Journal of Experimental Botany, Vol. 58, No. 13, pp. 3549–3566, 2007
doi:10.1093/jxb/erm201
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Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Transpiration efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility

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Received 6 June 2007; Revised 30 July 2007; Accepted 3 August 2007

Abstract

The response of whole-plant water-use efficiency, termed transpiration efficiency (*TE*), to variation in soil fertility was assessed in a tropical pioneer tree, *Ficus insipida* Willd. Measurements of stable isotope ratios ($\delta^{13}C$, $\delta^{18}O$, $\delta^{15}N$), elemental concentrations (C, N, P), plant growth, instantaneous leaf gas exchange, and whole-plant water use were used to analyse the mechanisms controlling *TE*. Plants were grown individually in 19 l pots with non-limiting soil moisture. Soil fertility was altered by mixing soil with varying proportions of rice husks, and applying a slow release fertilizer. A large variation was observed in leaf photosynthetic rate, mean relative growth rate (*RGR*), and *TE* in response to experimental treatments; these traits were well correlated with variation in leaf N concentration. Variation in *TE* showed a strong dependence on the ratio of intercellular to ambient CO_2 mole fractions (c_i/c_a); both for instantaneous measurements of c_i/c_a (R^2=0.69, P <0.0001, n=30), and integrated estimates based on C isotope discrimination (R^2=0.88, P <0.0001, n=30). On the other hand, variations in the leaf-to-air humidity gradient, unproductive water loss, and respiratory C use probably played only minor roles in modulating *TE* in the face of variable soil fertility. The pronounced variation in *TE* resulted from a combination of the strong response of c_i/c_a to leaf N, and inherently high values of c_i/c_a for this tropical tree species; these two factors conspired to cause a 4-fold variation among treatments in ($1-c_i/c_a$), the term that actually modifies *TE*. Results suggest that variation in plant N status could have important implications for the coupling between C and water exchange in tropical forest trees.

Key words: Carbon isotope, oxygen isotope, soil fertility, transpiration efficiency, tropical tree.

Introduction

Water-use efficiency at the whole-plant level, often referred to as transpiration efficiency (*TE*), is defined as the rate of biomass production of a plant relative to the rate of transpiration (Bacon, 2004). Although ecophysiologists frequently assess water-use efficiency at the leaf level, relatively few measurements of *TE* have been reported, largely due to the logistical challenges involved in obtaining such data. Nonetheless, the whole plant is clearly a meaningful organizational level at which to analyse controls over growth, CO_2 exchange, and water use (McCree, 1986; Meinzer and Goldstein, 1996). The *TE* effectively describes the coupling between whole-plant C and water exchange in terrestrial vegetation. Thus, a mechanistic understanding of the controls over *TE* is relevant to studies of plant competition, ecosystem function, and plant responses to climate change. In tropical forests, it was recently reported that seasonal drought significantly impacts tree community dynamics (Engelbrecht *et al*., 2007), suggesting that *TE* could play an important role in determining the performance and distribution of tropical tree species.

Leaf-level water-use efficiency generally increases in response to increasing leaf N concentration in C_3 plants (Wong, 1979; Toft *et al*., 1989; Duursma and Marshall, 2006). This is because more leaf N is usually associated with more photosynthetic capacity, which allows for a greater photosynthetic rate at a given rate of water loss. At the whole-plant scale, the *TE* of trees has also generally been observed to increase with increasing N availability (Guehl *et al*., 1995; Syvertsen *et al*., 1997;

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Livingston *et al*., 1999; Ripullone *et al*., 2004), although not always (Guehl *et al*., 1995; Hobbie and Colpaert, 2004). This trend also appears to apply for tropical trees: *TE* of *Ficus insipida* Willd., a fast-growing tropical pioneer tree capable of high rates of photosynthesis (Zotz *et al*., 1995), increased in response to fertilizer application (Winter *et al*., 2001); and a linear relationship was observed between *TE* and leaf N concentration in an experiment involving seven tropical tree species (Cernusak *et al*., 2007). Although there appears to be general agreement among experiments regarding the direction of response of tree *TE* to variation in soil fertility (Raven *et al*., 2004), the physiological mechanisms modulating the response are less well understood. For example, it was suggested that variation in respiratory C use (Guehl *et al*., 1995), or in the amount of unproductive water loss (Hobbie and Colpaert, 2004), could play important roles in determining the response of *TE* to soil fertility, in addition to the effects associated with leaf-level photosynthesis.

Since the revelation that C isotope fractionation correlates positively with the ratio of intercellular to ambient CO_2 mole fractions in C_3 plant leaves (Farquhar *et al*., 1982), analysis of $^{13}C/^{12}C$ in plant organic material has played an important role in water-use efficiency research (Bacon, 2004). It was later suggested that analysis of $^{18}O/^{16}O$ in plant organic material could also aid investigations of water-use efficiency by providing complementary information to that obtained from C isotope analyses (Farquhar *et al*., 1989; Sternberg *et al*., 1989). In this study, measurements of C and O isotope ratios were combined with measurements of plant elemental composition, growth, instantaneous leaf gas exchange, and whole-plant water use to analyse the mechanisms controlling the response of *TE* of the tropical pioneer tree *Ficus insipida* to variation in soil fertility.

Theory

At the leaf level, photosynthetic water-use efficiency can be expressed as the quotient of the diffusive fluxes of CO_2 and water vapour into and out of the leaf, respectively, during photosynthesis (Farquhar and Richards, 1984):

$$\frac{A}{E} = \frac{c_a - c_i}{1.6v} \quad (1)$$

where A is net photosynthesis (μmol CO_2 m^{-2} s^{-1}), E is leaf transpiration (mmol H_2O m^{-2} s^{-1}), c_a and c_i are atmospheric and intercellular CO_2 mole fractions (μmol mol^{-1}), 1.6 is the ratio of diffusivities for water vapour and CO_2 in air, and v is the leaf-to-air water vapour mole fraction difference (mmol mol^{-1}). A list of symbols used in the text is given in Table 1. Equation (1) 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). Thus, whole-plant transpiration efficiency (*TE*) can be defined as

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

where *TE* is mmol C fixed in plant biomass mol^{-1} H_2O transpired by the plant; 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 unproductive water loss relative to that associated with C uptake, i.e. nocturnal transpiration through partially open stomata and cuticular water loss by leaves and stems during the day and night.

The ratio of intercellular to ambient CO_2 mole fractions (c_i/c_a), shown in equation (2), also relates independently to C isotope discrimination ($\Delta^{13}C$). The $\Delta^{13}C$ for C_3 photosynthesis can be defined as (Farquhar *et al*., 1982; Farquhar and Richards, 1984; Hubick *et al*., 1986)

$$\Delta^{13}C = a - d + \left(b - a\right)\frac{c_i}{c_a} \quad (3)$$

where a is the $^{13}C/^{12}C$ fractionation caused by gaseous diffusion through stomata (4.4‰), and b is the fractionation caused by Rubisco, the primary carboxylating enzyme in C_3 plants (29‰). The term d summarizes collectively the fractionations caused by dissolution of CO_2 and liquid phase diffusion, photorespiration, and dark respiration (Farquhar *et al*., 1989). The effects of fractionations associated with these processes on the overall $\Delta^{13}C$ are small compared with that caused by Rubisco, but nonetheless significant (Brugnoli and Farquhar, 2000; Ghashghaie *et al*., 2003). The d in equation (3) substitutes for the following terms in the full model of $\Delta^{13}C$ for C_3 plants (Farquhar *et al*., 1989)

$$d = \left[\frac{(a - a_b)}{g_b} + \frac{(b - e_s - a_l)}{g_i}\right]\frac{A}{c_a} + \frac{\frac{eR_d}{k} + f\Gamma^*}{c_a} \quad (4)$$

where g_b and g_i are conductances to CO_2 (mol m^{-2} s^{-1}) of the leaf boundary layer and between the intercellular air spaces and sites of carboxylation, respectively. The a_b is the discrimination against $^{13}CO_2$ during diffusion through the leaf boundary layer (2.8‰), e_s is that during dissolution into water (1.1‰), and a_l is that during liquid phase diffusion (0.7‰). The R_d is day respiration (μmol CO_2 m^{-2} s^{-1}), e is the ^{13}C discrimination associated with day respiration, k is the carboxylation efficiency (mol CO_2 m^{-2} s^{-1}), f is the discrimination against ^{13}C associated with photorespiration, and Γ^* is the CO_2 compensation point in the absence of R_d (μmol mol^{-1}). The $\Delta^{13}C$ in

Table 1. *Symbols used in the text*

A	Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})
a	$^{13}C/^{12}C$ discrimination during diffusion through the stomatal pore
a_b	$^{13}C/^{12}C$ discrimination during diffusion through the leaf boundary layer
a_l	$^{13}C/^{12}C$ discrimination during liquid phase diffusion
b	$^{13}C/^{12}C$ discrimination by carboxylating enzymes during C_3 photosynthesis
C	Molar concentration of water (mol m^{-3})
c_a	Ambient CO_2 mole fraction (μmol mol^{-1})
c_i	Intercellular CO_2 mole fraction (μmol mol^{-1})
D	Diffusivity of $H_2^{18}O$ in water (m^2 s^{-1})
d	$^{13}C/^{12}C$ discrimination caused by processes other than a and b during C_3 photosynthesis
E	Transpiration rate (mmol H_2O m^{-2} s^{-1})
E_{grav}	Plant transpiration determined gravimetrically (mmol H_2O m^{-2} s^{-1})
E_{tot}	Cumulative transpiration over the course of an experiment (mol H_2O)
e	$^{13}C/^{12}C$ discrimination during dark respiration
e_s	$^{13}C/^{12}C$ discrimination during dissolution of CO_2 into water
f	$^{13}C/^{12}C$ discrimination during photorespiration
g_b	Boundary layer conductance to CO_2 (mol m^{-2} s^{-1})
g_c	Total conductance to CO_2 of stomata plus boundary layer (mol m^{-2} s^{-1})
g_i	Mesophyll conductance to CO_2 (mol m^{-2} s^{-1})
I	Intercept of the linear relationship between TE and $\Delta^{13}C$
k	Carboxylation efficiency during C_3 photosynthesis (mol CO_2 m^{-2} s^{-1})
L	Scaled effective path length in relation to ^{18}O advection/diffusion (m)
LA	Leaf area (m^2)
MTR	Mean transpiration rate (mol H_2O m^{-2} d^{-1})
m	Slope of the linear relationship between TE and $\Delta^{13}C$
N_{area}	Leaf N concentration expressed on an area basis (mmol N m^{-2})
N/P	Mass ratio of N to P in leaf dry matter
PFD	Photosynthetic photon flux density (μmol m^{-2} s^{-1})
P_{area}	Leaf P concentration expressed on an area basis (mmol P m^{-2})
p_{ex}	Proportion of O atoms exchanging with local water during cellulose synthesis
p_x	Proportion of unenriched source water in developing plant tissues
R_d	Leaf dark respiration rate (μmol CO_2 m^{-2} s^{-1})
RGR	Mean relative growth rate (mg g^{-1} d^{-1})
r_b	Boundary layer resistance (m^2 s mol^{-1})
r_s	Stomatal resistance (m^2 s mol^{-1})
T	Leaf temperature in K
TE	Transpiration efficiency (mmol C mol^{-1} H_2O)
TE_N	Transpiration efficiency of N acquisition (μmol N mol^{-1} H_2O)
T_L	Leaf temperature in C
t	Number of days in experiment
v	Leaf-to-air water vapour mole fraction difference (mmol mol^{-1})
w_a	Water vapour mole fraction of ambient air (mmol mol^{-1})
w_i	Water vapour mole fraction in the intercellular air spaces (mmol mol^{-1})
$\Delta^{13}C$	Photosynthetic $^{13}C/^{12}C$ discrimination
$\Delta^{13}C_L$	$^{13}C/^{12}C$ discrimination in leaf dry matter
$\Delta^{13}C_S$	$^{13}C/^{12}C$ discrimination in stem dry matter
$\Delta^{13}C_R$	$^{13}C/^{12}C$ discrimination in root dry matter
$\Delta^{13}C_{wp}$	$^{13}C/^{12}C$ discrimination in whole plant dry matter
$\Delta^{18}O$	$^{18}O/^{16}O$ enrichment relative to source water
$\Delta^{18}O_c$	$^{18}O/^{16}O$ enrichment of plant cellulose relative to source water
$\Delta^{18}O_e$	$^{18}O/^{16}O$ enrichment at the evaporative sites in leaves relative to source water
$\Delta^{18}O_L$	$^{18}O/^{16}O$ enrichment of leaf mesophyll water relative to source water
$\Delta^{18}O_p$	$^{18}O/^{16}O$ enrichment of plant dry matter relative to source water
$\Delta^{18}O_v$	$^{18}O/^{16}O$ enrichment of atmospheric water vapour relative to source water
$\delta^{13}C$	$^{13}C/^{12}C$ of a sample expressed relative to the Pee Dee Belemnite standard
$\delta^{13}C_a$	$^{13}C/^{12}C$ of atmospheric CO_2 expressed relative to the Pee Dee Belemnite standard
$\delta^{13}C_p$	$^{13}C/^{12}C$ of plant material expressed relative to the Pee Dee Belemnite standard
$\delta^{15}N$	$^{15}N/^{14}N$ of a sample expressed relative to that of air
$\delta^{18}O$	$^{18}O/^{16}O$ of a sample expressed relative to that of Vienna Standard Mean Ocean Water
$\delta^{18}O_p$	$\delta^{18}O$ of leaf dry matter
$\delta^{18}O_s$	$\delta^{18}O$ of source water
ε_{cp}	$\delta^{18}O$ difference between plant cellulose and plant dry matter
ε_k	$^{18}O/^{16}O$ fractionation during water vapour diffusion through stomata and boundary layer
ε_{wc}	Equilibrium $^{18}O/^{16}O$ fractionation between organic O and medium water
ε^+	Equilibrium $^{18}O/^{16}O$ fractionation between liquid water and vapour
ϕ_c	Proportion of fixed C respired to the atmosphere
ϕ_w	Ratio of unproductive to productive water loss
Γ^*	CO_2 compensation point of C_3 photosynthesis in the absence of R_d
$\wp$	Péclet number

equation (3) is defined with respect to atmospheric CO_2 as $\Delta^{13}C = R_a/R_p - 1$, where R_a is $^{13}C/^{12}C$ of atmospheric CO_2 and R_p is $^{13}C/^{12}C$ of plant material (Farquhar and Richards, 1984). In practice, $\Delta^{13}C$ is calculated from measured $\delta^{13}C$ values as

$$\Delta^{13}C = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 + \delta^{13}C_p} \quad (5)$$

where $\delta^{13}C_a$ is $\delta^{13}C$ of CO_2 in air, and $\delta^{13}C_p$ is that of plant material. For convenience, $\Delta^{13}C$ and $\delta^{13}C$ values are typically expressed as per mil (‰), meaning that they have been multiplied by the scaling factor 1000.

Equations (2) and (3) suggest that *TE* and $\Delta^{13}C$ share a mutual dependence on c_i/c_a. Combining the two equations yields (Hubick and Farquhar, 1989)

$$TE = \frac{c_a(1 - \phi_c)(b - d - \Delta^{13}C)}{1.6v(1 + \phi_w)(b - a)} \quad (6)$$

which, in turn, can be rearranged as

$$TE = -\Delta^{13}C\frac{c_a(1 - \phi_c)}{1.6v(1 + \phi_w)(b - a)} + \frac{c_a(1 - \phi_c)(b - d)}{1.6v(1 + \phi_w)(b - a)} \quad (7)$$

Equation (7) presents a linear relationship between *TE* and $\Delta^{13}C$ with slope $-m$ and intercept $m(b–d)$:

$$TE = -m\Delta^{13}C + m(b - d) \quad (8)$$

where $m = c_a(1–\phi_c)/[1.6v(1+\phi_w)(b–a)]$. Thus, as demonstrated previously (Hubick *et al*., 1986), the coefficients of a linear regression equation between *TE* and $\Delta^{13}C$ can be used to make inferences about parameters in equation (7) that are difficult to determine experimentally. Namely, the term d can be calculated as $d = b–(I/m)$, where I is the intercept and m is the negative slope of the relationship between *TE* and $\Delta^{13}C$, and ϕ_c can be calculated as $\phi_c = 1–1.6vm(1+\phi_w)(b–a)/c_a$. Note that these calculations assume that the terms for which m and I substitute in equation (7) are invariant over the range of $\Delta^{13}C$ for which the regression equation is fitted.

It was previously suggested that measurements of $^{18}O/^{16}O$ of plant organic material could prove useful in water-use efficiency studies by providing a means for making integrated estimates of v (Farquhar *et al*., 1989; Sternberg *et al*., 1989). The v is defined as $w_i–w_a$, where w_i is the water vapour mole fraction in the leaf intercellular air spaces and w_a is that in the surrounding atmosphere. The suggestion was that the following set of equations, describing the processes contributing to the $^{18}O/^{16}O$ of plant organic material, could be inverted to solve for w_i.

The terms w_a and w_i relate to steady-state leaf water ^{18}O enrichment at the sites of evaporation in leaves ($\Delta^{18}O_e$) in the following way (Craig and Gordon, 1965; Dongmann *et al*., 1974; Farquhar and Lloyd, 1993)

$$\Delta^{18}O_e = \varepsilon^+ + \varepsilon_k + \left(\Delta^{18}O_v - \varepsilon_k\right)\frac{w_a}{w_i} \quad (9)$$

where ε^+ is the equilibrium fractionation that occurs during the phase change from liquid water to vapour, ε_k is the kinetic fractionation that occurs during water vapour diffusion through stomatal pores and the leaf boundary layer, and $\Delta^{18}O_v$ is the ^{18}O enrichment of atmospheric water vapour with respect to water taken up by the roots (source water). The ^{18}O enrichment ($\Delta^{18}O$) is defined with respect to source water as $\Delta^{18}O = R/R_s - 1$, where R is $^{18}O/^{16}O$ of the sample of interest and R_s is that of source water. The equilibrium fractionation, ε^+, can be calculated as follows (Bottinga and Craig, 1969):

$$\varepsilon^+\left(‰\right) = 2.644 - 3.206\left(\frac{10^3}{T}\right) + 1.534\left(\frac{10^6}{T^2}\right) \quad (10)$$

where T is leaf temperature in K. The kinetic fractionation, ε_k, can be calculated as (Farquhar *et al*., 1989*b*)

$$\varepsilon_k(‰) = \frac{32r_s + 21r_b}{r_s + r_b} \quad (11)$$

where r_s and r_b are stomatal and boundary layer resistances to water vapour diffusion (m^2 s mol^{-1}), and 32 and 21 are associated fractionation factors scaled to per mil (Cappa *et al*., 2003).

The $\Delta^{18}O$ of leaf mesophyll water ($\Delta^{18}O_L$), the signature most relevant to production of plant organic material (Cernusak *et al*., 2003), can be related to $\Delta^{18}O_e$ as (Farquhar and Lloyd, 1993; Farquhar and Gan, 2003)

$$\Delta^{18}O_L = \frac{\Delta^{18}O_e(1 - e^{-\wp})}{\wp} \quad (12)$$

The $\wp$ is a Péclet number, defined as $EL/(CD)$, 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 is the diffusivity of $H_2^{18}O$ in water (m^2 s^{-1}). The D can be calculated as (Cuntz *et al*., 2007)

$$D = 119\times10^{-9}e^{\left(-\frac{637}{T-137}\right)} \quad (13)$$

where T is leaf temperature in K. The $\Delta^{18}O_L$ can in turn be related to the ^{18}O enrichment of plant cellulose ($\Delta^{18}O_c$) according to the following equation (Barbour and Farquhar, 2000):

$$\Delta^{18}O_c = \Delta^{18}O_L\left(1 - p_{ex}p_x\right) + \varepsilon_{wc} \quad (14)$$

where p_{ex} is the proportion of O atoms that exchange with local water in the developing plant tissue during cellulose synthesis, p_x is the proportion of unenriched source water

in the developing tissue, and ε_{wc} is the equilibrium fractionation between organic O and medium water. Finally, the ^{18}O enrichment of plant dry matter ($\Delta^{18}O_p$) can be related to that of plant cellulose by adding an additional fractionation factor (ε_{cp}) to account for the $\delta^{18}O$ difference between the two (Barbour and Farquhar, 2000);

$$\Delta^{18}O_p = \Delta^{18}O_c + \varepsilon_{cp} \quad (15)$$

Materials and methods

Plant material and experimental treatments

The experiment took place at the Smithsonian Tropical Research Institute, Santa Cruz Experimental Field Facility, Gamboa, Republic of Panama. The site is located at 9°07' N latitude, 79°42' W longitude, at an altitude of 28 m above sea level. Seeds of *Ficus insipida* Willd. (Moraceae) were collected from mature trees growing in the Panama Canal watershed. Seeds were germinated in trays containing a commercial potting soil in July 2005. Following germination, three seedlings each were transplanted into 40 pots, each of volume 19 l. Upon transplanting, a handful of soil was added to each pot together with roots taken from the base of the palm tree *Attalea butyracea* (Mutis ex L.f.) Wess. Boer. as a source of mycorrhizal inoculant. The pots were placed under a translucent rain shelter on plastic tables; they were elevated approximately 0.8 m above the concrete surface below the shelter. The shelter reduced incoming photon flux density (*PFD*) by approximately 20%. After an adjustment period of about 1 month, two seedlings were removed from each pot, leaving a uniform population of seedlings, with one seedling per pot. Among the 40 pots, five treatments were deployed, yielding eight pots per treatment. For each treatment, two pots were selected to serve as controls without plants; seedlings were removed from these pots. The discarded seedlings were used to measure seedling dry mass at the beginning of the experiment, estimated as 0.27 g.

The five soil fertility treatments consisted of varying mixtures of homogenous, dark topsoil and rice husks, with the high fertility treatment additionally receiving a one-time application of slow-release fertilizer. It was expected that the addition of rice husks to the soil mixture would reduce the soil fertility in two ways; both by diluting the nutrient content of the pot, and by adding a high C/N substrate that would tend to immobilize N and other nutrients, leading to greater deficiencies as the proportion of rice husks increased. The treatments were as follows, given as the volumetric percentage of air-dried topsoil in the topsoil/rice-husk mixture: 20, 40, 60, 80, 80+N. For the 80+N treatment, approximately 13 g of Osmocote-Plus controlled-release fertilizer (Scotts-Sierra, Maryville, OH, USA) was added to each pot. Due to the difference in density between the topsoil and rice husks, the dry mass of the topsoil/rice-husk mixture required to fill each 19 l pot varied by treatment: 5.4, 8.9, 12.4, 15.4, and 15.4 kg were placed in each pot for treatments 20, 40, 60, 80, and 80+N, respectively. The amount of water required to bring the pots to field capacity also varied slightly: 4.0, 4.5, 5.0, 5.0, and 5.0 kg of water were added to treatments 20, 40, 60, 80, and 80+N, respectively. 1.5 kg of gravel was added to the soil surface of each pot to reduce soil evaporation.

Plant water use

The pots were weighed at regular intervals from 23 August 2005 until plant harvest on 4 November 2005, a period of approximately 10 weeks. Pot weights were determined with a 64 kg capacity balance (Sartorius QS64B, Thomas, Swedesboro, NJ, USA). The pots were initially weighed once per week, but the frequency was increased to as much as three times per week when plant stature and water use increased toward the end of the experiment. Pot water loss in the interval between weight measurements did not exceed 2.5 kg; this value was only approached near the very end of the experiment and only for the largest plants. Woody tree seedlings typically do not show a reduction in daily transpiration rate until soil water content falls to approximately one-third the value at field capacity (Sinclair *et al*., 2005), so it is assumed that transpiration was not limited by soil water content at any time during the experiment. After weighing each pot, water was added until the initial weight at field capacity was restored. Plant transpiration over the course of the experiment was calculated as the difference between cumulative pot water loss and the mean water loss of the control pots for each treatment. Leaves, stems, and roots were oven-dried at 70 °C after harvest and weighed separately for each plant. Abscised leaves were collected during the experiment and their dry weight added to the plant dry weight for *TE* calculations. Leaf area at plant harvest was determined with an LI-3100 Leaf-Area Meter (Li-Cor Inc., Lincoln, NE, USA).

Meteorological conditions during the experiment were recorded every 15 min using an automated weather station (Campbell Scientific, Logan, UT, USA), as described previously (Winter *et al*., 2001, 2005). The mean daytime temperature, calculated between the h of sunrise and sunset, was 27.3 °C; mean daytime relative humidity was 81.5%; mean *PFD* was 670 μmol m^{-2} s^{-1}; and mean daytime wind speed was 0.4 m s^{-1}.

For three days prior to plant harvest, daily and nightly water use of each plant was measured. Pots were weighed prior to sunrise (05.30 h) and again following sunset (18.00 h).

Control pots were also weighed and control water loss subtracted from that of pots with plants to calculate plant water loss. Mean daily and nightly transpiration rates were expressed on a leaf area basis by dividing by the leaf area determined at plant harvest, which followed the third cycle of day/night measurements. The term ϕ_w for each plant was calculated as night-time plant water use divided by daytime plant water use.

Leaf gas exchange measurements

Gas exchange of the youngest, fully-expanded leaf of each plant was measured under light-saturating conditions (*PFD* >800 μmol m^{-2} s^{-1}) at both morning and midday on 20 October 2005 with an Li-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). Leaves were illuminated during measurements by natural sunlight. The mean *PFD* at the leaf surface during morning measurements was 1094±225 μmol m^{-2} s^{-1} (mean ±1 SD), whereas that during midday measurements was 1333±121 μmol m^{-2} s^{-1}. The mean *v* during morning measurements was 12.8±1.6 mmol mol^{-1}, and that during midday measurements was 17.2±1.4 mmol mol^{-1}. Mean leaf temperatures (T_L) during morning and midday measurements were 33.0±1.0 °C, and 36.0±0.5 °C, respectively. Dark respiration was measured on the youngest, fully-expanded leaf of each plant on 3 November 2005 between 19.30 h and 22.00 h. Mean leaf temperature during measurements was 25.9±0.2 °C.

Isotopic and elemental analyses

Leaf, stem, and root dry matter were ground to a fine powder for elemental and isotopic analyses. The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios of leaf, stem, and root dry matter were measured at the Idaho Stable Isotopes Laboratory at the University of Idaho, Moscow, ID, USA; the $^{18}O/^{16}O$ of leaf dry matter was measured at the Stable Isotope Core Laboratory, Washington State University, Pullman,

WA, USA. For $^{13}C/^{12}C$ and $^{15}N/^{14}N$ analyses, samples of approximately 3 mg were combusted in an NC2500 elemental analyser (CE Instruments, Milan, Italy), then swept by a helium carrier gas, via a continuous flow interface, into a Delta Plus isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany). In addition to $^{13}C/^{12}C$ and $^{15}N/^{14}N$, the C and N elemental concentrations of the sample material were determined from peak areas obtained from mass spectrometric measurements. The $^{18}O/^{16}O$ of leaf dry matter was measured on samples of approximately 1 mg on a Delta XP isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany), following pyrolysis in a high-temperature furnace (Thermoquest TC/EA, Finnigan MAT, Bremen, Germany). The C, N, and O stable isotope ratios were obtained in delta notation relative to standards of Pee Dee Belemnite, air, and Vienna Standard Mean Ocean Water, respectively.

Whole-plant $\delta^{13}C$ and $\delta^{15}N$ compositions were calculated by mass balance using the dry mass of each plant organ (leaves, stems, and roots), the C or N mass fraction, and the $\delta^{13}C$ or $\delta^{15}N$ composition. The $\Delta^{13}C$ was calculated from $\delta^{13}C$ values according to equation (5). The $\delta^{13}C_a$ was assumed to be –8‰, consistent with observed daytime $\delta^{13}C_a$ near Panama City, Panama (Winter and Holtum, 2002). The leaf $\Delta^{18}O_p$ was expressed with respect to the $\delta^{18}O$ of irrigation water (–4.0‰) according to the equation $\Delta^{18}O_p=(\delta^{18}O_p-\delta^{18}O_s)/(1+\delta^{18}O_s)$, where $\delta^{18}O_p$ is the $\delta^{18}O$ of leaf dry matter, and $\delta^{18}O_s$ is that of irrigation (source) water (Barbour *et al*., 2004).

In addition, elemental concentrations of P, K, Ca, Mg, Mn, and Zn were quantified in leaf dry matter. Approximately 200 mg of finely ground, oven-dried leaf material were digested at 380 °C in sulphuric acid and lithium sulphate with a selenium catalyst and hydrogen peroxide. Elemental concentrations were then determined on an inductively-coupled plasma optical-emission spectrometer (Perkin Elmer Inc., Wellesley, MA, USA).

Leaf temperature and leaf-to-air humidity gradient

Three different methods were used to estimate average values for T_L and v over the course of the experiment for the five soil fertility treatments. In the first method, a leaf energy balance model was used, details of which have been recently described (Barbour *et al*., 2000; Cernusak *et al*., 2003*a*). In the model, mean daytime air temperature, relative humidity, irradiance, and wind speed were used over the course of the experiment along with the transpiration rates measured gravimetrically just prior to plant harvest. The transpiration rates used in the analysis were those measured on 2 November 2005, when the mean daily *PFD* and mean daily air water vapour mole fraction deficit matched very closely the average values recorded over the course of the experiment (684 μmol m^{-2} s^{-1} and 6.6 mmol mol^{-1} versus 670 μmol m^{-2} s^{-1} and 6.6 mmol mol^{-1}, respectively). It was assumed that the incoming *PFD* was reduced by 20% by the translucent rain shelter covering the plants, and that the mean intercepted *PFD* for each plant was further reduced by 25% by self shading and non-horizontal leaf orientation. Thus, the *PFD* used in the leaf energy balance analysis was 400 μmol m^{-2} s^{-1}. The mean surface area of individual leaves for each treatment was used to calculate boundary layer conductance. The leaf energy balance model was used to predict mean T_L for each treatment, and v was calculated as the difference between the saturation vapour mole fraction at T_L and the average daytime air vapour mole fraction.

In the second method for estimating T_L and v, equation (2) was used, along with measured values of TE, c_i/c_a (based on measurements of $\Delta^{13}C$), and ϕ_w. It was assumed that c_a was constant at 375 μmol mol^{-1}, and that ϕ_c was constant at 0.4. Equation (2) for v was then solved. Average w_a was used to calculated w_i, and T_L was calculated as the dew point temperature at w_i.

The third method for estimating T_L and v was based on measurements of $\Delta^{18}O_p$ for leaf dry matter. Equations (9) to (15) were inverted to solve for w_i starting with values of $\Delta^{18}O_p$. The ε_{cp} was assumed to be –6.8‰ (Cernusak *et al*., 2004); ε_{wc} was assumed to be 27‰ (Sternberg and DeNiro, 1983); the term $p_{ex}p_x$ was assumed to be 0.38 (Cernusak *et al*., 2005); the L was assumed to be 0.015 m (Cernusak *et al*., 2002, 2005); the D was calculated to be 2.46×10^{-9} m^2 s^{-1}; the r_s values were taken from leaf gas exchange measurements; the r_b was calculated from mean leaf surface area and mean wind speed, as for the leaf energy balance analysis; the ε^+ was calculated to be 8.9‰; and the $\Delta^{18}O_v$ was assumed equal to $-\varepsilon^+$ (Farquhar *et al*., 2007). Although the parameters D and ε^+ are dependent on leaf temperature, estimates of v are relatively insensitive to small changes in these parameters. For example, calculating these parameters with a T_L of 25 °C versus 30 °C would shift our mean estimate of v from 8.1 to 8.0 mmol mol^{-1} in the case of D, and from 7.9 to 8.0 mmol mol^{-1} in the case of ε^+. Therefore, D and ε^+ were calculated assuming an approximate T_L of 28 °C. Final T_L was calculated from w_i as described above.

Statistical analysis

Ordinary least squares regression was used to evaluate the relationships between leaf isotopic characteristics, gas exchange characteristics, and leaf elemental concentrations. These analyses were used to determine the ability of an explanatory variable to predict variation in a response variable. However, to determine the regression coefficients for the relationship between TE and $\Delta^{13}C$ for estimations of d and ϕ_c, geometric mean regression was used. In this case, we were interested in the functional relationship between TE and $\Delta^{13}C$ rather than a causal, predictive relationship, the values of the regression coefficients were the primary focus of the analysis, and both parameters were measured with error, thereby indicating the use of geometric mean regression (Sokal and Rohlf, 1995). Analysis of variance was used to test for variation among treatments in physiological and morphological parameters. Tukey's method for pair-wise comparisons was used to test for significant differences between individual treatments.

Results

Plant growth and morphology

Plant growth and morphology differed among the soil fertility treatments (Table 2). Mean plant dry mass at the time of harvest ranged from 2.6 to 80.4 g from the lowest to the highest soil fertility treatment. Corresponding mean relative growth rates (*RGR*) ranged from 30.4 to 78.0 mg g^{-1} d^{-1}, respectively (Table 2). Differences in *RGR* among treatments were statistically significant for all pairs of treatments, except 40% and 60% soil. There was significant variation in root:shoot ratio, leaf area ratio, and specific leaf area among treatments, but this variation did not appear to be systematically related to the soil fertility treatments (Table 2).

Elemental composition

Whole-plant C concentration varied among treatments, but over a rather narrow range of 0.40–0.42 g g^{-1} (Table 3). Whole-plant N concentration, on the other hand, showed more pronounced variation among treatments, ranging

Table 2. *Morphological and physiological parameters for* Ficus insipida *plants according to soil fertility treatment*

Values are given as mean ±1 standard deviation. For each treatment, n=6. Values within a row followed by different letters are significantly different at P <0.05.

	Soil/rice-husk mixture (v/v)				
	20% Soil	40% Soil	60% Soil	80% Soil	80% Soil plus fertilizer
Plant dry mass at harvest (g)	2.6±1.0 a	11.8±3.3 a	7.8±2.0 a	27.4±5.2 b	80.4±12.3 c
Leaf area at harvest (cm^2)	247±92 a	1086±248 b	826±224 a,b	1885±285 c	7449±750 d
Root/shoot ratio ($g\ g^{-1}$)	0.68±0.28 a	0.45±0.06 a,b	0.43±0.05 b	0.58±0.07 a,b	0.46±0.07 a,b
Leaf area ratio ($m^2\ kg^{-1}$)	9.5±2.3 a	9.4±1.4 a	10.6±1.0 a	6.9±0.3 b	9.4±0.9 a
Specific leaf area ($m^2\ kg^{-1}$)	22.5±1.6 a	20.2±1.3 b,c	22.2±1.3 a,c	19.1±1.4 b	21.4±1.0 a,c
Mean relative growth rate ($mg\ g^{-1}\ d^{-1}$)	30.4±5.5 a	51.4±4.2 b	45.8±3.5 b	63.3±2.7 c	78.0±2.2 d
Gravimetric daytime transpiration ($mmol\ m^{-2}\ s^{-1}$)	1.92±0.18 a	1.58±0.09 b	1.70±0.09 b	1.32±0.08 c	1.14±0.13 c
Gravimetric night-time transpiration ($mmol\ m^{-2}\ s^{-1}$)	0.30±0.10 a	0.18±0.03 b,c	0.23±0.03 a,c	0.14±0.02 b	0.13±0.01 b
Ratio night-time/daytime transpiration (ϕ_w)	0.15±0.05 a	0.11±0.02 a,b	0.13±0.02 a,b	0.11±0.01 b	0.11±0.01 a,b
Net photosynthesis ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	11.0±2.2 a	15.1±1.5 b	16.4±1.7 b	15.4±1.3 b	22.4±1.7 c
Stomatal conductance to H_2O ($mol\ m^{-2}\ s^{-1}$)	0.59±0.06 a	0.72±0.08 a,b	0.76±0.10 b	0.75±0.08 b	0.81±0.09 b
Instantaneous transpiration ($mmol\ m^{-2}\ s^{-1}$)	8.4±0.5 a	9.1±0.4 a,b	8.6±1.0 a	9.0±0.8 a,b	9.9±0.5 b
Dark respiration rate ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	0.71±0.15 a	1.07±0.29 a,b	1.08±0.25 a,b	0.98±0.15 a,b	1.34±0.33 b
Ratio dark respiration/net photosynthesis	0.07±0.02 a	0.07±0.02 a	0.07±0.01 a	0.06±0.01 a	0.06±0.01 a
Whole-plant N isotope ratio ($\delta^{15}N$, ‰)	2.7±0.8 a	3.2±0.5 a	2.4±0.4 a	3.4±0.7 a	2.8±0.6 a
Transpiration efficiency: N ($\mu mol\ N\ mol^{-1}\ H_2O$)	17.8±4.3 a	35.8±3.9 b	39.5±4.0 b	41.8±4.6 b	107.0±18.5 c
Whole-plant C isotope discrimination ($\Delta^{13}C_{wp}$; ‰)	24.1±0.6 a	23.1±0.3 b	23.1±0.3 b	22.4±0.4 c	21.5±0.3 d
Leaf C isotope discrimination ($\Delta^{13}C_L$; ‰)	24.4±0.7 a	23.3±0.3 b	23.5±0.3 b	23.0±0.4 b	22.1±0.3 c
Stem C isotope discrimination ($\Delta^{13}C_S$; ‰)	24.5±0.6 a	23.1±0.3 b	23.0±0.3 b,c	22.4±0.5 c	21.5±0.5 d
Root C isotope discrimination ($\Delta^{13}C_R$; ‰)	23.7±0.5 a	22.9±0.3 a,b	22.7±0.6 b	21.8±0.3 c	20.4±0.5 d
Integrated c_i/c_a calculated from whole-plant $\Delta^{13}C$	0.96±0.02 a	0.92±0.01 b	0.92±0.01 b	0.89±0.01 c	0.86±0.01 d
Instantaneous c_i/c_a from gas exchange	0.88±0.02 a	0.86±0.01 a	0.86±0.01 a	0.86±0.01 a	0.82±0.01 b
Transpiration efficiency: C ($mmol\ C\ mol^{-1}\ H_2O$)	0.74±0.13 a	1.25±0.15 b	1.20±0.07 b	1.81±0.16 c	2.69±0.11 d
Leaf dry matter O isotope enrichment ($\Delta^{18}O_p$; ‰)	24.9±0.3 a	24.8±0.6 a	24.3±0.2 a	24.4±0.3 a	23.6±0.4 b

from 11.6 to 19.7 mg g^{-1} (Table 3). Leaf N concentration per unit leaf area (N_{area}) increased from 53.9 mmol m^{-2} to 90.4 mmol m^{-2} from the lowest to the highest soil fertility treatment (Table 3). Leaf P concentration showed an opposite trend to leaf N concentration, decreasing from the lowest to the highest soil fertility treatment (Table 3). Leaf Mg, Mn, and Zn concentrations followed similar trends to leaf P, decreasing from the lowest to the highest soil fertility (Table 3). Leaf K and Ca concentrations were less variable among treatments, although they showed weak tendencies to decrease (K) or increase (Ca) from the lowest to the highest soil fertility (Table 3).

The mass ratio of leaf N to P (N/P) increased across treatments from a mean of 4.7 for the lowest soil fertility to 16.4 for the highest soil fertility (Table 3). Variation in *TE* was closely correlated with variation in N/P (Fig. 1A). The relationship between the two parameters was slightly non-linear, such that the natural logarithm of N/P explained 90% of variation in *TE*, whereas N/P explained 87%. The relationship between *Ln*(N/P) and *TE* was $TE=1.42Ln(N/P)-1.28$ ($R^2=0.90$, P <0.0001, n=30). The *RGR* showed a non-linear response to variation in N/P; it increased up to N/P of about 15, then decreased slightly over the small range of values above 15 (Fig. 1B). Variation in *RGR* and *TE* was closely correlated (Fig. 1C). Again the relationship was slightly non-linear, such that the relationship between *RGR* and *Ln*(*TE*) was slightly stronger ($R^2=0.95$, P <0.0001, n=30)

Table 3. *Elemental composition of the dry matter of* Ficus insipida *plants according to soil fertility treatment*

Values are given as the mean ±1 standard deviation. For each treatment, n=6. Values within a row followed by different letters are significantly different at P <0.05.

	Soil/rice-husk mixture (v/v)				
	20% Soil	40% Soil	60% Soil	80% Soil	80% Soil plus fertilizer
Whole-plant C concentration (g g^{-1})	0.40±0.01 a	0.42±0.01 b,c	0.41±0.01 a,c	0.42±0.01 b,c	0.42±0.01 b,c
Whole-plant N concentration (mg g^{-1})	11.9±2.0 a	14.2±1.6 a,b	16.0±1.6 b	11.6±1.0 a	19.7±3.7 c
Whole-plant C/N ratio (g g^{-1})	34.3±5.5 a,b	29.7±3.7 b,c	26.0±2.4 c,d	36.7±2.7 a	21.9±3.0 d
Leaf N/P ratio (g g^{-1})	4.7±0.9 a	6.0±0.8 a	5.9±1.0 a	7.9±0.7 b	16.4±1.1 c
Leaf N per unit area (mmol m^{-2})	53.9±6.6 a	68.9±6.4 b	67.7±6.9 b	62.0±4.4 a,b	90.4±10.8 c
Leaf P per unit area (mmol m^{-2})	5.34±1.07 a	5.22±0.44 a	5.22±0.49 a	3.55±0.38 b	2.49±0.20 c
Leaf N concentration (mg g^{-1})	16.9±2.1 a	19.4±2.0 a,b	21.0±2.3 b	16.5±1.2 a	27.1±3.7 c
Leaf P concentration (mg g^{-1})	3.75±0.90 a	3.27±0.47 a	3.58±0.31 a	2.09±0.20 b	1.65±0.14 b
Leaf K concentration (mg g^{-1})	27.4±2.1 a,b	26.2±1.9 a,b	29.1±1.8 b	25.1±3.1 a	24.5±2.6 a
Leaf Ca concentration (mg g^{-1})	14.4±1.4 a,b	13.6±0.6 b	14.7±2.0 a,b	16.8±1.7 a	15.4±2.0 a,b
Leaf Mg concentration (mg g^{-1})	2.89±0.37 a	2.18±0.28 b,c	2.64±0.44 a,b	2.25±0.27 b,c	2.04±0.18 c
Leaf Mn concentration (μg g^{-1})	154±7 a	134±14 a,b	133±16 a,b	132±28 a,b	118±15 b
Leaf Zn concentration (μg g^{-1})	28.4±3.2 a	21.9±2.9 b	23.1±4.5 a,b	16.6±4.6 b,c	15.1±3.5 c

than that between *RGR* and *TE* (R^2=0.92, P <0.0001, n=30).

Leaf P_{area} showed a strong positive, linear dependence on mean daytime transpiration rate, also expressed on a leaf area basis, with E_{grav} explaining 74% of variation in leaf P_{area} (Fig. 2A). Similar positive, linear dependencies on E_{grav} were also observed for leaf Zn per unit area (R^2=0.45, P <0.0001, n=29) and leaf Mg per unit area (R^2=0.23, P <0.005, n=29).

Leaf gas exchange

Photosynthesis of the youngest, fully-expanded leaf of each plant at saturating irradiance increased with increasing soil fertility; mean treatment values ranged from 11.0 to 22.4 μmol CO_2 m^{-2} s^{-1} from the lowest to the highest soil fertility treatment (Table 2). These values represent averages of measurements made during morning and midday. Leaf dark respiration also increased from low to high soil fertility, with treatment means ranging from 0.71 to 1.34 μmol CO_2 m^{-2} s^{-1} (Table 2). The ratio of leaf dark respiration to leaf net photosynthesis, on the other hand, was invariant among treatments (Table 2). Leaf net photosynthesis, leaf dark respiration, and whole-plant relative growth rate were linearly related to leaf N_{area} (Fig. 3). The ratio of leaf dark respiration to leaf net photosynthesis, in contrast, showed no correlation with N_{area} (P=0.67, n=30).

Gravimetric transpiration

Mean daytime transpiration rates, determined gravimetrically, varied among soil fertility treatments. Mean treatment daytime E_{grav} ranged from 1.92 to 1.14 mmol m^{-2} s^{-1}, and generally decreased from the lowest soil fertility treatment to the highest (Table 2). Mean night-time E_{grav} followed a similar pattern (Table 2). The term ϕ_w, describing night-time transpiration as a proportion of daytime transpiration, ranged from 0.15 to 0.11 from low to high soil fertility treatments, respectively (Table 2). Mean daytime E_{grav} showed a negative, linear dependence on N_{area}. The equation describing the relationship was E_{grav}= –0.01N_{area}+2.3 (R^2=0.29, P=0.001, n=29).

Stable isotope composition

Mean values of $\Delta^{13}C_L$, $\Delta^{13}C_S$, $\Delta^{13}C_R$, and $\Delta^{13}C_{wp}$ for the five soil fertility treatments are shown in Table 2. All $\Delta^{13}C$ values decreased from lowest to highest soil fertility (Table 2). The general pattern among the different plant tissues was $\Delta^{13}C_L$>$\Delta^{13}C_S$>$\Delta^{13}C_R$ (Table 2). Average whole-plant $\delta^{15}N$ values spanned a relatively narrow range from 2.4‰ to 3.4‰, and did not differ significantly among treatments (Table 2). Leaf $\Delta^{18}O_p$ was significantly lower for the highest soil fertility treatment than for the other treatments, and tended to decrease with increasing soil fertility (Table 2). The $\Delta^{18}O_p$ was negatively

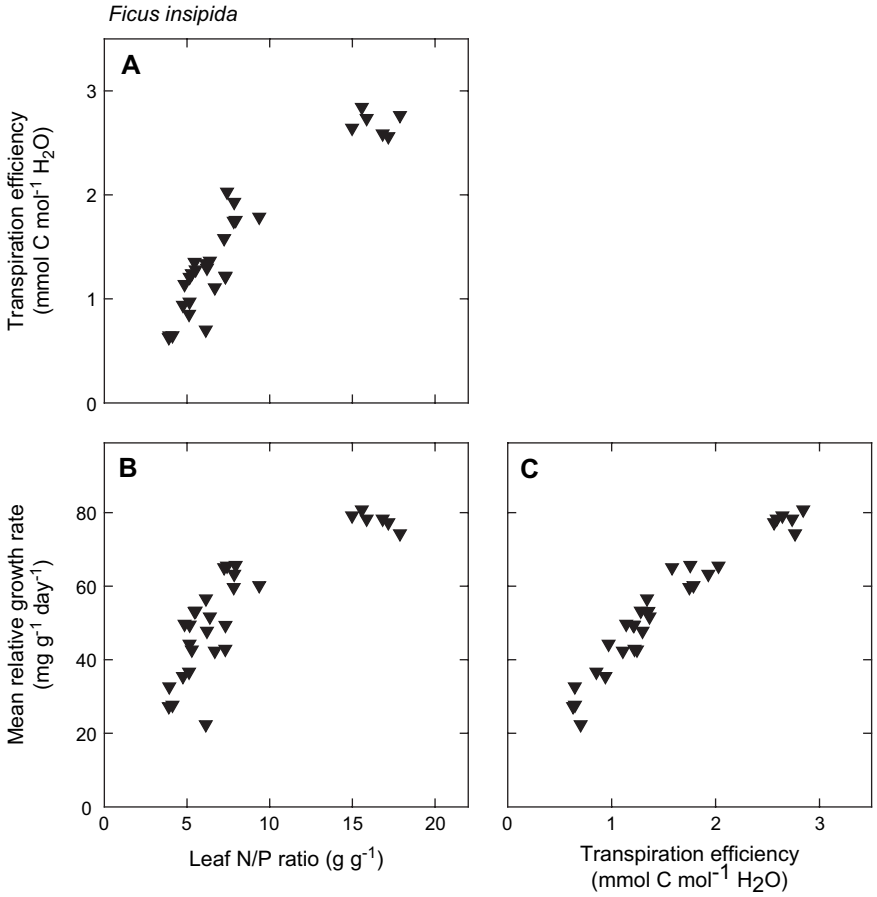

Fig. 1. Transpiration efficiency (A) and mean relative growth rate (B) plotted against leaf N/P mass ratio, and mean relative growth rate plotted against transpiration efficiency (C) for *Ficus insipida* plants subject to varying soil fertility.

correlated with mean daytime E_{grav} across all treatments (Fig. 2B).

Leaf temperature and leaf-to-air humidity gradient

Estimates of T_L and v generated by the three different methods are summarized in Table 4. The leaf energy balance model estimated a variation of 1.5 °C in T_L across the soil fertility treatments. Values ranged from 27.1 °C to 28.6 °C from the lowest to the highest soil fertility treatment; corresponding values for v ranged from 6.2 mmol mol^{-1} to 9.5 mmol mol^{-1} (Table 4). The second method that was used to estimate T_L and v, which assumed a constant value of 0.4 for ϕ_c, predicted less variation among treatments; values ranged from 26.9 °C to 27.8 °C and 5.8 mmol mol^{-1} to 7.8 mmol mol^{-1}, respectively. The third method, based on measurements of $\Delta^{18}O_p$, predicted a similar range of values to the leaf energy balance model, but with values trending in the opposite direction across treatments; i.e., decreasing from the lowest to the highest soil fertility. For the $\Delta^{18}O_p$ method, values ranged from 28.4 °C to 27.0 °C for T_L and from 9.0 mmol mol^{-1} to 6.0 mmol mol^{-1} for v (Table 4).

Transpiration efficiency

The *TE* varied strongly among soil fertility treatments (Table 2). Variation in *TE* was closely correlated with variation in $\Delta^{13}C_{wp}$ (Fig. 4). The equation relating *TE* to $\Delta^{13}C_{wp}$, determined by geometric mean regression, was $TE = -0.72\Delta^{13}C_{wp}+18.0$. These coefficients were similar to those determined by ordinary least-squares regression (–0.68 and 17.0 for m and I, respectively). The d calculated from m and I values of –0.72 and 18.0 was 4.0‰. Long-term, integrated c_i/c_a estimated from $\Delta^{13}C_{wp}$, using d=4.0‰, varied strongly among treatments (Table 2). Treatment means ranged from 0.96 to 0.86 from low to high soil fertility. The $\Delta^{13}C_{wp}$ was well correlated with c_i/c_a determined from instantaneous gas exchange measurements (Fig. 5), as was *TE* (R^2=0.69, P <0.0001, n=30). Instantaneous c_i/c_a was lower than $\Delta^{13}C_{wp}$-based c_i/c_a for all treatments (Table 2). Instantaneous c_i/c_a, $\Delta^{13}C_{wp}$, and *TE* each correlated well with leaf N_{area} (Fig. 6A, B, C). These parameters also correlated strongly with the light-saturated photosynthetic rate of the youngest, fully-expanded leaf of each plant (Fig. 6D, E, F). There were weaker correlations between stomatal conductance and

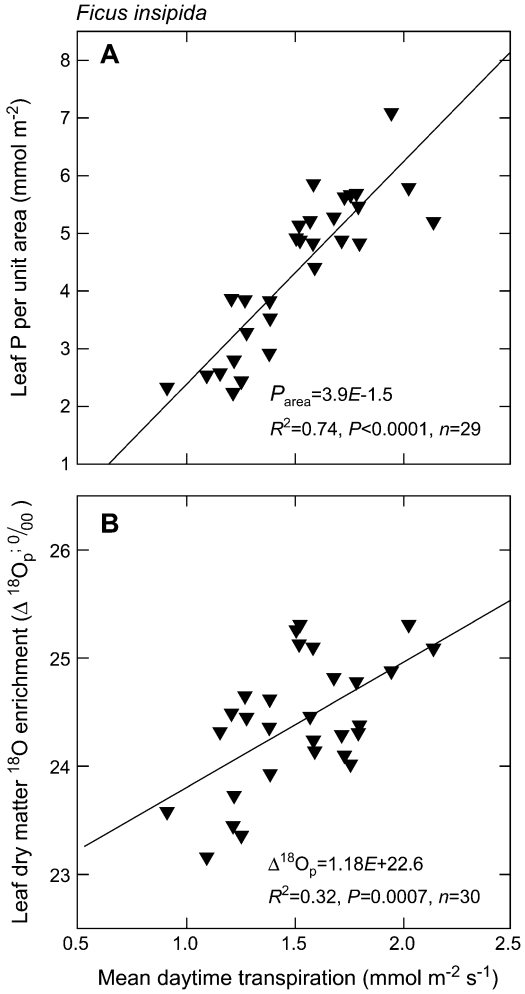

Fig. 2. Leaf P per unit area (A) and leaf dry matter ^{18}O enrichment (B) plotted against mean daytime transpiration rate for *Ficus insipida* plants. Transpiration was determined gravimetrically for whole plants on 1–3 November 2005; plant leaf area was determined after harvest on 4 November 2005.

c_i/c_a, $\Delta^{13}C_{wp}$, and *TE*; however, these correlations were opposite in sign to what would be expected if variation in stomatal conductance were controlling variation in these parameters (Table 2).

The transpiration efficiency of N acquisition (TE_N), calculated as whole-plant N increment divided by cumulative plant water loss, increased from 17.8 μmol N mol^{-1} H_2O for the lowest soil fertility treatment to 107.0 μmol N mol^{-1} H_2O for the highest soil fertility treatment (Table 2). The *TE* increased linearly as a function of TE_N according to the equation $TE=0.020TE_N+0.58$ ($R^2=0.84$, $P<0.0001$, $n=30$).

A sensitivity analysis is shown in Table 5 illustrating the predicted effect of changing the input parameters in equation (2) on *TE*. The ranges of input values used in the analysis were based on observations for the five soil fertility treatments for the parameters c_i/c_a, v, and ϕ_w; for

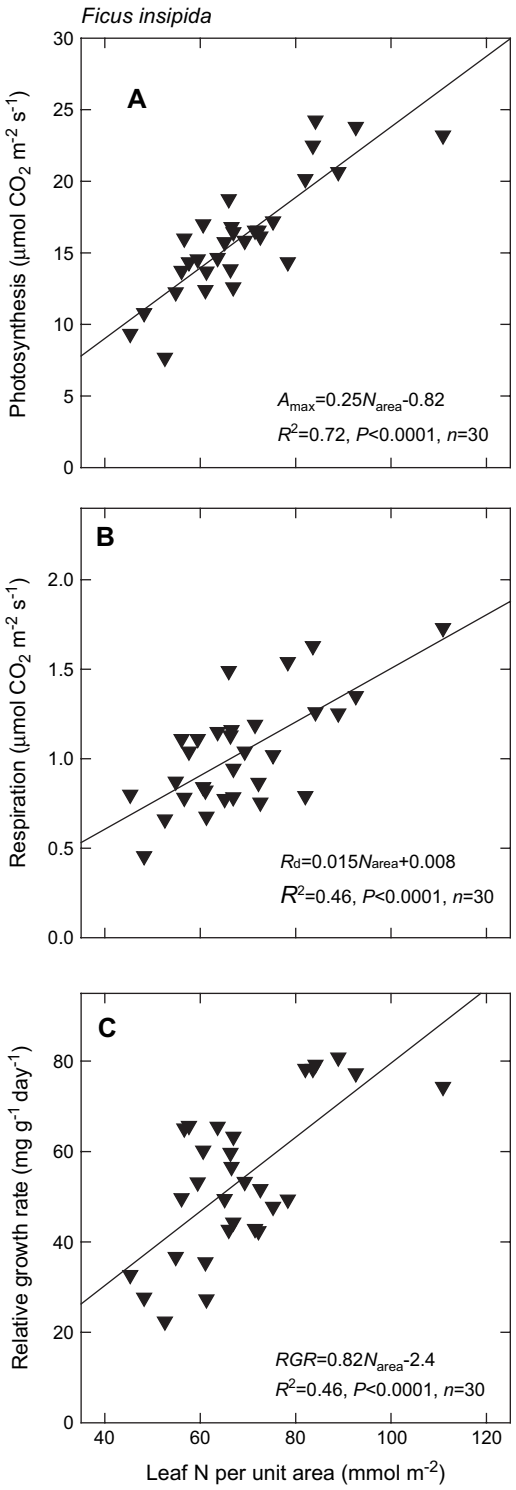

Fig. 3. Light-saturated photosynthesis (A), leaf dark respiration (B), and mean relative growth rate (C) plotted against leaf N per unit area for plants of *Ficus insipida* subject to varying soil fertility. Leaf photosynthesis was measured during morning and midday on 20 October 2005; mean photon flux density was ~1200 μmol m^{-2} s^{-1}. Leaf dark respiration was measured on 3 November 2005; mean leaf temperature was ~26 °C. Gas-exchange measurements were made on the youngest fully-expanded leaf for each plant.

Table 4. *Estimates for average leaf temperature (T_L) and average leaf to air water vapour mole fraction difference (*v*) over the course of the experiment for the five soil fertility treatments*

Three different methods were used to estimate T_L and *v*. The first method employed a leaf energy balance model. The second method relied on an assumption of constant ϕ_c across treatments. The third method was based on leaf dry matter $\Delta^{18}O_p$.

Method of estimation	Parameter	Soil/rice-husk mixture (v/v)				
		20% Soil	40% Soil	60% Soil	80% Soil	80% Soil plus fertilizer
Leaf energy balance	T_L (°C)	27.1	27.7	27.5	28.4	28.6
	v (mmol mol^{-1})	6.2	7.5	7.1	9.0	9.5
Constant ϕ_c	T_L (°C)	26.9	27.7	27.8	27.7	27.3
	v (mmol mol^{-1})	5.8	7.6	7.8	7.4	6.7
Leaf ^{18}O enrichment	T_L (°C)	28.4	28.4	27.9	27.9	27.0
	v (mmol mol^{-1})	9.0	9.1	7.9	8.0	6.0

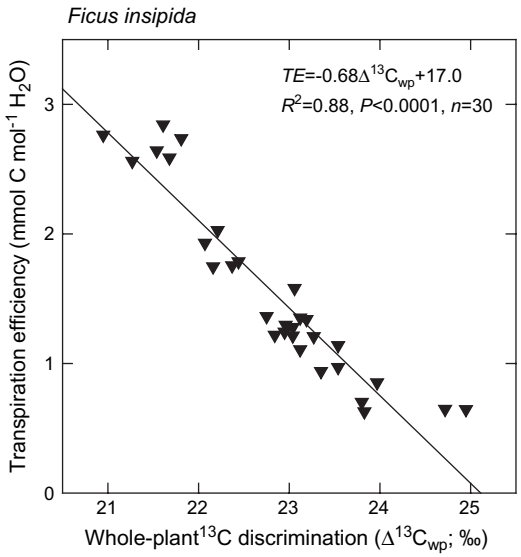

Fig. 4. Transpiration efficiency (*TE*) plotted as a function of whole-plant C isotope discrimination ($\Delta^{13}C_{wp}$) for *Ficus insipida* plants subject to varying soil fertility. The $\Delta^{13}C_{wp}$ was calculated from measurements of $\delta^{13}C$ and C mass in leaves, stems, and roots. The $\delta^{13}C$ of ambient air was assumed to be –8‰.

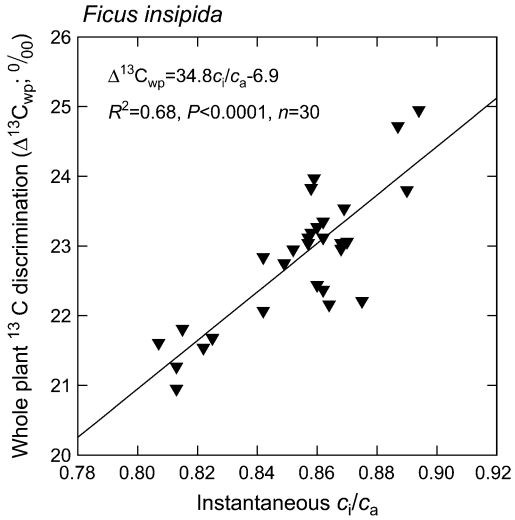

Fig. 5. Whole-plant C isotope discrimination ($\Delta^{13}C_{wp}$) plotted as a function of the ratio of intercellular to ambient CO_2 mole fractions (c_i/c_a) for plants of *Ficus insipida* subject to varying soil fertility. The c_i/c_a was calculated from instantaneous leaf gas exchange measurements made during the morning and at midday on 20 October 2005. The $\Delta^{13}C_{wp}$ was calculated from measurements of $\delta^{13}C$ and C mass in leaves, stems, and roots. The $\delta^{13}C$ of ambient air was assumed to be –8‰.

the parameters ϕ_c and c_a, the selected ranges represent best guesses at likely limits of their variation. Table 5 suggests that the variation that was observed in *TE* was largely driven by variation in c_i/c_a.

Discussion

Large variation in growth and whole-plant water-use efficiency of a tropical pioneer tree in response to variation in soil fertility was observed under non-limiting soil moisture conditions. Analyses of elemental concentrations in dry matter of the experimental plants indicated that treatment differences in *RGR* and *TE* resulted largely from variation in N availability. Leaf photosynthesis and dark respiration rates were well correlated with leaf N_{area}, as was *RGR* (Fig. 3). Variation in *TE* was linearly correlated with variation in c_i/c_a, both for instantaneous measurements of c_i/c_a, and for integrated estimates based on $\Delta^{13}C_{wp}$ (Figs 4, 5). The c_i/c_a, $\Delta^{13}C_{wp}$, and *TE*, in turn, were well correlated with variation in leaf N_{area} and leaf photosynthesis (Fig. 6). The response of *TE* to soil fertility was largely caused by variation in c_i/c_a; on the other hand, variations in *v*, ϕ_c and ϕ_w probably played only minor roles in modulating *TE* in response to soil fertility (Table 5). The variation in c_i/c_a resulted from variation in photosynthetic capacity caused by variation in leaf N_{area}, rather than from variation in stomatal conductance (Table 2; Fig. 6).

Elemental composition

Of the mineral elements quantified in the leaves of the experimental plants, leaf N showed the strongest positive relationship with *RGR*. The only other element to be

positively correlated with *RGR* was Ca. However, leaf Ca per unit area explained only 13% of variation in *RGR*, whereas leaf N per unit area explained 46%. Thus, it is concluded that plant growth was primarily constrained by N availability. Of the other measured elements, P, Mg, and Zn showed positive linear correlations with E_{grav}, suggesting that these elements were absorbed in relatively constant proportion to the water flux into the roots. The relationship with leaf P was particularly striking, with variation in E_{grav} explaining 74% of variation in leaf P_{area} (Fig. 2A). This relationship was surprising, given that P is generally thought to be relatively insoluble in soils. Supply of a source of mycorrhizal inoculant at planting and the high organic matter content of the soil due to the addition of rice husks may have played some role in enabling the relationship between leaf P_{area} and E_{grav} in our experiment. In contrast to the relationship between leaf P_{area} and E_{grav}, there was a negative correlation between leaf N_{area} and E_{grav} across the soil fertility treatments. Variation in leaf N_{area} explained 29% of variation in E_{grav}. Increases in transpiration in response to low N availability have also been observed in other tree species (Guehl *et al*., 1995; Livingston *et al*., 1999), and transpirational control of N accumulation was previously demonstrated in a mistletoe/tree complex (Marshall *et al*., 1994).

A non-linear response of *RGR* to leaf N/P was observed (Fig. 1B). The maximum *RGR* occurred near N/P of 15, which agrees well with the prediction that N and P should be in balanced supply at N/P between 14 and 16 (Koerselman and Meuleman, 1996). Koerselman and Meuleman (1996) suggested that at N/P <14, growth would be N limited, whereas at N/P >16, growth would be P limited. This further reinforces the notion that variation in *RGR* and *TE* in our experiment was primarily caused by differential N availability.

It was found that N/P was an excellent predictor of variation in *TE* (Fig. 1A); the natural logarithm of N/P explained 90% of variation in *TE*. For comparison, $\Delta^{13}C_{wp}$ explained 88% of variation in *TE*. The close correlation between N/P and *TE* stemmed from the relationships between leaf N and photosynthetic rate (Fig. 3A) and leaf P and transpiration rate (Fig. 2A). Whether such a relationship will hold up more generally outside our experimental conditions is unknown.

C isotope discrimination and c_i/c_a

Estimating c_i/c_a from $\Delta^{13}C$ according to equation (3) requires an estimate of *d*. The method described in the theory section for estimating *d*, based on the coefficients of a regression analysis between *TE* to $\Delta^{13}C$, assumes that the terms for which the slope and intercept coefficients substitute are invariant over the range of the analysis. This assumption was not strictly met in our experiment; for example, there were probably subtle variations in *v* and ϕ_w among treatments (Tables 2, 4). However, if the assumption were strongly violated, one would expect either to see curvature in the relationship between *TE* and $\Delta^{13}C_{wp}$, or a large degree of scatter. In fact, the relationship that was observed appeared linear with little scatter: variation in $\Delta^{13}C_{wp}$ explained 88% of variation in *TE* in a least-squares linear regression (Fig. 4). There were, however, two data points that appeared to depart slightly from the linear trend; these two points had the highest $\Delta^{13}C_{wp}$ values in the dataset (Fig. 4). Repeating the analysis with these two data points excluded would result in an estimate for *d* of 4.4‰, whereas the estimate for the full data set was 4.0‰. Although this difference is not large, it nonetheless highlights the sensitivity of our method for estimating *d* to variations in the values of the regression coefficients.

Few direct estimates of *d* exist in the literature, although assessment of *d* is implicit in the determination of mesophyll conductance from instantaneous measurements of $\Delta^{13}C$ and c_i/c_a (Evans *et al*., 1986). Hubick *et al*. (1986) estimated *d* to be approximately 3‰, based on the simultaneous measurements in wheat of $\Delta^{13}C$ and c_i/c_a (Evans *et al*., 1986). The *d* was later estimated to be near zero for barley (Hubick and Farquhar, 1989), and approximately 1‰ for peanut (Hubick, 1990). Thus, our estimate of 4.0‰ for *d* in *Ficus insipida* is slightly higher than values previously reported for crop plants. In the method used here to calculate *d* [i.e., $d=b–I/m$ from equation (8)], the value of *d* clearly depends on the assumed value of *b*. Fortunately, any change in the assumed value of *b* has only a minor effect on $\Delta^{13}C$-based estimates of c_i/c_a. This is because any change in the assumed value of *b* will be offset by a similar change in the calculated value of *d*. Thus, changing the assumed value of *b* from 29‰ to 27‰ in our analysis would only change the mean $\Delta^{13}C$-based estimate of c_i/c_a from 0.913 to 0.906.

It is clear from equation (4) that *d* is a complex parameter, and the general trend in the literature has been to drop it from equation (3) when making long-term, integrated estimates of c_i/c_a from measurements of $\Delta^{13}C$ in plant tissues. However, based on our analysis, it is suggested that it may not be prudent to omit *d* from equation (3). Assuming $b=29‰$, excluding *d* from the calculations would shift the mean $\Delta^{13}C_{wp}$-based estimate of c_i/c_a in our experiment from 0.91 to 0.75. Whereas if we assumed $b=27‰$, the mean Δ-based estimate of c_i/c_a would shift from 0.91 to 0.82 if *d* were omitted. If these c_i/c_a estimates were then used to predict *TE* from equation (2), the shift caused by omitting *d* would equate to an approximately 3-fold increase in predicted *TE* at $b=29‰$, and a doubling of predicted *TE* at $b=27‰$.

It was observed that instantaneous measurements of c_i/c_a were consistently lower than $\Delta^{13}C_{wp}$-based estimates

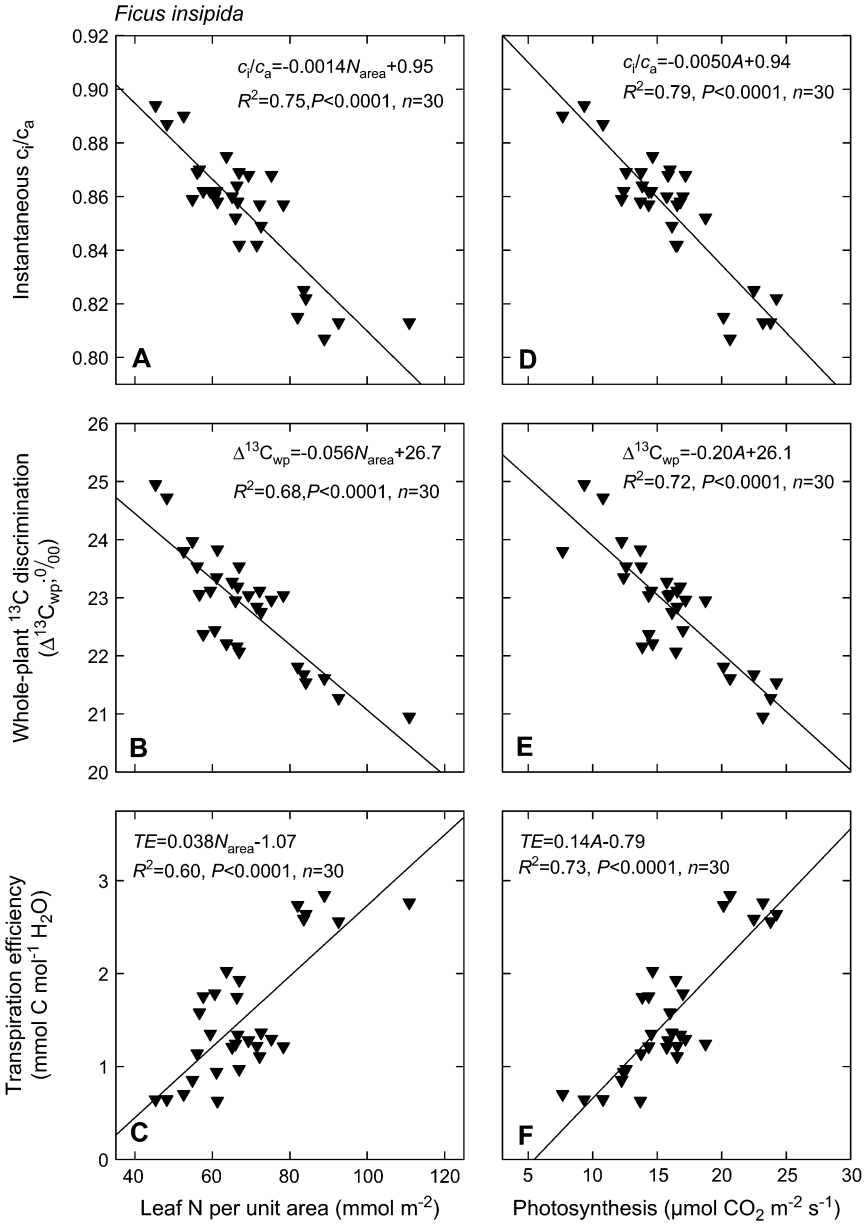

Fig. 6. Ratio of intercellular to ambient CO_2 mole fractions, c_i/c_a, determined from instantaneous gas-exchange measurements (A), whole-plant C isotope discrimination, $\Delta^{13}C_{wp}$ (B), and transpiration efficiency, *TE* (C) plotted against leaf N per unit area. Similarly, c_i/c_a (D), $\Delta^{13}C_{wp}$ (E), and *TE* (F) plotted against light-saturated net photosynthetic rate of the youngest fully-expanded leaf.

across treatments (Table 2). The mean instantaneous estimate for all treatments combined was 0.85, whereas the mean $\Delta^{13}C_{wp}$-based estimate was 0.91. This discrepancy may have partly resulted from differences between *v* and *PFD* averaged over the course of the experiment, as compared with values in the cuvette during instantaneous measurements (8 mmol mol^{-1} versus 15 mmol mol^{-1} for *v*, respectively; and 400 μmol m^{-2} s^{-1} versus 1200 μmol m^{-2} s^{-1} for *PFD*, respectively). However, at least part of the discrepancy between instantaneous c_i/c_a and $\Delta^{13}C_{wp}$-based c_i/c_a relates to the fact that c_i/c_a is calculated differently from instantaneous gas exchange measurements than from isotopic measurements, as described by GD Farquhar (unpublished presentation, BASIN meeting, Marshall, USA, 2004). The equations used to calculate c_i from instantaneous measurements are based on a ternary system of gases: CO_2, water vapour, and air (Jarman, 1974). The c_i is calculated as $c_i=[(g_c–E/2)c_a–A]/(g_c+E/2)$, where g_c is the total conductance to CO_2 of stomata plus boundary layer (Caemmerer and Farquhar, 1981). This calculation takes into account not only collisions between CO_2 and air, but also collisions between CO_2 and water vapour. By contrast, the equations presented in the theory section of this paper describing the relationship between

Table 5. *A sensitivity analysis of the dependence of transpiration efficiency (TE) on c_a, ϕ_c, c_i/c_a, v, and ϕ_w*

Symbols are defined as follows: c_a, ambient CO_2 mole fraction; ϕ_c, the proportion of net photosynthesis used for respiration; c_i/c_a, the ratio of intercellular to ambient CO_2 mole fractions; v, the leaf-to-air water vapour mole fraction difference; and ϕ_w, unproductive water loss as a proportion of productive water loss. Values of the input parameters were varied over their ranges one at a time, and the change in *TE* calculated according to equation (2). Parameters that were not being varied during each calculation were fixed at the median value in the given range.

Parameter	Range of values		Change in *TE* (mmol C mol^{-1} H_2O)
c_a (μmol mol^{-1})	370	380	–0.04
ϕ_c	0.35	0.45	0.25
c_i/c_a	0.86	0.96	1.66
v (mmol mol^{-1})	6.0	9.0	0.62
ϕ_w	0.11	0.15	0.05

c_i/c_a and $\Delta^{13}C$ do not take into account collisions between CO_2 and water vapour. Instead, c_i is simply defined as $c_i=c_a–A/g_c$. If the data from the instantaneous measurements are recalculated using this latter definition of c_i, a mean value for c_i/c_a of 0.88 is obtained, which cuts in half the observed difference between instantaneous and $\Delta^{13}C_{wp}$-based estimates of c_i/c_a.

In our analysis of the relationship between *TE* and $\Delta^{13}C$, the whole-plant $\Delta^{13}C$ was used rather than that of a single tissue, such as leaves. Using $\Delta^{13}C_L$ in place of $\Delta^{13}C_{wp}$ has only a small effect on our results. It would shift our estimate of d from 4.0‰ to 3.8‰, and would shift the mean $\Delta^{13}C$-based estimate of c_i/c_a from 0.91 to 0.92.

Unproductive water loss

The unproductive water loss described by the term ϕ_w in equation (2) comprises non-stomatal water loss during the day and night, and stomatal water loss at night. Of the two, the expectation was that transpiration at night through partially open stomata would dominate. Conductances of leaf cuticles are on the order of 1–2 mmol m^{-2} s^{-1} (Kerstiens, 1996), as are surface conductances of branches and stems (Cernusak and Marshall, 2000; Cernusak *et al*., 2001). In contrast, it is not uncommon to observe nocturnal stomatal conductances ranging from 20 to more than 100 mmol m^{-2} s^{-1} (Donovan *et al*., 1999; Snyder *et al*., 2003; Bucci *et al*., 2004; Barbour *et al*., 2005; Seibt *et al*., 2007). The mean nocturnal stomatal conductance that was observed during the dark respiration measurements was 124±43 mmol m^{-2} s^{-1} (mean ±1 SD). However, because nocturnal v is typically low (mean value of 1.3 mmol mol^{-1} in our experiment), as are nocturnal wind speeds, nocturnal transpiration is still likely to be small in comparison to daytime transpiration. A mean ϕ_w of 0.12 was observed in our experiment, indicating that nocturnal transpiration was equal to 0.12 times daytime transpiration. Expressed as a percentage of total transpiration, nocturnal transpiration was 11% on average. This can be compared to observations of nocturnal transpiration as a percentage of total transpiration of 5% for *Eucalyptus grandis* (Benyon, 1999), 10% for *Betula papyrifera* (Daley and Phillips, 2006), and 13–28% for woody species in Brazilian savanna (Bucci *et al*., 2004, 2005). The mean ϕ_w of 0.12 that was observed is similar to the value of 0.18 cited by Hubick and Farquhar (1989).

Data reviewed above suggest maximum likely values for ϕ_w of about 0.4. By contrast, values for ϕ_w ranging from 3.3 to 5.6 were reported for an experiment with seedlings of *Pinus sylvestris* (Hobbie and Colpaert, 2004). This would suggest that nocturnal transpiration was 3.3–5.6 times greater than daytime transpiration. The possibility is suggested that these surprisingly high estimates of ϕ_w may have resulted from omitting d from equation (3), which was used to calculate c_i/c_a from measurements of $\Delta^{13}C$. Mean c_i/c_a for the *Pinus sylvestris* seedlings was estimated to be 0.65. This value can be compared to the mean $\Delta^{13}C$-based c_i/c_a estimate of 0.91 for our experiment with *Ficus insipida*. The *Pinus sylvestris* seedlings were grown under similar conditions to those in our experiment: low irradiance (300 μmol photons m^{-2} s^{-1}), low v (7.3 mmol mol^{-1}), and low N availability. The relative insensitivity of equation (2) to variations in ϕ_w, as shown in Table 5, means that if the equation is inverted to solve for ϕ_w, as was done in the experiment with *Pinus sylvestris*, the predicted values of ϕ_w can be highly sensitive to any bias in terms such as c_i/c_a.

Although significant variation in ϕ_w was observed among treatments in our experiment, the range of variation was small, with treatment means ranging from 0.11 to 0.15 (Table 1). This narrow range of values occurred over a large gradient in soil fertility, as evidenced by the large variation among treatments in *RGR* (Table 2). Compared with other terms in equation (2), such as c_i/c_a and v, the ϕ_w played a very minor role in our experiment in modulating *TE* (Table 5).

Leaf-to-air humidity gradient

Three different methods were employed to estimate v for the experimental treatments. Whereas the three methods yielded a similar range of results, there were some differences in the direction of variation proceeding from low soil fertility to high soil fertility (Table 4). In using the leaf energy balance model to estimate v, it was necessary to assume a relationship between the mean intercepted irradiance at the leaf level and the irradiance measured outside the canopy. It was assumed that the former would be 0.75 times the latter for all treatments. This assumption is probably not realistic because the increasing plant size and leaf area going from low to high soil fertility (Table 2) would have been accompanied by

increased self shading, and increasingly non-horizontal leaf orientation. Therefore, the mean intercepted irradiance at the leaf surface was likely less at high soil fertility than at low soil fertility. Thus, the leaf energy balance model may have overestimated v, especially for the highest soil fertility treatment, where the leaf area per plant was substantially greater than in the other treatments (Table 2).

The $\Delta^{18}O_p$ method, based on equations (9) to (15), provides a means for estimating v that could have considerable advantages over the other two. Namely, it provides an integrated measurement over the life of the plant, based on a single isotopic analysis of plant organic material. The disadvantage of this method is that it requires assumed values for a large number of parameters. Some of these parameters, such as L and ε_{cp}, have been measured only for a small number of species, so the extent to which they can be generalized is largely unknown (Barbour, 2007). Nonetheless, it is encouraging that it was possible to obtain estimates of v that agreed rather well with the other two methods of estimation (Table 4).

The final method that was used to estimate v was one that involved assuming a constant value for ϕ_c. It has been suggested that the term ϕ_c should be a relatively conservative parameter in terrestrial plants (McCree and Troughton, 1966; Gifford, 1994, 2003; Dewar *et al*., 1999; Thornley and Cannell, 2000). Estimates of ϕ_c for individual plants are typically in the range of about 0.35 to 0.45 (McCree, 1986; Gifford, 2003). The slope of the regression between TE and Δ suggests a mean ϕ_c for our experiment near the midrange value of 0.4. Using the slope coefficient and the relationship with ϕ_c described in the theory section, a mean ϕ_c of 0.4 would correspond to a mean v across treatments of 7.0 mmol mol^{-1}, close to the mean values for v estimated by the leaf energy balance model and the $\Delta^{18}O_p$ method (Table 4). Although using the slope coefficient in this way does not allow us to test for variation among treatments in ϕ_c, no significant variation was observed among treatments at the leaf level in the ratio of dark respiration/net photosynthesis, and systematic variation across treatments in leaf area ratio was not observed (Table 2). Thus, it is suggested that it is possible that there was relatively little variation in ϕ_c in our experiment.

Farquhar *et al*. (1989) noted that v in equation (2) should actually be weighted by conductance, because a particular v results in greater water loss when conductance is large than when it is small. This consideration suggests an added complexity to v that may not be well represented in the leaf energy balance or $\Delta^{18}O_p$ models. Solving equation (2) for v, using measured data, and assuming c_a of 375 μmol mol^{-1} and ϕ_c of 0.40, resulted in a smaller range of values across treatments than the other two methods (Table 4). Although this method of estimating v requires assuming values for c_a and ϕ_c, the possible ranges of these two parameters are reasonably well constrained. Therefore, we suggest that v predictions based on this method probably provided the most reliable estimates under our experimental conditions.

O isotope enrichment

A positive correlation was observed between $\Delta^{18}O_p$ and mean daytime E_{grav} (Fig. 2B). Sheshshayee *et al*. (2005) recently observed similar positive correlations for different genotypes of groundnut and rice. In their experiments, plant transpiration was measured gravimetrically and expressed as mean transpiration rate (*MTR*), where $MTR=E_{tot}/[(LA_1+LA_2)0.5t]$, where E_{tot} is cumulative transpiration over the course of the experiment, LA_1 and LA_2 are leaf area at the beginning and end of the experiment, and t is number of days in the experiment. If our transpiration data are expressed as *MTR* and $\Delta^{18}O_p$ plotted against it, a positive linear relationship is also observed, in which $\Delta^{18}O_p=0.017MTR+23.2$ ($R^2=0.45$, $P<0.0001$, $n=30$).

Farquhar *et al*. (2007) recently reviewed theory underlying steady-state leaf water enrichment, and posed the following question: as E increases, $\Delta^{18}O$ should also increase, true or false? Equations (9) through (13) suggest the following response: when the source of variation in E is evaporative demand, $\Delta^{18}O$ should increase with increasing E; conversely, when the source of variation in E is stomatal, $\Delta^{18}O$ should decrease with increasing E (Farquhar *et al*., 2007). At first glance, data presented in Fig. 2B appear to be in disagreement with this generalized response. This is because the plants were grown side by side during the same time period, so that the evaporative demand of the bulk atmosphere was the same for all plants. However, closer examination of the data in Table 2 reveals that the source of variation in E_{grav} among treatments was not stomatal. By contrast, increasing stomatal conductance across soil fertility treatments was actually associated with decreasing E_{grav}. The decreasing E_{grav} was probably associated with decreasing v and increasing canopy boundary layer resistance at high soil fertility as compared to low soil fertility. The increased leaf area per plant with increasing soil fertility would have caused both of these factors, through increased self-shading, and therefore lower canopy-averaged T_L, and increased canopy boundary layer development. As seen in equations (9) and (11), these processes would also result in decreasing $\Delta^{18}O_e$, due to greater w_a/w_i and lower ε_k. The decrease in $\Delta^{18}O_e$ accompanying lower E would be countered by a relative increase in $\Delta^{18}O_L$, as seen in equation (12); however, in our data set, this relatively subtle shift caused by the Péclet effect was apparently not sufficient to overcome the effect of lower $\Delta^{18}O_e$, such that the net result was a decrease in $\Delta^{18}O_p$ with decreasing E_{grav}.

Uncoupling is commonly observed between cuvette-based measurements of stomatal conductance and transpiration measured at the whole plant level for tropical trees (Meinzer *et al*., 1996). In our experiment, cuvette-based measurements of E were 5-fold higher than corresponding values for E_{grav} (Table 2). The discrepancy can be partly accounted for by variation in v, which was 2-fold greater in the cuvette than the average value in ambient air during E_{grav} measurments. The remaining variation relates to differences in leaf boundary layer resistance between the cuvette and the ambient environment, and possibly to variation in stomatal conductance between the youngest, fully-expanded leaf, on which gas exchange measurements were made, and the integrated average over the whole canopy. This uncoupling, in addition to the surprising result of the positive correlation between $\Delta^{18}O_p$ and E_{grav}, demonstrates the importance of measuring physiological processes at the whole-plant scale, in addition to the leaf scale.

Conclusions

It was observed that c_i/c_a was the primary control over variation in *TE* in response to soil fertility in a pioneer tree grown under tropical field conditions (Table 5). It is suggested that c_i/c_a may be a particularly important control over whole-plant water-use efficiency in fast-growing tropical trees, where c_i/c_a may generally be high, as evidenced by low $\delta^{13}C$ (Guehl *et al*., 1998; Martinelli *et al*., 1998; Bonal *et al*., 2000*a*, *b*; Holtum and Winter, 2005). When c_i/c_a is high, the impact of any change in c_i/c_a on *TE* is amplified. This is because the relevant term in equation (2) is not actually c_i/c_a, but rather $(1–c_i/c_a)$. For example, a change in c_i/c_a from 0.95 to 0.90, while seemingly trivial, will cause a doubling of *TE*, all else being equal.

The strong response of c_i/c_a and *TE* to leaf N_{area} in tropical trees, as observed here and previously (Cernusak *et al*., 2007), could have important implications for the management of tropical forest plantations, particularly where managers aim to maximize biomass production relative to stand water use; in addition, it could have important implications for the coupling of C and water cycles of tropical vegetation subject to anthropogenic alterations in N availability.

Acknowledgements

We thank Milton Garcia, Rodrigo Nuñez, Tania Romero, and Aurelio Virgo for technical assistance, and Bob Brander, Ben Miller, and Ben Harlow for assistance with isotopic analyses. We thank two anonymous reviewers for helpful comments on the manuscript. This research was funded by the AW Mellon Foundation and the Smithsonian Tropical Research Institute. LAC was supported by a Smithsonian Institution Postdoctoral Fellowship.

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