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Research paper

Transpiration modulates phosphorus acquisition in tropical tree seedlings

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Several experiments were conducted with tropical tree and liana seedlings in which transpiration ratio and leaf phosphorus to carbon ratio (P:C) were measured. Transpiration ratio was expressed as kg H_2O transpired g^{-1} C incorporated into plant biomass, and leaf P:C as mg P g^{-1} C. Leaf P:C was positively correlated with transpiration ratio across 19 species for plants grown under similar conditions ($R^2 = 0.35$, $P < 0.01$, $n = 19$). For five species in the dataset, multiple treatments were imposed to cause intra-specific variation in transpiration ratio. Within four of these five species, leaf P:C correlated positively with transpiration ratio. The slope and strength of the correlation varied among species. In one experiment, whole-plant P:C was measured in addition to leaf P:C. Patterns of correlation between whole-plant P:C and transpiration ratio were similar to those between leaf P:C and transpiration ratio. Together, these observations suggest that transpiration can influence the rate of P uptake from soil in tropical tree and liana seedlings. We suggest that this occurs through transport of inorganic phosphate and organic P compounds to root surfaces by transpiration-induced mass flow of the soil solution. The positive correlation between leaf P:C and transpiration ratio suggests that leaf P:C could decline in tropical forests as atmospheric CO_2 concentration rises, due to decreasing transpiration ratios.

Keywords: elevated CO_2, mass flow, phosphorus concentration, water-use efficiency.

Introduction

Phosphorus (P) availability is generally assumed to impose a significant constraint on productivity in tropical forests (Grubb 1977, Vitousek 1984, Herbert and Fownes 1995, McGroddy et al. 2004, Reich and Oleksyn 2004), although there are exceptions to this, for example, in montane forests and early successional forests (Tanner et al. 1998, Davidson et al. 2007, LeBauer and Treseder 2008). Because P acquisition is an important determinant of tropical tree growth, an understanding of acquisition strategies and mechanisms is important for predicting responses of tropical forest productivity to global change phenomena and other anthropogenic perturbations.

Nutrients in the soil solution can be transported to root surfaces either by diffusion or by mass flow (Barber 1995, Tinker and Nye 2000, McDonald et al. 2002, Cramer et al. 2008, Cramer and Hawkins 2009, Cramer et al. 2009). Mass flow of the soil solution occurs as a consequence of plant transpiration. Under steady-state conditions, and with all else being equal, an increased rate of water uptake by roots will result in a higher solute concentration at the root surface (Nye and Tinker 1977). Increasing the concentration of a solute at the root surface could lead to an increased rate of solute transport across the membranes of root cells in three ways: by an increased diffusive flux, an increased convective flux or an increased rate of active uptake (Dalton et al. 1975, Fiscus 1975, Fiscus and Kramer 1975). Thus, transpiration can play a role in modulating nutrient uptake by delivering nutrients to root surfaces through mass flow, and by influencing the convective flux of solutes into root cells if the reflection coefficient of root cell membranes for that solute is less than unity.

Phosphorus is mainly absorbed into root cells as inorganic phosphate, P_i (Bieleski 1973, Schachtman et al. 1998). The P_i

concentrations in soil solution are typically low, in the order of 1 μM (Bieleski 1973, Barber 1995). For this reason, it has generally been assumed that transpiration-induced mass flow of the soil solution will play only a small role in supplying P to root surfaces (Bieleski 1973). A rough calculation demonstrates why this is the case. Assuming a $[P_i]$ in the soil solution of 1 μM, a transpiration ratio of 1 kg H_2O g^{-1} C and a plant biomass P:C of 3 mg P g^{-1} C, mass flow of the soil solution would deliver to root surfaces ~1% of the total P taken up by the plant. However, in some soil solutions P_i concentrations may be substantially higher than 1 μM (Barber 1995). In addition, mass flow may transport dissolved organic compounds to root surfaces, where soil phosphatase activity can be several-fold higher than in bulk soil (Richardson et al. 2005). Extracellular phosphatase enzymes are capable of hydrolyzing some organic P compounds, thereby releasing P_i that can be subsequently transported across root cell membranes.

Transpiration ratio is a measurement of plant water-use efficiency that has been employed since early research on the topic (Woodward 1699, Lawes 1850, Briggs and Shantz 1914, Shantz and Piemeisel 1927). It describes the rate of water loss from a plant for a given amount of dry matter production. Transpiration ratio typically decreases with increasing atmospheric CO_2 concentration (Morison 1985, Eamus 1991, Farquhar 1997, Winter et al. 2001), decreases with increasing soil fertility (Guehl et al. 1995, Livingston et al. 1999, Raven et al. 2004, Ripullone et al. 2004, Cernusak et al. 2007*b*) and decreases with decreasing water supply (Hubick 1990, Zhang and Marshall 1994, Sun et al. 1996, Cernusak et al. 2009).

In conducting research into the water-use efficiency of tropical trees, we have noted that leaf P concentration tends to correlate with plant transpiration rate (Cernusak et al. 2007*b*, 2010, Garrish et al. 2010). In this paper, we bring together data from several previous experiments and a new experiment to test the hypothesis that P:C of tropical tree and liana seedlings correlates with their transpiration ratios, expressed as kg H_2O transpired g^{-1} C incorporated into plant biomass. Comparison of these two parameters provides a meaningful test of the relationship between transpiration and plant P uptake because both are expressed on a common basis, i.e. per unit C gained by the plant. A positive correlation between these two parameters would provide evidence for a previously unappreciated role of transpiration in modulating P uptake in tropical trees and lianas.

Materials and methods

The data presented in this paper are derived from a series of experiments that took place at the Santa Cruz Experimental Field Facility, Smithsonian Tropical Research Institute, Gamboa, Panama (9°07′N, 79°42′W). Comparisons between leaf P:C and transpiration ratio for these experiments are presented for the first time here. Results relating to growth, water-use efficiency and other physiological processes have been presented previously for four of the experiments (Cernusak et al. 2007*a*, 2007*b*, 2008, 2009, 2010).

A fifth experiment was also conducted, in which the method for determining transpiration ratio was the same as that implemented in the previous experiments. In the fifth experiment, plants were grown in two glasshouses: one had CO_2 concentration similar to ambient (400 ppm) and the other had elevated CO_2 concentration (700 ppm). The elevated CO_2 concentration was maintained by releasing CO_2 gas from a high-pressure cylinder. The glasshouses were air conditioned, with the air conditioners programmed to turn on when air temperature exceeded 30 °C.

Twenty seedlings each of *Swietenia macrophylla* and *Ormosia macrocalyx* were grown in each of the two glasshouses. Seeds were collected from trees growing in the Panama Canal watershed. Seeds were collected from several mature individuals of each species. Seedlings were grown individually in 19 l pots. Each pot contained 13.5 kg dry homogenized soil mixture. The soil mixture comprised a 3:2 volumetric mixture of dark, air-dried forest topsoil and air-dried rice husks. The pots were saturated with water and drained overnight to establish the pot water content at field capacity, which was determined to be 5.0 kg. The soil surface of each pot was then covered with 1.5 kg gravel to reduce evaporation. Ten control pots with no plants were placed in each glasshouse to estimate pot water loss due to evaporation from the soil surface.

Two fertilizer and two water supply treatments were implemented in each glasshouse. At the beginning of the experiment, 10 of the 20 pots for each species were randomly chosen to receive ~12 g Osmocote-Plus controlled-release fertilizer (Scotts-Sierra, Maryville, OH, USA). The fertilizer contained by weight 15% N, 9% P and 12% K, and had an estimated release time of 5–6 months. Five fertilized and five unfertilized pots from each species were then randomly allocated to receive reduced water supply. All pots started the experiment watered to field capacity. Those receiving high water supply were weighed each week and re-watered to near field capacity. Later in the experiment, pots were weighed and re-watered at shorter intervals, depending on water loss rates. Pots receiving low water supply were allowed to dry down to pot water contents of less than 1.5 kg, or ~30% of field capacity, over several weeks. Thereafter they were weighed and re-watered to this pot water content each week, or at shorter intervals, as necessary. Pots were weighed to the nearest 5 g with a 64 kg capacity balance (Sartorius QS64B, Thomas, Swedesboro, NJ, USA). Drain holes at the bases of the pots were sealed for the duration of the experiment.

Pots were placed in the ambient and elevated CO_2 concentration glasshouses on 8 January 2007, ~1 month after seedlings had germinated. Within each glasshouse, the pots were

placed on plastic tables, such that they were elevated ~1 m above the glasshouse floor. Initial plant dry mass was estimated by harvesting five representative individuals of each species. Mean values were 0.3 and 0.2 g for *O. macrocalyx* and *S. macrophylla*, respectively. Plants were harvested on 26 April 2007, after >3 months growth at either ambient or elevated CO_2 concentration.

Cumulative plant water use over the course of the experiment was calculated as the sum of pot water loss minus the average sum of water loss from the control pots. Pot water loss was determined for each weekly or sub-weekly interval in the experiment from the gravimetric measurements described above. Harvested plants were dried to constant mass at 70 °C. The transpiration ratio of each plant was calculated as $TR = E_t/(m_{C2} - m_{C1} + l_C)$, where m_{C1} and m_{C2} are the plant carbon masses at the beginning and end of the experiment, l_C is the carbon mass of leaves abscised over the course of the experiment and E_t is the sum of water transpired over the course of the experiment.

In the experiment of Cernusak et al. (2009), the same fertilizer and watering regime treatments as described above were implemented. It differed in that plants were grown under an open-air rain shelter, and therefore at ambient CO_2 concentration. The species included in that experiment were *S. macrophylla*, *Tectona grandis* and *Platymiscium pinnatum*. In the experiment of Cernusak et al. (2007*b*), seedlings of *Ficus insipida* were grown under an open-air rain shelter in volumetric mixtures of topsoil and rice husks ranging from 1:4 to 4:1, and with an additional treatment of 4:1 plus slow-release fertilizer. In the experiment of Cernusak et al. (2008), seedlings were grown in a 3:2 volumetric mixture of topsoil and rice husks. Plants were well watered and grown under an open-air rain shelter. Species included in that experiment were *Calophyllum longifolium*, *Cupressus lusitanica*, *Clusia pratensis*, *Gouania lupuloides*, *Hieronyma alchorneoides*, *Luehea seemannii*, *Mikania leiostachya*, *Pinus caribaea*, *P. pinnatum*, *Stigmaphyllon hypargyreum*, *S. macrophylla*, *T. grandis*, *Thuja occidentalis* and *Tabebuia rosea*. The experiment of Cernusak et al. (2007*a*) was similar, except that plants were grown in a 3:2 volumetric mixture of topsoil and leaf litter. Species included in that experiment were *Dalbergia retusa*, *F. insipida*, *Pachira quinata*, *P. pinnatum*, *Pseudobombax septenatum*, *S. macrophylla* and *T. grandis*.

In all experiments, dry matter of each plant was ground to a fine, homogeneous powder in a Cyclotec 1093 sample mill with a 0.5 mm screen (Foss, Eden Prairie, MN, USA). Carbon concentrations were measured by combusting ~3 mg plant material in an elemental analyzer (ECS 4010, Costech Analytical Technologies, Valencia, CA, USA) coupled to an isotope ratio mass spectrometer (Delta XP, Finigan MAT, Bremen, Germany). For the experiments of Cernusak et al. (2007*b*, 2008), leaf dry matter was analyzed for P concentration by acid digestion and detection on an inductively coupled plasma optical-emission spectrometer (Perkin Elmer Inc., Wellesley, MA, USA). Samples were prepared by digesting ~200 mg plant material under pressure at 180 °C in PTFE vessels with 2 ml of concentrated nitric acid. For the experiments of Cernusak et al. (2007*a*, 2009), and for the fifth experiment described above, plant dry matter was analyzed for P concentration by ashing at 550 °C, followed by dissolution in 1 M H_2SO_4, with phosphate detection by automated molybdate colorimetry using a Lachat Quickchem 8500 (Hach Ltd, Loveland, CO, USA).

Relationships between leaf or whole-plant P:C and transpiration ratio were analyzed by least-squares linear regression. Normality and the distribution of residuals in the regression analyses were improved by log transforming plant P:C and transpiration ratio prior to analysis. Thus, all regression analyses were performed on log-transformed data. However, we present untransformed data in all figures, for ease of interpretation by the reader. Results were considered to be statistically significant at $P < 0.05$. Statistical analyses were carried out in Systat 12 (Systat Software, Inc., Chicago, IL, USA).

Results

The relationship between leaf P:C and transpiration ratio of 19 tropical tree and liana species is shown in Figure 1. Plants

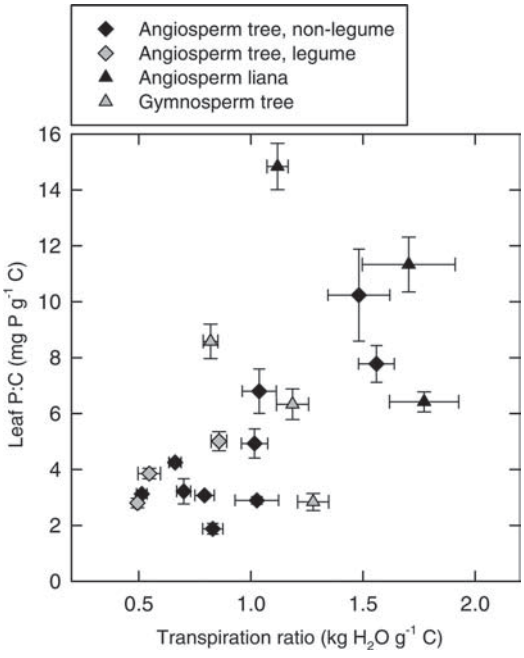

Figure 1. Leaf P:C for seedlings of 19 tropical tree and liana species plotted against transpiration ratio. Each data point represents the mean of several plants for one species. Error bars are one standard error. The identity and number of individuals included for each species are given in Table 1. Different symbols denote different functional groups. Plants were grown in unfertilized soil under well-watered conditions.

Table 1. A list of species included in the analysis presented in Figure 1. Mean leaf N:C, P:C and N:P, and mean transpiration ratio are given for each species, with the standard deviation shown in parentheses. Functional groups are angiosperm tree (AT), angiosperm liana (AL) and gymnosperm tree (GT).

Species	Family	Functional group	Number of plants	Leaf N:C (mg N g^{-1} C)	Leaf P:C (mg P g^{-1} C)	Leaf N:P (g N g^{-1} P)	Transpiration ratio (kg H_2O g^{-1} C)
Calophyllum longifolium	Clusiaceae	AT	6	22.8 (3.5)	1.88 (0.47)	12.4 (1.5)	0.83 (0.11)
Clusia pratensis	Clusiaceae	AT	6	29.5 (3.9)	3.22 (1.10)	9.7 (2.1)	0.70 (0.08)
Cupressus lusitanica	Cupressaceae	GT	8	21.6 (1.8)	6.33 (1.54)	3.6 (0.8)	1.19 (0.20)
Dalbergia retusa	Fabaceae	AT	7	68.9 (11.3)	2.80 (0.45)	24.6 (1.4)	0.49 (0.03)
Ficus insipida	Moraceae	AT	12	49.1 (7.0)	6.80 (2.75)	8.1 (2.5)	1.04 (0.26)
Gouania lupuloides	Rhamnaceae	AL	6	47.6 (3.5)	11.33 (2.40)	4.4 (0.9)	1.70 (0.51)
Hieronyma alchorneoides	Phyllanthaceae	AT	6	31.9 (3.8)	4.93 (1.28)	6.8 (1.5)	1.02 (0.14)
Luehea seemannii	Tiliaceae	AT	7	37.4 (4.0)	7.78 (1.74)	5.0 (1.4)	1.56 (0.21)
Mikania leiostachya	Asteraceae	AL	6	41.9 (7.3)	6.42 (0.88)	6.6 (1.2)	1.77 (0.38)
Ormosia macrocalyx	Fabaceae	AT	5	58.5 (5.1)	5.01 (0.76)	11.9 (2.5)	0.86 (0.08)
Pachira quinata	Malvaceae	AT	8	40.5 (4.1)	4.25 (0.36)	9.5 (0.9)	0.66 (0.08)
Pinus caribaea	Pinaceae	GT	9	22.0 (8.0)	2.84 (0.92)	7.6 (1.4)	1.28 (0.21)
Platymiscium pinnatum	Fabaceae	AT	18	64.3 (13.9)	3.85 (0.79)	16.8 (3.1)	0.55 (0.21)
Pseudobombax septenatum	Malvaceae	AT	7	32.6 (3.3)	3.12 (0.36)	10.4 (0.4)	0.51 (0.06)
Stigmaphyllon hypargyreum	Malpighiaceae	AL	7	51.4 (5.1)	14.84 (2.19)	3.5 (0.5)	1.12 (0.12)
Swietenia macrophylla	Meliaceae	AT	17	35.0 (3.3)	2.89 (0.56)	12.4 (2.0)	1.03 (0.40)
Tabebuia rosea	Bignoniaceae	AT	6	33.9 (5.9)	3.07 (0.13)	11.1 (2.2)	0.79 (0.11)
Tectona grandis	Verbenaceae	AT	11	26.0 (2.8)	10.24 (5.45)	3.7 (2.5)	1.48 (0.46)
Thuja occidentalis	Cupressaceae	GT	8	28.2 (2.8)	8.58 (1.74)	3.4 (0.8)	0.82 (0.09)

included in this analysis were grown under similar conditions. Forest topsoil was mixed either with rice husks or with leaf litter at a ratio of 3:2 on a volumetric basis. Plants were unfertilized and well watered. The identity and number of individuals for each species are detailed in Table 1. Leaf P:C was significantly correlated with transpiration ratio across these 19 species (Figure 1). The regression equation relating the two parameters was $\log_{10}(\text{leaf P:C}) = 0.85 \log_{10}(\text{TR}) + 0.72$ ($R^2 = 0.35$, $P < 0.01$, $n = 19$), where TR is transpiration ratio in kg H_2O g^{-1} C and leaf P:C is in mg P g^{-1} C.

Leaf P:C also correlated with transpiration ratio within species. Variation in transpiration ratio was induced in these experiments by growing plants in fertilized or unfertilized soil, at high or low water supply, under ambient or elevated CO_2 concentration, and in variable mixtures of forest topsoil and rice husks. These data are shown in Figures 2–5. For the pioneer tree *F. insipida*, transpiration ratio explained 77% of variation in leaf P:C (Figure 2). The regression equation relating leaf P:C to transpiration ratio for *F. insipida* was $\log_{10}(\text{leaf P:C}) = 0.82 \log_{10}(\text{TR}) + 0.79$ ($R^2 = 0.77$, $P < 0.001$, $n = 36$).

Leaf P:C correlated significantly with transpiration ratio in *S. macrophylla* (Figure 3). This analysis included seedlings grown at both ambient and elevated CO_2 concentrations. The regression equation relating leaf P:C to transpiration ratio for *S. macrophylla* was $\log_{10}(\text{leaf P:C}) = 0.36 \log_{10}(\text{TR}) + 0.44$ ($R^2 = 0.34$, $P < 0.001$, $n = 71$).

Data for *T. grandis* were clumped at high and low transpiration ratios, such that the distribution of data along the transpiration ratio axis was not continuous (Figure 4). For this reason, we did not perform a regression analysis of data for this species. However, it can clearly be seen without the aid of statistical analysis that the group of plants that had higher transpiration ratios also had higher leaf P:C.

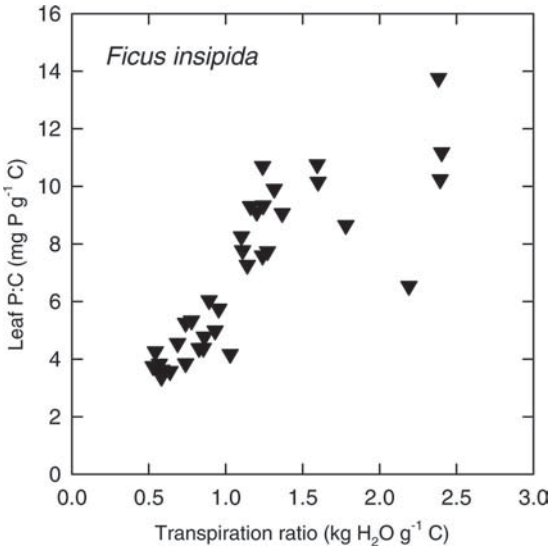

Figure 2. Leaf P:C plotted against transpiration ratio for seedlings of *F. insipida*. Each data point represents one plant. Plants were grown in varying mixtures of topsoil and rice husks, and with or without added fertilizer. Growth conditions are described in detail by Cernusak et al. (2007*a*, 2007*b*).

For the leguminous tree species *P. pinnatum*, transpiration ratio explained 21% of variation in leaf P:C (Figure 5). The regression equation relating leaf P:C to transpiration ratio

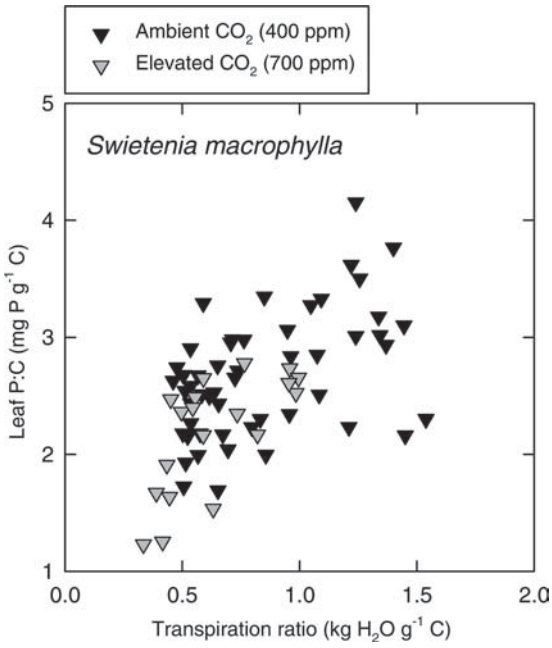

Figure 3. Leaf P:C plotted against transpiration ratio for seedlings of *S. macrophylla*. Each data point represents one plant. Plants were grown with or without added fertilizer, at high or low water supply, and at ambient or elevated CO_2 concentration. Growth conditions are described in detail by Cernusak et al. (2007*a*, 2008, 2009, 2010).

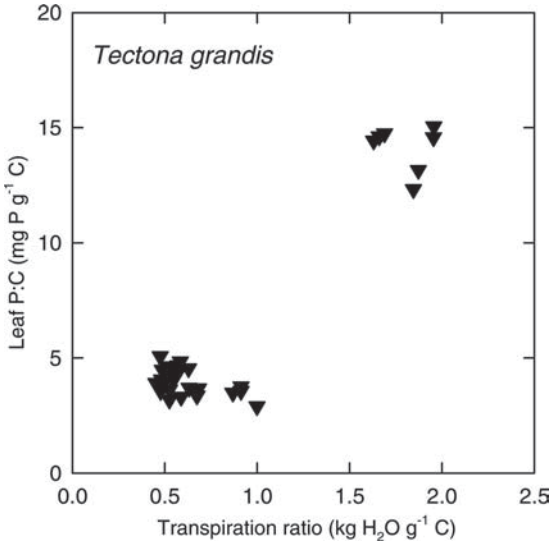

Figure 4. Leaf P:C plotted against transpiration ratio for seedlings of *T. grandis*. Each data point represents one plant. Plants were grown with or without added fertilizer, and at high or low water supply. Growth conditions are described in detail by Cernusak et al. (2007*a*, 2008, 2009).

for *P. pinnatum* was $\log_{10}(\text{leaf P:C}) = 0.34 \log_{10}(\text{TR}) + 0.69$ ($R^2 = 0.21$, $P < 0.01$, $n = 33$). For a second leguminous tree species, *O. macrocalyx*, there was no correlation between leaf P:C and transpiration ratio ($P = 0.45$, $n = 40$). This species was grown with and without fertilizer, at high and low water

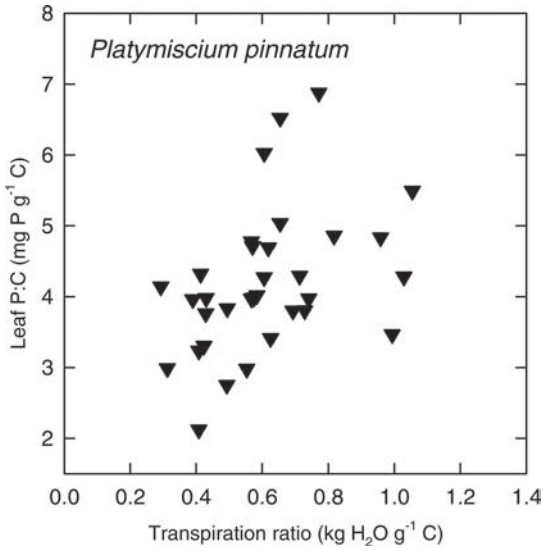

Figure 5. Leaf P:C plotted against transpiration ratio for seedlings of *Platymiscium pinnatum*. Each data point represents one plant. Plants were grown with or without added fertilizer, and at high or low water supply. Growth conditions are described in detail by Cernusak et al. (2007*a*, 2008, 2009).

supply, and at ambient and elevated CO_2 concentration. Overall, *O. macrocalyx* showed a range of variation in transpiration ratio that was similar to that of *P. pinnatum*, and therefore narrower than that of *F. insipida*, *S. macrophylla* and *T. grandis* (Figures 2–5).

Thus, of the five species for which plants were grown in multiple treatments designed to induce variation in transpiration ratio, four showed significant correlations between leaf P:C and transpiration ratio. Slopes of the log–log relationships between the two parameters differed among species, ranging from 0.34 for *P. pinnatum* to 0.82 for *F. insipida*.

For all plants in the dataset combined, transpiration ratio explained 30% of variation in leaf P:C. The combined relationship was as follows: $\log_{10}(\text{leaf P:C}) = 0.67 \log_{10}(\text{TR}) + 0.68$ ($R^2 = 0.30$, $P < 0.001$, $n = 307$). There was more variation in leaf P:C at high than at low transpiration ratios, such that the distribution of residuals in the regression analysis was clearly improved by log-transforming the two parameters.

For one of the experiments included in the dataset, we measured whole-plant P:C in addition to leaf P:C. This experiment involved measurements on 39 individuals of *S. macrophylla* and on 40 individuals of *O. macrocalyx* grown in fertilized and unfertilized soil, at high and low water supply, and under elevated and ambient CO_2 concentration. For these data, we tested whether correlations between whole-plant P:C and transpiration ratio were stronger or weaker than correlations between leaf P:C and transpiration ratio. We found that the strength of the correlations was similar between either whole-plant P:C or leaf P:C and transpiration ratio. For *S. macrophylla*, transpiration ratio explained 60% of variation in leaf P:C

($P < 0.001$, $n = 39$) and 58% of variation in whole-plant P:C ($P < 0.001$, $n = 39$). For *O. macrocalyx*, transpiration ratio explained 2% of variation in leaf P:C ($P = 0.45$, $n = 40$) and 0.3% of variation in whole-plant P:C ($P = 0.73$, $n = 40$).

Discussion

We observed that leaf P:C correlated with transpiration ratio across species when grown under similar conditions, and within species when growth conditions were varied to induce a range of transpiration ratios. For the subset of data for which whole-plant P:C was measured, correlations between whole-plant P:C and transpiration ratio were similar to those between leaf P:C and transpiration ratio. This suggests that transpiration ratio correlates with P uptake at the whole-plant level. Correlations between leaf P concentration and transpiration ratio have previously been observed in herbaceous plants (Masle et al. 1992). The results of that study were similar to ours in that species differed in the slope and strength of the correlation between the two parameters. Of the five species investigated by Masle et al. (1992), three exhibited significant correlations between leaf P concentration and transpiration ratio or carbon isotope discrimination, a proxy for transpiration ratio. In our study, significant correlations between leaf P:C and transpiration ratio were observed in four of the five species for which different treatments were imposed to induce variation in transpiration ratio. The relationship that we observed between leaf P:C and transpiration ratio across the full dataset was robust, considering the number of species included and the range of treatments applied to the experimental plants and soils, including drought, fertilizer application, mixture of the soil with variable amounts of plant residues (leaf litter and rice husks), and growth under ambient and elevated CO_2 concentration.

It is likely that the forest topsoil used in our experiments had relatively high P availability compared with many tropical forest soils. Soil obtained from the same forest area had a mean plant-available P concentration of 15 μg P g^{-1} dry soil, determined by extraction in Bray solution (30 mM NH_4F and 25 mM HCl). This extractable P concentration is in the high range of values typically observed for neotropical rainforest soils (Clinebell et al. 1995). Further, the addition of leaf litter or rice husks to the soil mixture used in our experiments could have increased the concentration of organic P compounds dissolved in the soil solution (Vincent et al. 2010). Based on these considerations, we suggest that the total P concentration of the soil solution could have been sufficiently high in our experiments, such that P transported to root surfaces by mass flow would have represented a significant fraction of total P taken up by the plants.

Although it has not traditionally been included in calculations of P transport to roots by mass flow (Bieleski 1973, Barber 1995), organic P may play a significant role in this process. Organic P typically represents 30–80% of total soil P, including in tropical forest soils (Dalal 1977, Anderson 1980, Turner and Engelbrecht 2011). Some organic P compounds are mobile in the soil solution, being less strongly sorbed to metal oxides than P_i (Hoffman and Rolston 1980, Frossard et al. 1989, Turner and Haygarth 2001, Condron et al. 2005, Richardson et al. 2005, Turner 2008). Soil solution concentrations of organic P can be substantially higher than P_i concentrations (Ron Vaz et al. 1993, Shand et al. 1994). Thus, it seems likely that organic P will be transported to the root surfaces of transpiring plants by mass flow. Plant roots produce a variety of phosphatase enzymes, which can be located in cell walls or exuded into the rhizosphere (Richardson et al. 2005). These phosphatase enzymes can hydrolyze organic P, thereby releasing P_i, which can then be absorbed by roots. In addition, it was recently reported that root cells may be able to directly absorb DNA fragments from the soil solution (Paungfoo-Lonhienne et al. 2010).

The mean leaf P:C across all experimental plants in our dataset was 4.8 mg P g^{-1} C, and the mean leaf N:P was 12.3 g N g^{-1} P. This mean leaf P:C is in the high range of leaf P:C observed in tropical rainforests (Reich and Oleksyn 2004, Townsend et al. 2007). According to a simple interpretation of leaf N:P (Koerselman and Meuleman 1996, Aerts and Chapin 2000, Güsewell 2004), the mean value for our experimental plants would suggest that they were N limited rather than P limited. Although it is clear that interpretation of leaf N:P may be more complex (Ågren 2008, Craine et al. 2008, Cernusak et al. 2010, Garrish et al. 2010), these observations together suggest that the plants in our experiment were not P deprived. Therefore, our results indicate that when P availability is relatively high, tropical trees may be able to acquire a significant fraction of their P through transpiration-induced mass flow of P to root surfaces. Under such circumstances, a plant could achieve a savings on the resources that it would otherwise invest in specialized structures to promote P uptake, such as root hairs and mycorrhizas. Conversely, if P availability were relatively low, acquisition of P by mass flow would decrease, necessitating increased allocation of resources toward alternative strategies for P acquisition.

Mycorrhizas play an important role in the P nutrition of vascular plants (Bolan 1991, Lambers et al. 2008, Plassard and Dell 2010). Most of the tropical tree and liana species examined in this study were likely associated with arbuscular mycorrhizal fungi (Wang and Qiu 2006), with the exception of *Pinus caribaea*, which could have supported ectomycorrhizas (Tedersoo et al. 2007). In the absence of quantitative data on mycorrhizal associations in our experiments, however, we are unable to draw conclusions about how relationships between transpiration ratio and leaf P:C might be influenced by mycorrhizal infection.

The relationships that we observed between leaf P:C and transpiration ratio in tropical tree and liana seedlings could have implications for P acquisition in tropical forests as the atmospheric CO_2 concentration rises. Transpiration ratio generally decreases with increasing atmospheric CO_2 concentration (Morison 1985, Eamus 1991, Farquhar 1997, Winter et al. 2001, Long et al. 2004, Holtum and Winter 2010). If correlations between transpiration ratio and P uptake are widespread in tropical forests, decreasing transpiration ratios could lead to decreasing rates of P uptake under rising atmospheric CO_2 concentration. This could lead to an increasing constraint on productivity by P availability in tropical forests. A deeper understanding of the mechanism linking leaf P:C to transpiration ratio is critical to be able to incorporate such a process into analyses of tropical forest biogeochemistry.

In the dataset analyzed for this paper, two species were grown at both ambient and elevated CO_2 concentrations. For *S. macrophylla*, both transpiration ratio ($P < 0.001$, $n = 39$) and leaf P:C ($P < 0.001$, $n = 39$) decreased at elevated compared with ambient CO_2 concentration. For *O. macrocalyx*, on the other hand, transpiration ratio decreased at elevated compared with ambient CO_2 concentration ($P < 0.001$, $n = 40$), but leaf P:C did not differ between the two treatments ($P = 0.82$, $n = 40$). This suggests that leaf P:C of some tropical tree species may decline in response to decreasing transpiration ratio caused by increasing atmospheric CO_2 concentration, whereas that of other species may be unaffected. This could have consequences for competitive interactions among species.

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