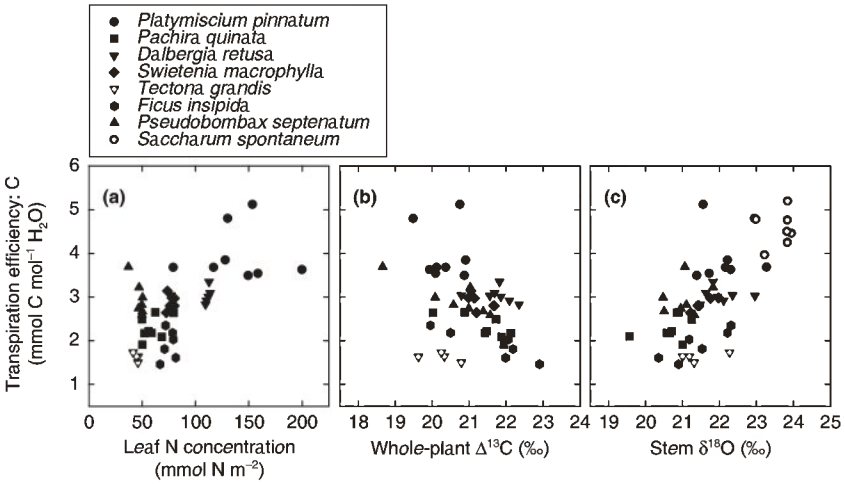

Fig. 3 Transpiration efficiency of carbon gain plotted against (a) leaf nitrogen concentration per unit leaf area; (b) whole-plant carbon-isotope discrimination; (c) stem $\delta^{18}O$. The Δ of *Saccharum spontaneum* is not plotted in (b) because of the fundamental difference in Δ between C_3 and C_4 plants; this species is not shown in (a) because its leaf area was not measured; in (c) the $\delta^{18}O$ of roots is shown for this species because it does not have a morphologically distinct stem.

higher N concentrations than either stems ($P < 0.0001$, $n = 46$) or roots ($P < 0.0001$, $n = 53$). Mean values for leaves, stems and roots were 1.9, 0.7 and 0.8%, respectively.

The TE_C was linearly related to leaf N concentration expressed on a leaf-area basis (Fig. 3a). The equation relating the two parameters was $TE_C = 0.014N_A + 1.62$ ($R^2 = 0.40$, $P < 0.0001$, $n = 46$), where TE_C is in mmol C mol^{-1} H_2O and leaf N per unit leaf area (N_A) is in mmol N m^{-2}. The N_A, in turn, was linearly related to TE_N (Fig. 4a). The equation relating the two was $N_A = 0.80TE_N + 26.0$ ($R^2 = 0.73$, $P < 0.0001$, $n = 46$), where TE_N is in μmol N mol^{-1} H_2O. Similarly, TE_C was positively correlated with TE_N (Fig. 4b). The TE_C appeared to be a saturating function of TE_N. The relationship could be described by the nonlinear equation $TE_C = 4.24(1 - \exp(-0.02TE_N))$ ($R^2 = 0.61$, $n = 53$).

Stable isotope compositions

There was variation among species in whole-plant C-isotope discrimination, Δ (Table 4). Mean values for the C_3 tree species ranged from 20.3‰ for *T. grandis* and *P. pinnatum* to 21.7‰ for *D. retusa*. The mean value for *S. spontaneum*, was 3.0‰, reflecting the large variation in Δ between the C_3 and C_4 photosynthetic pathways. Across all C_3 species, leaf Δ was larger than that of both stems ($P < 0.0001$, $n = 46$) and roots ($P < 0.0001$, $n = 46$); mean values were 21.9, 20.5 and 20.6‰ for leaves, stems and roots, respectively. We calculated the difference between leaf Δ and that for heterotrophic tissues (stems plus roots) for each species (Table 4). This difference varied significantly among species, with values for the C_3 trees ranging from 2.6‰ for *Swietenia macrophylla* to 0.2‰ for *F. insipida*. The value for *S. spontaneum*, −1.2‰, differed significantly from those of all C_3 species.

We show the relationship between TE_C and whole-plant Δ for the C_3 tree species in Fig. 3b. The TE_C was significantly related to Δ when analysed across all species, although Δ explained a relatively small amount of variation in TE_C. The regression equation relating the two parameters was $TE_C = -0.35\Delta + 10.2$ ($R^2 = 0.13$, $P = 0.008$, $n = 46$). To analyse further the relationship between TE_C and Δ, we performed an ANCOVA, taking species as independent variable and Δ as covariate. The analysis indicated that the interaction term

Table 4 Carbon, nitrogen and oxygen isotope composition of dry matter for plant organs and whole plants of each species

Species	C-isotope discrimination (Δ, ‰)					N-isotope ratio (δ^{15}N, ‰)				O-isotope ratio (δ^{18}O, ‰)	
	Leaves	Stems	Roots	Whole plant	Leaves – (stems + roots)	Leaves	Stems	Roots	Whole plant	Leaves	Stems
Dalbergia retusa	22.4 (0.6)	21.3 (0.5)	21.3 (0.4)	21.7 (0.5)a	1.1 (0.4)b,d	0.4 (0.7)	0.5 (0.6)	3.2 (0.7)	0.8 (0.6)a	22.7 (0.8)	22.0 (0.5)b,c
Ficus insipida	21.7 (1.2)	21.3 (1.3)	21.8 (0.9)	21.6 (1.1)a,c	0.2 (0.3)c	0.7 (0.4)	1.8 (0.5)	3.0 (0.2)	1.5 (0.3)a,c,d	23.2 (0.7)	21.4 (0.8)a,c
Pachira quinata	22.0 (0.6)	21.1 (0.8)	21.1 (0.7)	21.4 (0.7)a,c	0.9 (0.4)c,d	2.9 (0.4)	2.2 (0.4)	3.3 (0.4)	2.8 (0.2)b,d	24.7 (0.6)	20.7 (0.5)a
Platymiscium pinnatum	21.5 (0.4)	19.7 (0.6)	19.7 (0.6)	20.3 (0.5)b	1.8 (0.6)a,b	3.0 (1.4)	0.4 (1.9)	0.4 (1.3)	1.9 (1.4)a,b	24.1 (1.0)	22.2 (0.7)b,c
Pseudobombax septenatum	21.8 (0.7)	20.0 (1.1)	20.2 (1.3)	20.6 (1.0)a,b	1.7 (0.7)b	1.3 (0.7)	1.2 (0.4)	1.7 (0.3)	1.3 (0.5)a,c	21.1 (0.4)	21.0 (0.5)a
Swietenia macrophylla	22.8 (0.3)	20.2 (0.5)	20.4 (0.3)	21.3 (0.3)a,b	2.6 (0.5)a	3.7 (1.3)	1.3 (0.6)	2.7 (0.4)	3.0 (0.9)b	20.1 (0.8)	21.5 (0.3)a,c
Tectona grandis	21.1 (0.5)	19.6 (0.4)	19.7 (0.5)	20.3 (0.5)b,c	1.4 (0.2)b,d	0.7 (0.3)	0.5 (0.9)	1.0 (0.8)	0.7 (0.4)a	21.9 (0.6)	21.4 (0.6)a,c
Saccharum spontaneum	2.6 (0.5)	N/A	3.8 (0.6)	3.0 (0.5)d	−1.2 (0.6)e	2.4 (0.9)	N/A	3.1 (0.7)	2.5 (0.9)b,c	24.9 (0.3)	23.6 (0.4)d

Values are means (SD). Whole-plant values were calculated by mass balance using the dry mass and mass fraction of the element of interest in each plant organ. C-isotope composition was expressed as discrimination by assuming a value for atmospheric CO_2 of −8‰. δ^{15}N values are referenced to air as a standard; those for δ^{18}O to Vienna Standard Mean Ocean Water. For C-isotope discrimination, the difference between leaves and heterotrophic plant tissues (sum of stems and roots) is also given. For δ^{18}O of *Saccharum spontaneum*, roots were analysed in place of stems as this grass species does not have a morphologically distinct stem. Values within a column followed by different letters are statistically different at $P < 0.05$.

Fig. 4 (a) Leaf nitrogen concentration; (b) transpiration efficiency of carbon gain plotted against the transpiration efficiency of N acquisition. *Saccharum spontaneum* is not plotted in (a) because its leaf area was not measured.

between species and Δ was not significant ($P = 0.98$, $n = 46$), suggesting that the slopes of the relationships between TE_C and Δ did not differ among species. The next step of the analysis indicated that there was significant variation in TE_C associated with both species ($P < 0.0001$) and with Δ ($P = 0.0004$), suggesting that TE_C varied significantly with Δ, and that there was variation among species in the intercept of the relationship between TE_C and Δ. Overall, the model accounted for 88% of variation in TE_C. As further evidence of uncoupling between TE_C and Δ at the species level, a linear regression of species means for the two parameters was not significant ($P = 0.71$, $n = 7$).

Whole-plant $\delta^{15}N$ varied significantly among species (Table 4). Values ranged from 0.8‰ for the legume species *D. retusa* to 3.0‰ for *S. macrophylla*. The other legume species, *P. pinnatum*, had an intermediate value of 1.9‰. Differences between plant tissues appeared to vary depending on species. For example, in *D. retusa*, roots were heavier by approx. 2.7‰ compared with stems or leaves, whereas in *P. pinnatum* leaves were heavier by 2.6‰ compared with roots or stems. A visual examination of the root systems of the two legume species showed *D. retusa* to be well nodulated, whereas *P. pinnatum* had only a few conspicuous nodules.

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

We performed regression analyses to determine whether the patterns observed for species-level variation in TE_C, Δ and $\delta^{18}O$ resulted from small differences in environmental conditions experienced by each species, caused by variation in harvest dates (Table 1). The mean TE_C for the C_3 tree species showed no correlation with mean midday VPD. This was true regardless of whether mean midday VPD was calculated over the 45 d preceding plant harvest ($P = 0.61$, $n = 7$), or over the entire experimental period for each species ($P = 0.65$, $n = 7$). Similarly, mean Δ for the C_3 tree species showed no correlation with mean midday VPD, whether calculated over the 45 d preceding plant harvest ($P = 0.97$, $n = 7$) or over the entire experimental period ($P = 0.38$, $n = 7$). Mean species $\delta^{18}O$ showed no correlation with mean midday relative humidity calculated over the 45 d preceding plant harvest ($P = 0.77$, $n = 8$), and no correlation with that calculated over the entire experimental period for each species ($P = 0.73$, $n = 8$). Relative humidity is the environmental parameter most likely to cause variation in $\delta^{18}O$ in this context. We conclude that the species-level trends that we observed in TE_C, Δ and $\delta^{18}O$ were not caused by variation in the environmental conditions experienced by each species during growth.

Discussion

We observed large variation in TE_C under well watered conditions among a suite of tropical tree species employing the C_3 photosynthetic pathway. This variation in TE_C among species could not be accounted for by considering subtle differences in environmental conditions experienced during growth by each species caused by different harvest dates. The range of values was striking, with TE_C of *P. pinnatum* approaching that achieved by a concurrently grown C_4 grass (Fig. 2a). In contrast, the TE_C of *T. grandis* was about one-third that of the C_4 grass. We did not observe a correlation between RGR and TE_C, suggesting that high water-use efficiency need not come at the expense of slow growth. Across species, TE_C was positively correlated with N_A (Fig. 3a), negatively correlated with whole-plant Δ (Fig. 3b), and positively correlated with stem dry matter $\delta^{18}O$ (Fig. 3c). In the following discussion, we first relate our results to observations elsewhere in the literature of whole-plant water-use efficiency for C_3 trees; we then rely on the above-stated correlations to draw inferences about the physiological mechanisms driving species-level variation in TE_C.

The variations in whole-plant water-use efficiency that we observed are consistent with previous results that are available for some of the same species. Winter *et al.* (2005) reported mean values for TR of *S. macrophylla* and *T. grandis* of 256 and 373 g H_2O g^{-1} DM, respectively. If we convert data from the current study to TR to match their data, we obtain mean values of 230 and 396 g H_2O g^{-1} DM, respectively, in close agreement with the previous results. For the C_4 grass *Zea mays*, Winter *et al.* (2005) reported a mean TR of 132 g H_2O g^{-1} DM, whereas we observed a mean value for *S. spontaneum*, also a C_4 grass, of 136 g H_2O g^{-1} DM. For the C_3 tree *F. insipida*, Winter *et al.* (2001a) observed TR ranging from 229 to 309 g H_2O g^{-1} DM for plants grown under conditions comparable with those of the current study (ambient CO_2, unfertilized soil, no open-top chamber). In comparison, we observed a mean value of 332 g H_2O g^{-1} DM for this species. Thus both the relative ranking and absolute values for whole-plant water-use efficiency observed in this study agree well with previous results.

There are few other reports of variation among C_3 tree species in whole-plant water-use efficiency. Variation was observed between *Quercus robur* and *Pinus pinaster*, but only at low nutrient availability (Guehl *et al.*, 1995). *Quercus petraea* was observed to consistently have a higher TE_C than *P. pinaster*, even under varying atmospheric CO_2 concentration and soil moisture (Guehl *et al.*, 1994). Data presented by Pate & Dawson (1999) suggest variation in TR between mallee eucalypts (species names not provided) and *Eucalyptus globulus*.

Examination of equation 2 suggests that variation in TE_C, for plants grown in a common environment, can occur during leaf-level gas exchange, because of variation in p_i/p_a and/or v. Other potential sources of variation are ϕ_c, the proportion of C fixed during photosynthesis that is subsequently lost by respiration, and ϕ_w, the proportion of water lost from the plant that is not associated with photosynthesis. Variation in p_i/p_a is often identified as a primary source of variation in TE_C (Farquhar & Richards, 1984; Farquhar *et al.*, 1989b; Hubick & Farquhar, 1989). In some studies with trees, ϕ_c has also been suggested to be an important determinant (Guehl *et al.*, 1994; Osório *et al.*, 1998; Matzner *et al.*, 2001); and in one case ϕ_w was suggested to have influenced TE_C significantly (Hobbie & Colpaert, 2004). While we did not quantify these processes in our experiment, the measurements of elemental and isotopic composition allow us to draw some inferences about the processes responsible for the variation that we observed in TE_C.

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

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

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

$$TE_C = -\Delta[p_a(1-\phi_c)]/[1.6v(1+\phi_w)(b-a)] + [p_a(1-\phi_c)(b-d)]/[1.6v(1+\phi_w)(b-a)] \quad \text{Eqn 6}$$

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

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

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

We observed variation among species in dry matter $\delta^{15}N$ (Table 4). The range of species means that we observed for whole-plant $\delta^{15}N$ (0.7 to 3.0‰) was slightly less than the

corresponding range of values for leaf $\delta^{15}N$ (0.4 to 3.7‰). This range, in turn, is similar to the range of leaf $\delta^{15}N$ observed among 21 species in an Amazonian rainforest in French Guyana (−0.3 to 3.5‰; Guehl *et al.*, 1998), and to that observed for 32 species in lowland tropical forest in Panama (−1 to 5‰ with 88% of values between 0 and 4‰; Santiago *et al.*, 2004). Given the homogeneity of the plant culture conditions in our experiment, this amount of variation is surprising. One of the legume species in our study, *D. retusa*, had clearly visible, abundant bacterial nodules on its roots, whereas the other legume species, *P. pinnatum*, had far fewer visible nodules. *Dalbergia retusa* had leaf $\delta^{15}N$ nearest to 0‰ among all of the species, whereas *P. pinnatum* had the second highest leaf $\delta^{15}N$ at 3.0‰. Based on these data, one might suggest that *D. retusa* obtained a much greater proportion of biologically fixed, atmospheric N than *P. pinnatum*. However, the two species displayed similarly high values for the transpiration efficiency of N acquisition (Fig. 2b), which set them clearly apart from the other C_3 tree species. The $\delta^{15}N$ data are difficult to reconcile with this observation. Moreover, $\delta^{15}N$ values for the nonleguminous species covered essentially the same range as those for the legumes. As in the study in French Guyana (Guehl *et al.*, 1998), we conclude that dry matter $\delta^{15}N$ did not provide a straightforward indication of biological N fixation in our experiment.

We observed that mean RGR correlated with both SLA and LAR among the C_3 tree species. This result is consistent with observations among herbaceous plant species (Poorter & Remkes, 1990) and observations of two tropical tree species when exposed to variable environmental conditions (Winter *et al.*, 2000; Winter *et al.*, 2001b). The lack of correlation that we observed between RGR and TE_C is also similar to previous results comparing RGR and PWUE (Poorter & Farquhar, 1994).

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

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

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