

# Leaf nitrogen to phosphorus ratios of tropical trees: experimental assessment of physiological and environmental controls

Lucas A. Cernusak<sup>1,2</sup>, Klaus Winter<sup>1</sup> and Benjamin L. Turner<sup>1</sup>

<sup>1</sup>Smithsonian Tropical Research Institute, PO Box 0843-03092, Balboa, Ancon, Republic of Panama; <sup>2</sup>Present address: School of Environmental and Life Sciences, Charles Darwin University, Darwin, NT 0909, Australia

## Summary

Author for correspondence:

Lucas A. Cernusak  
Tel: +61 8 8946 7630  
Email: lucas.cernusak@cdu.edu.au

Received: 28 August 2009  
Accepted: 8 October 2009

*New Phytologist* (2010) **185**: 770–779  
doi: 10.1111/j.1469-8137.2009.03106.x

**Key words:** leaf nitrogen concentration, leaf nitrogen to phosphorus ratio, leaf phosphorus concentration, relative growth rate, water-use efficiency.

- We investigated the variation in leaf nitrogen to phosphorus ratios of tropical tree and liana seedlings as a function of the relative growth rate, whole-plant water-use efficiency, soil water content and fertilizer addition.
- First, seedlings of 13 tree and liana species were grown individually in 38-l pots prepared with a homogeneous soil mixture. Second, seedlings of three tree species were grown in 19-l pots at high or low soil water content, and with or without added fertilizer containing nitrogen, phosphorus and potassium.
- For plants grown under common soil conditions, leaf nitrogen to phosphorus ratios showed a unimodal, or hump-shaped, relationship with the relative growth rate. The leaf nitrogen to phosphorus ratio increased in response to low soil water content in three species, and increased in response to fertilizer addition in two of the three species. Across all species and treatments, the leaf nitrogen to phosphorus ratio was positively correlated with the water-use efficiency.
- The results suggest that the within-site variation among tropical tree species in the leaf nitrogen to phosphorus ratio may be caused by associations between this ratio and the relative growth rate. Modification of the soil environment changed the leaf nitrogen to phosphorus ratio, but underlying associations between this ratio and the relative growth rate were generally maintained. The observed correlation between the leaf nitrogen to phosphorus ratio and water-use efficiency has implications for linking nutrient stoichiometry with plant transpiration.

## Introduction

The nitrogen to phosphorus (N : P) ratio in terrestrial plant leaves can provide important information about nutrient limitations to primary productivity (Sterner & Elser, 2002; Ågren, 2008). For example, it has been suggested that leaf N : P ratios above a given threshold (*c.* 16 on a mass basis) indicate phosphorus limitation to biomass production, and those below a given threshold (*c.* 14 on a mass basis) indicate nitrogen limitation (Koerselman & Meuleman, 1996; Aerts & Chapin, 2000; Tessier & Raynal, 2003; Güsewell, 2004). This offers a powerful tool for ecological and physiological investigations by providing a straightforward means of characterizing the relative availability of nitrogen vs phosphorus at a given site. However, some terrestrial ecosystems appear not to conform to this expectation (Craine *et al.*,

2008), suggesting that the interpretation of terrestrial plant N : P ratios may be more complex.

At a basic level, it is clear that terrestrial plants exercise some level of homeostatic control over their N : P ratios. For example, the N : P ratio of plants grown experimentally reflects the N : P supply ratio of the nutrient solution fed to the plants, but the range of values in the former is several fold less than that of the latter (Güsewell & Koerselman, 2002). Thus, the challenge to the interpretation of plant N : P ratios in nature is to understand the relative partitioning of control between intrinsic physiology and external environment (Ågren, 2008). A recent study has illustrated this point with respect to tropical rainforests, finding a large variation in leaf N : P ratio among tropical tree species at a given site (Townsend *et al.*, 2007); the authors concluded that

the dominant control over leaf N : P ratios in the tropics is probably the identity of the species or mixture of species under examination. What causes the variation in leaf N : P ratios of different tropical tree species growing in the same environment? The answer to this question is important for the interpretation of leaf N : P ratios in tropical forests, and would provide general insight into the rapidly emerging field of ecological stoichiometry (Sterner & Elser, 2002). It may also contribute towards a better understanding of the effect of species diversity on relationships between nitrogen and phosphorus availability and biomass production in tropical forests (Kitayama, 2005).

Ågren (2004) proposed a conceptual model for the understanding of the C : N : P stoichiometry of photoautotrophic organisms. The model is predicated on the basis that photosynthetic organisms require proteins to carry out photosynthesis and growth. On the other hand, they require ribosomes to synthesize proteins. Proteins are nitrogen rich and phosphorus poor, whereas ribosomes contain approximately equal amounts of protein and rRNA, which is phosphorus rich; this gives them a very low N : P mass ratio of *c.* 3.3 (Sterner & Elser, 2002). Thus, photosynthetic organisms require nitrogen-rich proteins to capture carbon and grow, but also require phosphorus-rich ribosomes to synthesize proteins. According to these constraints, the growth rate of a photosynthetic organism can be described as (Ågren, 2004):

$$\frac{dC}{dt} = \phi_{CN} N_p, \quad \text{Eqn 1}$$

$$\frac{dN_p}{dt} = \phi_{NP} P_{ri}, \quad \text{Eqn 2}$$

(*C*, amount of carbon in the plant; *t*, time; *N<sub>p</sub>*, amount of nitrogen in proteins used for growth; *P<sub>ri</sub>*, amount of phosphorus in ribosomes used for protein synthesis;  $\phi_{CN}$  and  $\phi_{NP}$ , rate factors). Plants contain nitrogen and phosphorus in compounds other than those used for growth and protein synthesis, respectively, such that the total amounts of nitrogen (*N*) and phosphorus (*P*) in the plant can be expressed as:

$$N = N_p + \beta_N C, \quad \text{Eqn 3}$$

$$P = P_{ri} + \beta_P C, \quad \text{Eqn 4}$$

( $\beta_N$  and  $\beta_P$ , proportionality constants). Eqns 3 and 4 state that the amounts of nitrogen and phosphorus in the plant not associated with growth and protein synthesis, respectively, but still necessary for normal functioning, are proportional to the amount of plant carbon. Under stable and

balanced growth, such that the plant has a constant relative chemical composition and a constant relative growth rate ( $\mu$ ), the following definitions exist:

$$\mu = \frac{1}{C} \frac{dC}{dt} = \frac{1}{N} \frac{dN}{dt} = \frac{1}{P} \frac{dP}{dt} = \frac{1}{M} \frac{dM}{dt}, \quad \text{Eqn 5}$$

(*M*, total plant mass). Equations 1–5 can be used to predict plant C : N : P stoichiometry as a function of  $\mu$  (Ågren, 2004):

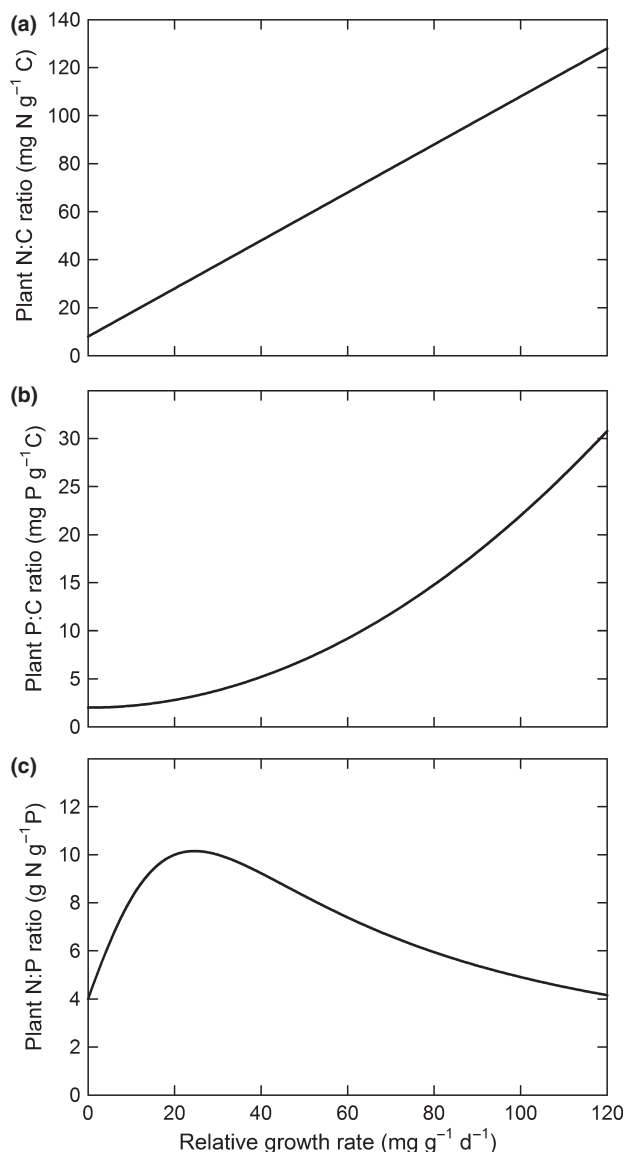
$$\frac{N}{C} = \frac{\mu}{\phi_{CN}} + \beta_N, \quad \text{Eqn 6}$$

$$\frac{P}{C} = \frac{\mu^2}{\phi_{CN}\phi_{NP}} + \beta_P, \quad \text{Eqn 7}$$

$$\frac{N}{P} = \frac{\mu\phi_{NP} + \beta_N\phi_{CN}\phi_{NP}}{\mu^2 + \beta_P\phi_{CN}\phi_{NP}}. \quad \text{Eqn 8}$$

Equation 6 predicts that, for nitrogen-limited growth, the N : C ratio should increase linearly as a function of  $\mu$ . On the other hand, Eqn 7 predicts that, for phosphorus-limited growth, the P : C ratio should increase curvilinearly as a quadratic function of  $\mu$ . Eqn 8 predicts that the whole-plant critical N : P ratio, where both nitrogen and phosphorus are simultaneously limiting growth, is expected to show a unimodal relationship with  $\mu$ , increasing at low values of  $\mu$  to a maximum, and then decreasing as  $\mu$  increases further. Example predictions based on Eqns 6, 7 and 8 are shown in Fig. 1. Although the model described above (Ågren, 2004) predicts the N : P ratio on a whole-plant basis, leaf N : P ratios have been shown to correlate with those in other plant organs (Kerkhoff *et al.*, 2006), and the model may therefore provide useful insight into the variation in the N : P ratios of leaves. If excess uptake of nitrogen or phosphorus takes place when the supply of one of these nutrients limits growth but the supply of the other does not, plant N : P ratios would be expected to differ from the prediction of Eqn 8. Variation in the leaf N : P ratio, however, might be partly buffered against such an effect if the storage of excess nitrogen or phosphorus were to take place mostly in the roots and stems in woody perennial plants (e.g. Dyckmans & Flessa, 2001).

Cernusak *et al.* (2007) observed that the leaf N : P ratios in seedlings of a tropical pioneer tree species, *Ficus insipida*, correlated positively with the whole-plant water-use efficiency (WUE). The WUE can be expressed in mass units of  $\text{g C kg}^{-1} \text{H}_2\text{O}$ , and is a measure of the amount of carbon accumulated in the plant biomass for a given amount of water transpired to the atmosphere. The plant N : P ratio might be expected to correlate with WUE according to the



**Fig. 1** Representative predictions of relationships between the plant N : C (a), P : C (b) and N : P (c) ratios and the relative growth rate. Predicted relationships are for the case in which the nutrient or nutrients involved are limiting growth. Predictions are according to the C : N : P model of Ågren (2004), using the following parameter values:  $\phi_{CN} = 1 \text{ g C g}^{-1} \text{ N d}^{-1}$ ;  $\phi_{NP} = 0.5 \text{ g P g}^{-1} \text{ N d}^{-1}$ ;  $\beta_N = 8 \text{ mg N g}^{-1} \text{ C}$ ;  $\beta_P = 2 \text{ mg P g}^{-1} \text{ C}$ .

following argument: plant carbon gain relates positively to the amount of nitrogen in proteins associated with growth, as shown above in Eqn 1; on the other hand, the transport of phosphorus to the surfaces of roots in the soil, where it can be subsequently absorbed by the plant, partly depends on the mass flow of the soil solution resulting from transpiration by the plant (Barber, 1995; Tinker & Nye, 2000; Cernusak *et al.*, 2007; Scholz *et al.*, 2007; Cramer *et al.*, 2008, 2009). Thus, carbon uptake should correlate with  $N_p$ , and phosphorus uptake should correlate with transpira-

tion. Consequently, it could be hypothesized that the N : P ratio would correlate with the C : T ratio, where T is the cumulative transpiration, and C : T is equal to WUE. There are some limitations to this line of reasoning that should be recognized. These include the observation that WUE appears to increase with increasing phosphorus availability, not just with increasing nitrogen availability (Raven *et al.*, 2004), and the relative immobility of phosphorus in soils, which might minimize its transport to root surfaces by mass flow (Barber, 1995; Tinker & Nye, 2000). Fortunately, the hypothesis is amenable to experimental testing.

In this article, we report the relationship between the leaf N : P ratio and  $\mu$  for seedlings of 13 tropical tree and liana species grown in a homogeneous soil environment. In addition, we examined the effects of a variation in soil water content and fertilizer addition on the leaf N : P ratios in seedlings of three tropical tree species with contrasting  $\mu$  values. Finally, we tested the hypothesis that the leaf N : P ratio correlates with WUE across a diverse range of species and soil conditions.

## Materials and Methods

Plants were grown at the Santa Cruz Experimental Field Facility of the Smithsonian Tropical Research Institute in Gamboa, Panama (9°07'N, 79°42'W). The altitude at the site is *c.* 28 m above sea level. Leaf N : P ratios considered in this article are from plants grown for two experiments. In the first experiment, several individuals each of 13 tropical tree and liana species were grown in 38-l pots, with one plant in each pot, for several months. The pots were filled with a homogenized soil mixture, comprising 60% by volume of dark, air-dried topsoil and 40% by volume of air-dried rice husks. The rice husks were added to the soil mixture to improve soil structure and drainage. Soil water content throughout the experiment was maintained between field capacity and 60% of field capacity, such that the plants were well watered. This experiment included three coniferous tree species [*Cupressus lusitanica* Mill. (Cupressaceae), *Pinus caribaea* Morelet (Pinaceae) and *Thuja occidentalis* L. (Cupressaceae)], seven angiosperm tree species [*Calophyllum longifolium* Willd. (Clusiaceae), *Clusia pratensis* Seem. (Clusiaceae), *Hyeronima alchorneoides* Allemão (Euphorbiaceae), *Luehea seemannii* Triana & Planch. (Tiliaceae), *Swietenia macrophylla* King (Meliaceae), *Tabebuia rosea* (Bertol.) A. DC. (Bignoniaceae), *Tectona grandis* Linn. (Verbenaceae)] and three angiosperm liana species [*Gouania lupuloides* (L.) Urb. (Rhamnaceae), *Mikania leiostachya* Benth. (Asteraceae), *Stigmaphyllon hypargyreum* Triana & Planch. (Malphiaceae)]. Additional information about the experiment can be found in Cernusak *et al.* (2008).

The second experiment employed three angiosperm tree species [*Platymiscium pinnatum* (Jacq.) Dugand (Fabaceae), *S. macrophylla* and *T. grandis*]. Twenty seedlings of each

species were planted individually in 19-l pots. The pots were filled with a homogenized soil mixture. The soil mixture for this experiment comprised 80% by volume of dark, air-dried topsoil and 20% by volume of air-dried rice husks. At the beginning of the experiment, 10 of the 20 pots for each species were randomly chosen to receive *c.* 12 g Osmocote-Plus controlled-release fertilizer (Scotts-Sierra, Maryville, OH, USA). The fertilizer contained by weight 15% nitrogen, 9% phosphorus and 12% potassium, and had an estimated release time of 5–6 months at a temperature of 32°C according to the manufacturer. Five fertilized and five unfertilized pots from each species were then randomly allocated to receive a reduced water supply. All pots started the experiment watered to field capacity. Those receiving the full water allocation 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 the water loss rates. Those receiving the reduced water allocation were allowed to dry down to pot water contents of < 2.5 kg, or *c.* 40% 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). Additional information about the experiment can be found in Cernusak *et al.* (2009).

The initial plant dry mass at the beginning of the experiments was estimated by harvesting several representative individuals of each species. At the conclusion of the experiments, leaves, stems and roots were harvested separately and dried to constant weight at 70°C. The mean  $\mu$  value of each plant was calculated as  $\mu = [\log_e(M_2) - \log_e(M_1)]/t$ , where  $\log_e(M_2)$  and  $\log_e(M_1)$  are the natural logarithms of plant dry mass at the end and beginning of the experiment, respectively, and  $t$  is the duration of the experiment (Blackman, 1919). Cumulative plant water use over the course of the experiments was determined by weighing the pots at weekly, or subweekly, intervals, as necessary. Pots were closed such that they did not drain during the experiments. At each weighing, the pots were replenished with water to a previously determined target weight. Pots without plants were deployed to estimate soil evaporation. The WUE was calculated as  $(C_2 - C_1 + C_L)/T$ , where  $C_2$  and  $C_1$  are the plant carbon masses at the end and beginning of the experiment, respectively,  $C_L$  is the leaf litter carbon mass abscised during the experiment and  $T$  is the cumulative transpiration.

The leaf 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). Samples of *c.* 3 mg were analyzed for carbon and nitrogen concentration with an elemental analyzer (ECS 4010; Costech Analytical Technologies, Valencia, CA, USA) coupled to an isotope ratio mass spectrometer (Delta XP; Finigan MAT,

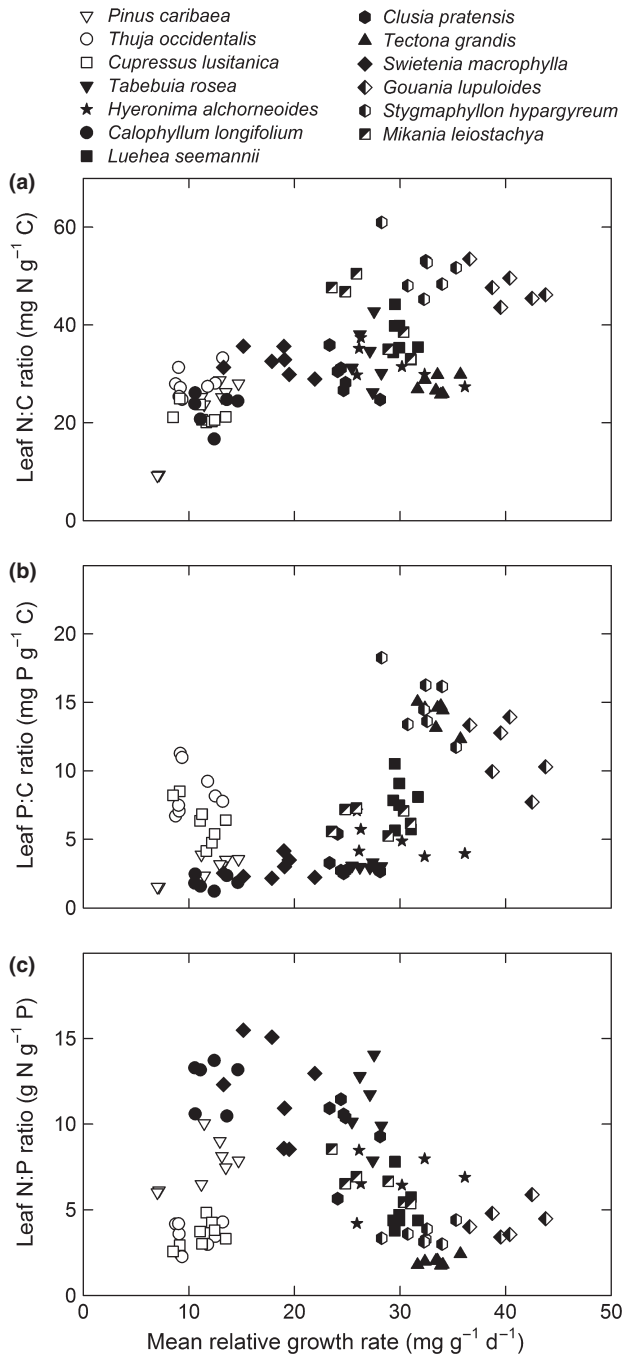
Bremen, Germany). In the first experiment, leaf dry matter was analyzed for phosphorus concentration by acid digestion and detection on an inductively coupled plasma optical emission spectrometer (Perkin Elmer Inc., Wellesley, MA, USA). Leaf samples were prepared by digesting *c.* 200 mg of sample material under pressure in polytetrafluoroethylene vessels with 2 ml of concentrated nitric acid. In the second experiment, leaf dry matter was analyzed for phosphorus concentration by ashing at 550°C, followed by dissolution in 1 M H<sub>2</sub>SO<sub>4</sub>, with phosphate detection by automated molybdate colorimetry using a Lachat Quickchem 8500 (Hach Ltd, Loveland, CO, USA). Species' mean leaf nitrogen and phosphorus concentrations for the first experiment have been reported previously (Cernusak *et al.*, 2008), but the variation in the leaf N : P ratio was not assessed as a function of  $\mu$  or WUE in that paper. Leaf phosphorus concentrations and leaf N : P ratios for the second experiment are presented for the first time here.

The dependence of the leaf N : C, P : C and N : P ratios on  $\mu$  for the first experiment was assessed using the linear and nonlinear regression routines in Systat 12.0 (SPSS, Chicago, IL, USA). Variation among species and treatments in leaf N : C, P : C and N : P ratios for the second experiment was assessed by analysis of variance. The number of observations was 60, the degree of freedom for species was two, the degrees of freedom for nutrient and water treatments were one each and the degree of freedom error was 48. Pair-wise comparisons among species following analyses of variance were made according to Tukey's method. Results were considered to be statistically significant at  $P < 0.05$ .

## Results

The leaf N : C ratio varied linearly as a function of  $\mu$  across the dataset for the first experiment (Fig. 2a), consistent with the prediction of Eqn 6. The  $\mu$  value explained 44% of the variation in the leaf N : C ratio. The regression equation relating the two was leaf N : C =  $0.671\mu + 17.1$  ( $R^2 = 0.44$ ,  $P < 0.001$ ,  $n = 88$ ), where the leaf N : C ratio is in  $\text{mg g}^{-1}$  and  $\mu$  is in  $\text{mg g}^{-1} \text{d}^{-1}$ . On the other hand, the leaf P : C ratio varied as a nonlinear function of  $\mu$  (Fig. 2b), qualitatively consistent with the prediction of Eqn 7. The nonlinear regression equation relating the leaf P : C ratio to  $\mu^2$  was leaf P : C =  $0.0053\mu^2 + 3.55$  ( $R^2 = 0.31$ ,  $n = 88$ ), where the leaf P : C ratio is in  $\text{mg g}^{-1}$  and  $\mu$  is in  $\text{mg g}^{-1} \text{d}^{-1}$ . For comparison, a linear regression of the leaf P : C ratio with  $\mu$  as independent variable had  $R^2$  of 0.27. The leaf N : P ratio also varied as a nonlinear function of  $\mu$  (Fig. 2c). The leaf N : P ratio increased with increasing  $\mu$  at low values of  $\mu$ , reached a maximum, and then decreased as  $\mu$  increased further. This unimodal pattern of variation was qualitatively consistent with that predicted by Eqn 8. A nonlinear regression, taking the form of





**Fig. 2** Leaf N : C (a), P : C (b) and N : P (c) ratios plotted as functions of the relative growth rate for 13 tropical tree and liana species. Open symbols, coniferous species; filled symbols, angiosperm tree species; semi-filled symbols, angiosperm liana species.

Eqn 8, resulted in the following equation: leaf N : P =  $[(258\mu - 1510)/(\mu^2 + 62)]$  ( $R^2 = 0.23$ ,  $n = 88$ ).

Mean values for the second experiment are shown in Table 1 for the leaf N : C, P : C and N : P ratios for each species by treatment combination. Analysis of variance indicated significant variation among species in the leaf N : C,

P : C and N : P ratios ( $P < 0.001$ ). The leaf N : C ratio was higher in *P. pinnatum* than in *T. grandis* or *S. macrophylla*, and the leaf P : C ratio was highest in *P. pinnatum*, intermediate in *T. grandis* and lowest in *S. macrophylla*. In general, treatment effects on the leaf N : C ratio were more pronounced than treatment effects on the leaf P : C ratio. The leaf N : C ratio increased significantly in response to low soil water content and fertilizer addition ( $P < 0.001$ ). By contrast, the leaf P : C ratio decreased slightly in response to low soil water content and increased slightly in response to fertilizer addition, but these effects were only moderately significant ( $P = 0.04$  and  $P = 0.06$ , respectively). The leaf N : P ratio varied significantly by nutrient treatment ( $P < 0.001$ ) and by water treatment ( $P < 0.001$ ). The mean leaf N : P ratio was lowest in *T. grandis*, whereas *S. macrophylla* and *P. pinnatum* showed similar mean values. There was a marked increase in the leaf N : P ratio with decreasing soil water content, and this pattern was consistent across all three species (Table 1). The leaf N : P ratio also increased in response to fertilizer addition. There was a significant interaction between nutrient treatment and species ( $P < 0.001$ ), such that *S. macrophylla* showed a strong response of the leaf N : P ratio to fertilizer addition, whereas *T. grandis* showed a weaker response and *P. pinnatum* showed no response (Table 1).

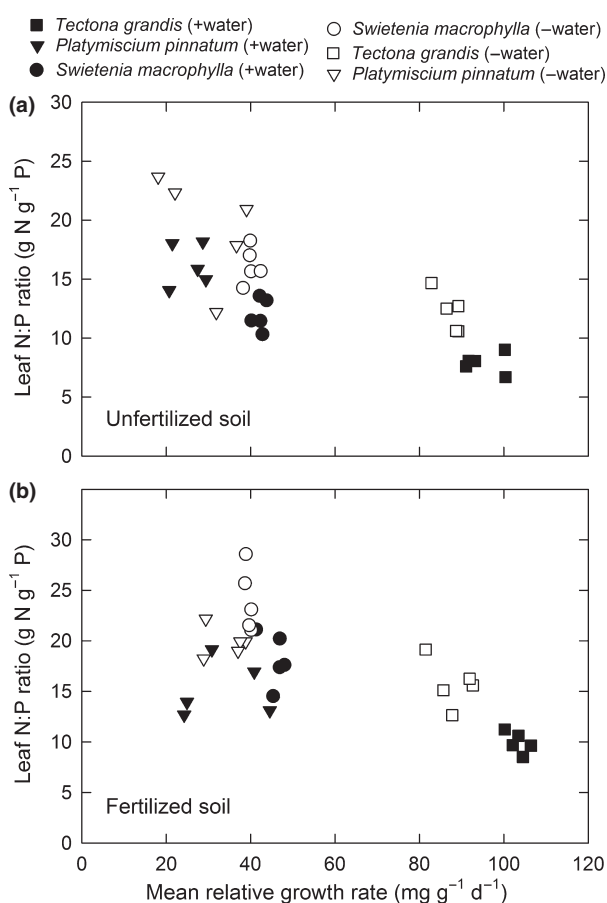
The leaf N : P ratio from the second experiment is shown as a function of  $\mu$  in Figs 3 and 4. *Platymiscium pinnatum* and *S. macrophylla* were slower growing species compared with *T. grandis*. Variation in the nitrogen and phosphorus availability of the soil environment, associated with either a variation in soil water content or fertilizer addition, caused the leaf N : P ratios to shift up or down, with the shift caused by the treatment superimposed on the background variation in the leaf N : P ratio associated with the variation in  $\mu$ . The effect of soil water content on the leaf N : P ratio can be seen in Fig. 3, for both unfertilized (Fig. 3a) and fertilized (Fig. 3b) soil. Similarly, the effect of fertilizer addition on the leaf N : P ratio can be seen in Fig. 4, at both high (Fig. 4a) and low (Fig. 4b) soil water contents. In general, for a given soil treatment in the second experiment, the leaf N : P ratio showed a relationship with  $\mu$  qualitatively consistent with that predicted by Eqn 8.

When data from both experiments were combined, the leaf N : P ratio showed a positive correlation with WUE (Fig. 5). Across the combined dataset, WUE explained 42% of the variation in the leaf N : P ratio. The regression equation relating the two was leaf N : P =  $8.28\text{WUE} + 0.26$  ( $R^2 = 0.42$ ,  $P < 0.001$ ,  $n = 148$ ). The positive correlation between the leaf N : P ratio and WUE resulted from the contributions of both a positive correlation between leaf nitrogen concentration and WUE ( $R^2 = 0.15$ ,  $P < 0.001$ ,  $n = 148$ ) and a negative correlation between leaf phosphorus concentration and WUE ( $R^2 = 0.24$ ,  $P < 0.001$ ,  $n = 148$ ).

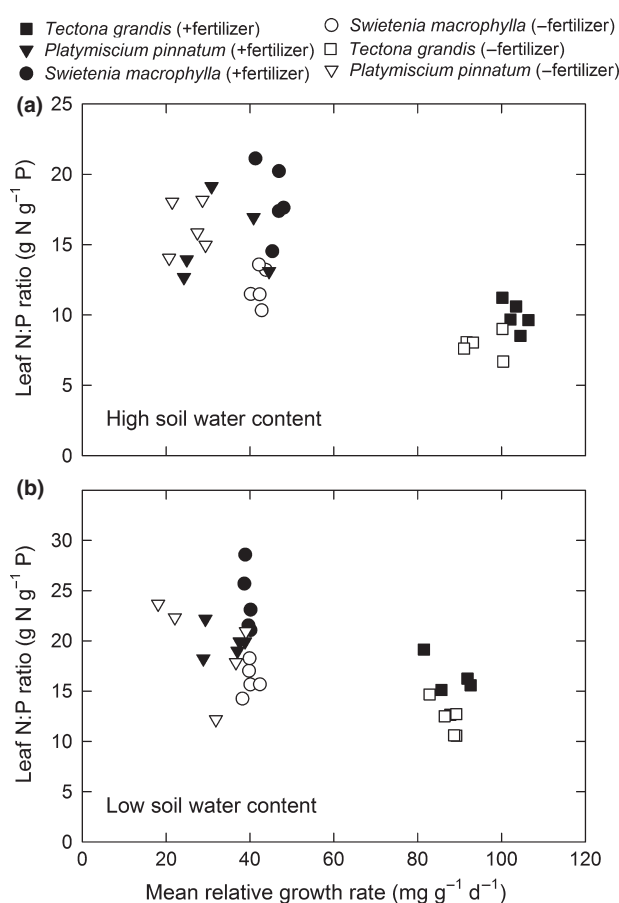
**Table 1** Leaf N : C, P : C and N : P ratios for three tropical tree species grown at high or low soil water content (SWC) and with or without added fertilizer

Treatment	<i>Tectona grandis</i>			<i>Swietenia macrophylla</i>			<i>Platymiscium pinnatum</i>		
	Leaf N : C	Leaf P : C	Leaf N : P	Leaf N : C	Leaf P : C	Leaf N : P	Leaf N : C	Leaf P : C	Leaf N : P
Unfertilized, high SWC	28.5 (0.9)	3.65 (0.42)	7.9 (0.8)	30.1 (2.2)	2.54 (0.42)	12.0 (1.4)	75.4 (9.7)	4.67 (0.58)	16.2 (1.8)
Unfertilized, low SWC	47.3 (7.2)	3.88 (0.38)	12.2 (1.7)	37.5 (1.9)	2.33 (0.28)	16.2 (1.5)	77.0 (14.9)	4.20 (1.57)	19.4 (4.6)
Fertilized, high SWC	45.2 (5.4)	4.56 (0.24)	9.9 (1.0)	48.5 (3.1)	2.70 (0.35)	18.2 (2.6)	78.3 (12.4)	5.25 (0.97)	15.2 (2.8)
Fertilized, low SWC	64.9 (3.3)	4.20 (0.67)	15.7 (2.3)	51.7 (3.2)	2.20 (0.39)	24.0 (3.1)	86.9 (13.1)	4.37 (0.53)	19.9 (1.5)

Values are the means for five plants in each treatment, with one standard deviation given in parentheses.



**Fig. 3** Leaf N : P ratios of three tropical tree species plotted against the relative growth rate for unfertilized (a) and fertilized (b) soil. Open symbols, plants grown at low soil water content; filled symbols, plants grown at high soil water content.

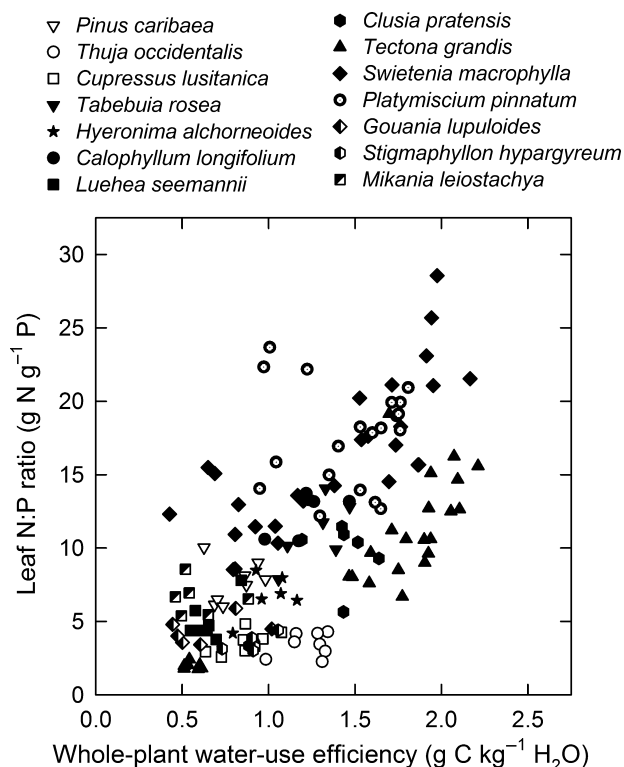


**Fig. 4** Leaf N : P ratios of three tropical tree species plotted against the relative growth rate for plants grown at high (a) and low (b) soil water contents. Open symbols, plants grown in unfertilized soil; filled symbols, plants grown in fertilized soil.

## Discussion

The results presented in Figs 2–4 clearly demonstrate a strong association between the leaf N : P ratio and  $\mu$  for the tropical tree and liana seedlings employed in this study,

when grown in a common soil environment. The relationships observed between the leaf N : P ratio and  $\mu$  were qualitatively consistent with a theoretical prediction, suggesting that the plant N : P ratio should increase at low  $\mu$ , reach a maximum, and then decrease as  $\mu$  continues to



**Fig. 5** Leaf N : P ratios plotted against whole-plant water-use efficiency for all plants in this study, including those grown under different soil conditions. Open symbols, coniferous species; filled symbols, angiosperm tree species; semi-filled symbols, angiosperm liana species.

increase (Ågren, 2004). Data from our second experiment suggest that the leaf N : P ratio can increase or decrease depending on the soil environment, but that species-level associations between the leaf N : P ratio and  $\mu$  are generally maintained (Figs 3,4). In addition, we observed a positive correlation between the leaf N : P ratio and WUE for the combined dataset including all species and soil treatments, suggesting that the relationship between these two parameters may be general.

Support for the C : N : P model reviewed in the Introduction section was previously presented for a temperate tree species, *Betula pendula* (Ågren, 2004). In that example, variation in  $\mu$  occurred within a single species as a result of a varying nutrient supply rate. The data presented here suggest that a similar relationship may also hold for multiple tropical species growing in a homogeneous soil environment when the variation in  $\mu$  is mainly caused by species identity. Thus, variation in  $\mu$  may partly explain the large within-site variation among tropical tree species in leaf N : P ratios observed in tropical rainforests (Santiago & Wright, 2007; Townsend *et al.*, 2007; Hättenschwiler *et al.*, 2008).

Equations 6–8 predict the C : N : P stoichiometry as a function of  $\mu$  for situations in which the nutrient or nutri-

ents concerned are limiting growth (Ågren, 2004). Thus, Eqn 8 predicts a hump-shaped relationship between the plant N : P ratio and  $\mu$  when nitrogen and phosphorus simultaneously limit growth. It is unlikely that this assumption would have been met for all species in our experiments. For example, Fig. 2b suggests that the leaf P : C ratio tends to decrease with increasing  $\mu$  for the coniferous species in the first experiment, which suggests that phosphorus is not limiting growth in these plants. Thus, the relationship between the leaf P : C ratio and  $\mu$  appears to differ for angiosperm vs coniferous species. Nevertheless, for plants grown in a given soil treatment, the leaf N : P ratio still appeared to vary as a function of  $\mu$  in a manner qualitatively consistent with that predicted by Eqn 8. Additional data will be necessary to further test the generality of this observation.

Negative correlations between the leaf N : P ratio and  $\mu$  have been observed previously among some vascular plant species (Güsewell, 2004; Niklas *et al.*, 2005; Niklas, 2006; Matzek & Vitousek, 2009). These results are consistent with our observations for species at all but the lowest  $\mu$  values, which occurred in the coniferous species (Fig. 2). Data for the angiosperm species in Figs 2–4 are still suggestive of a hump-shaped relationship between the leaf N : P ratio and  $\mu$ , but for the overwhelming majority of the range of  $\mu$  observed, the relationship between the leaf N : P ratio and  $\mu$  was negative. Thus, careful experimentation will be required to determine whether the strongly hump-shaped relationship observed in Fig. 2, when both coniferous and angiosperm species are considered, is a general pattern among terrestrial plants, or whether conifers behave qualitatively differently from angiosperms with respect to leaf C : N : P stoichiometry. Variation between these two groups in the leaf N : P ratio has also been observed in large, multispecies comparisons over broad spatial scales (McGroddy *et al.*, 2004; Reich & Oleksyn, 2004; Wright *et al.*, 2005).

The only potentially N<sub>2</sub>-fixing species in our experiments was *P. pinnatum*. Thus, Fig. 2 only contains data for non-N<sub>2</sub>-fixing species. Consistent with the ecophysiology of legumes generally (McKey, 1994), *P. pinnatum* had the highest leaf N : C ratios observed in the study (Table 1). It also had the lowest mean  $\mu$  value in the second experiment (Figs 3,4), suggesting a different relationship between the leaf N : C ratio and  $\mu$  than in the non-N<sub>2</sub>-fixing species. Interestingly, however, although the leaf N : C ratio of *P. pinnatum* was markedly higher than that of the other two species in the second experiment, its leaf N : P ratio was similar to that of *S. macrophylla*, a non-N<sub>2</sub>-fixing species with a similar  $\mu$  value (Figs 3, 4). This observation is consistent with data compiled from several tropical rainforests (Townsend *et al.*, 2007), where leguminous trees tend to have higher leaf nitrogen concentrations than do nonlegumes, but do not have markedly different leaf N : P ratios.

It has been suggested recently that nitrogen fixation by legumes in tropical forests could increase phosphorus acquisition by enabling the production of nitrogen-rich extracellular phosphatase enzymes (Houlton *et al.*, 2008); this is one of a variety of possible interactions between nitrogen and phosphorus acquisition that might contribute towards the regulation of the N : P ratios in these species.

The slow-release fertilizer employed for the second experiment had an N : P mass ratio of less than two. However, rather than decreasing in response to fertilizer addition, the leaf N : P ratio either increased (*T. grandis* and *S. macrophylla*) or showed little response (*P. pinnatum*), as shown in Table 1 and Fig. 4. This probably reflected the difference in buffering capacity of the soil for nitrate vs phosphate, forms of nitrogen and phosphorus likely to be important for absorption by roots. Nitrate does not adsorb to soil particles, whereas phosphate is strongly adsorbed. As a result, nitrate released from the fertilizer would probably be in the soil solution, available for transport to root surfaces by diffusion and mass flow. By contrast, the buffer power ( $B$ ) for phosphate can be on the order of  $10^2$ – $10^3$  (Barber, 1995), where  $B$  is defined as  $dS/dS_1$ , with  $S$  being the concentration of exchangeable, or labile, solute per unit volume of soil, and  $S_1$  the liquid concentration of the solute in the soil solution. Thus,  $10^2$ – $10^3$   $\mu\text{mol}$  phosphate would have to be added to 1 l of soil to increase the phosphate concentration of the soil solution by  $1 \mu\text{mol l}^{-1}$ . Compared with the situation for nitrate, this indicates that the phosphate concentration of the soil solution would change relatively little with phosphate addition to the soil.

The leaf N : P ratio increased in response to declining soil water content in all three species in the second experiment (Fig. 3). This pattern can be at least partly explained by differential changes in the mobility of nitrate vs phosphate in response to declining soil water content. The effective diffusion coefficient ( $D_e$ ) for a solute in soil can be calculated as  $D_e = D_1 f_i \theta / B$ , where  $D_1$  is the diffusion coefficient of the solute in free solution,  $f_i$  is the impedance factor and  $\theta$  is the volumetric water content of the soil (Tinker & Nye, 2000). For an anion such as nitrate, that is generally not adsorbed to soil particles, the quotient  $\theta/B$  will tend towards unity, such that  $D_e = D_1 f_i$ . The ratio of the effective diffusion coefficients for nitrate and phosphate ( $D_{eN}/D_{eP}$ ) can thus be described as  $D_{eN}/D_{eP} = D_{1N} f_{iN} / (D_{1P} f_{iP} \theta / B_P)$ , where subscripts 'N' and 'P' refer to nitrate and phosphate, respectively. If  $f_i$  is assumed to vary similarly as a function of  $\theta$  for both anions (Barber, 1995), it cancels from the right side of the equation. Then, to the extent that  $D_{1N}/(D_{1P}/B_P)$  can be reasonably assumed to be constant,  $D_{eN}/D_{eP}$  will vary as a function of  $1/\theta$ . Thus, as  $\theta$  declines,  $D_{eN}/D_{eP}$  will increase, and the mobility of nitrate relative to that of phosphate will increase, making nitrate relatively more available for absorption by roots. These considerations, combined with the results in Fig. 3, suggest that it

may be useful to take the variation in soil water content into account when interpreting the variation in leaf N : P ratios among tropical woody plants.

When the data from the two experiments in this study were combined, we observed a positive correlation between the leaf N : P ratio and WUE. This result is consistent with previous observations for seedlings of a tropical pioneer tree, *Ficus insipida* (Cernusak *et al.*, 2007). If data from that study and the present study are combined, the relationship between the leaf N : P ratio and WUE is even stronger, with WUE explaining 47% of the variation in the leaf N : P ratio ( $R^2 = 0.47$ ,  $P < 0.001$ ,  $n = 178$ ). As pointed out in the Introduction section, we suggest that this relationship results from correlations between plant nitrogen concentration and WUE (Guehl *et al.*, 1995; Livingston *et al.*, 1999; Raven *et al.*, 2004; Ripullone *et al.*, 2004; Cernusak *et al.*, 2007), and between plant transpiration and the transport of phosphorus to root surfaces by mass flow (Barber, 1995; Tinker & Nye, 2000; Cernusak *et al.*, 2007; Scholz *et al.*, 2007; Cramer *et al.*, 2008, 2009). The correlation shown in Fig. 5 includes data from a range of species and soil conditions, including fertilizer addition, manipulation of soil water content and variable mixtures of rice husks with soil (40% by volume in the first experiment vs 20% in the second). Thus, the relationship between the leaf N : P ratio and WUE may be general, but more data are needed to test this hypothesis. If supported, the idea could have important implications for ecosystem analysis, because it links plant C : N : P stoichiometry with plant transpiration, and therefore integrates carbon, nutrient and hydrological cycles.

Although phosphorus is generally considered to be relatively immobile in soils, some phosphorus-containing molecules may be more amenable to transport by mass flow than others. Soil organic phosphorus can play an important role in the phosphorus nutrition of forests growing on strongly weathered soils (Tiessen *et al.*, 1994; Johnson *et al.*, 2003; Turner *et al.*, 2007), which are common in the tropics. Soil organic phosphorus occurs in a diverse range of compounds that differ markedly in their physical and chemical behavior in soils. For example, many simple phosphate monoesters (e.g. sugar phosphates and mononucleotides) and phosphate diesters (e.g. nucleic acids and phospholipids) are generally weakly adsorbed to soil particles (Condrón *et al.*, 2005). Mass flow of the soil solution caused by plant transpiration may therefore play an important role in transporting these molecules towards root surfaces, where extracellular phosphatase enzymes can catalyze the hydrolysis of ester-bonded phosphorus to release phosphate, making it available for absorption into the root. The physical and chemical characteristics of the soil matrix interacting with phosphorus-containing molecules will also probably play an important role in determining the extent of phosphorus transport by mass flow. Many more data are needed



to adequately assess the role of transpiration in the modulation of phosphorus uptake in terrestrial plants in general (Cramer *et al.*, 2009), and this is particularly true for tropical woody plants.

We conclude that the variation in the leaf N : P ratios of tropical tree and liana seedlings can be associated with the variation in physiological processes ( $\mu$  and WUE) and in the external soil environment (soil water content and fertilizer addition). This suggests both internal and external controls over leaf N : P ratios of tropical trees and lianas, as recognized previously for other terrestrial plant species (Güsewell, 2004; Ågren, 2008). We observed a hump-shaped relationship between the leaf N : P ratio and  $\mu$  for seedlings grown in a common soil environment, where the variation in  $\mu$  was mainly associated with species' identity. Further experiments are needed to test the generality of this pattern. Modification of the external soil environment caused the leaf N : P ratio to shift, whereas the underlying variation associated with  $\mu$  was generally maintained. Thus, for the assessment of the nutrient status among sites based on the leaf N : P ratios, it would be prudent to sample the same species or suite of species at each site. Finally, the positive correlation observed between the leaf N : P ratio and whole-plant WUE implies that the C : N : P stoichiometry may be linked to hydraulic processes in terrestrial plants.

## Acknowledgements

We thank Jorge Aranda, Milton Garcia, Aurelio Virgo, Lisa Petheram, Carlos Martinez, Tania Romero and Aneth Sarmiento for technical assistance in carrying out the experiments. Lucas A. Cernusak was supported by a Tupper Postdoctoral Fellowship from the Smithsonian Tropical Research Institute and by an Australian Postdoctoral Fellowship from the Australian Research Council.

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