

RESEARCH PAPER

Nitrogen to phosphorus ratio of plant biomass versus soil solution in a tropical pioneer tree, *Ficus insipida*

Valerie Garrish¹, Lucas A. Cernusak^{2,*}, Klaus Winter³ and Benjamin L. Turner³

¹ Department of Geography, McGill University, Montréal, Québec H3A-1B1, Canada

² School of Environmental and Life Sciences, Charles Darwin University, Darwin, Northern Territory 0909, Australia

³ Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Republic of Panama

* To whom correspondence should be addressed. E-mail: lucas.cernusak@cdu.edu.au

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Abstract

It is commonly assumed that the nitrogen to phosphorus (N:P) ratio of a terrestrial plant reflects the relative availability of N and P in the soil in which the plant grows. Here, this was assessed for a tropical pioneer tree, *Ficus insipida*. Seedlings were grown in sand and irrigated with nutrient solutions containing N:P ratios ranging from <1 to >100. The experimental design further allowed investigation of physiological responses to N and P availability. Homeostatic control over N:P ratios was stronger in leaves than in stems or roots, suggesting that N:P ratios of stems and roots are more sensitive indicators of the relative availability of N and P at a site than N:P ratios of leaves. The leaf N:P ratio at which the largest plant dry mass and highest photosynthetic rates were achieved was ~11, whereas the corresponding whole-plant N:P ratio was ~6. Plant P concentration varied as a function of transpiration rate at constant nutrient solution P concentration, possibly due to transpiration-induced variation in the mass flow of P to root surfaces. The transpiration rate varied in response to nutrient solution N concentration, but not to nutrient solution P concentration, demonstrating nutritional control over transpiration by N but not P. Water-use efficiency varied as a function of N availability, but not as a function of P availability.

Key words: Carbon isotope ratio, N:P ratio, nutrient supply, transpiration, tropical tree, water-use efficiency.

Introduction

Analysis of nitrogen to phosphorus (N:P) ratios in plant biomass offers a simple and cost-effective tool for assessing constraints on terrestrial plant productivity associated with N and P availability (Koerselman and Meuleman, 1996; Aerts and Chapin, 2000; Tessier and Raynal, 2003; Güsewell, 2004). It was suggested that N:P mass ratios <14 indicate limitation by N and those >16 indicate limitation by P (Koerselman and Meuleman, 1996). On the other hand, plants with N:P ratios outside these ranges do not always respond to fertilization with the nutrient indicated to be in limiting supply (Güsewell *et al.*, 2003; Craine *et al.*, 2008). In addition, significant variation has been observed in N:P ratios among species growing at a common site, presumably with access to similar N and P supplies in the soil environment (Townsend *et al.*, 2007; Ågren, 2008;

Cernusak *et al.*, 2010). Therefore, meaningful interpretation of the plant biomass N:P ratio requires a more refined understanding of its environmental and physiological controls. An important component of environmental control is the N:P ratio of the soil solution (Güsewell, 2004). The extent to which the plant N:P ratio reflects that of the soil solution is a function of the homeostatic regulation exerted by the plant over its N:P stoichiometry.

Sterner and Elser (2002) suggested a method for analysing the extent of homeostatic regulation that an organism exerts with respect to a resource that it consumes. The method involves plotting the consumer stoichiometry on the y-axis of a graph against resource stoichiometry on the x-axis. The relationship between consumer and resource stoichiometry can then be described as

$$\frac{dy}{dx} = \frac{y}{Hx} \quad (1)$$

where H is a regulatory coefficient >1 that describes the extent of homeostasis. As H approaches infinity, the change in consumer stoichiometry as a function of a change in resource stoichiometry approaches zero, or perfect homeostasis. Equation 1 can be integrated to give

$$y = cx^{\frac{1}{H}} \quad (2)$$

where c is a constant. Visualization and analysis of H can then be simplified by linearizing Equation 2 using logarithms:

$$\log y = \log c + \frac{1}{H} \log x \quad (3)$$

The regulatory coefficient, H , can thus be calculated as the inverse of the slope of the relationship between consumer stoichiometry and resource stoichiometry plotted on a log–log scale.

In this study, the dependence of the biomass N:P ratio of a tropical pioneer tree, *Ficus insipida* Willd. (Moraceae), on variation in the soil solution N:P ratio was tested. Leaves, stems, and roots were each assessed independently, to determine whether organ-specific variation exists in the extent of homeostatic control over biomass N:P. In addition, the experimental design allowed for investigation of the responses of several physiological processes to variation in N and P availability in this species. These processes included growth, biomass allocation, photosynthesis, stomatal conductance, transpiration, and water-use efficiency.

Materials and methods

Plant material and experimental treatments

The experiment took place at the Smithsonian Tropical Research Institute, Santa Cruz Experimental Field Facility, Gamboa, Panama. The site is located at 9°07' N, 79°42' W, and is at an altitude of 28 m above sea level. Seeds of *F. insipida* were collected from the forest floor in the Panama Canal watershed. Seeds were germinated in trays containing a commercial potting mix in July 2007. Following germination, 70 seedlings were transplanted into 7.0 l pots, with one seedling in each pot. Each pot contained a volumetric mixture of 95% non-calcareous sand and 5% soil from the A horizon of a nearby forest. The sand was supplied locally and had a Mehlich-3-extractable P concentration (Mehlich, 1984) of 4.8 mg P kg⁻¹ sand, whereas the topsoil contained 26.4 mg P kg⁻¹ soil. It was expected that the high sand content of the mixture would reduce the cation exchange capacity, such that [N] and [P] of the soil solution in the pots would be similar to those of nutrient solutions fed to the plants.

The pots were placed on plastic tables under a translucent rain shelter, which elevated them ~0.8 m above the concrete surface below the shelter. The shelter reduced incoming photon flux density (PFD) by ~20%. The plants were rotated around the shelter every 2 weeks. Nutrient solution treatments were initiated after an adjustment period of 1 week. The plants were harvested after 9 weeks of treatment with nutrient solutions, on 14 November 2007.

The seven nutrient solution treatments, including an additional null or control treatment, were applied to eight pots each for a total of 64 experimental pots. Seedlings from the six remaining pots were harvested to estimate the seedling dry mass at the beginning of the experiment (0.013 g) and the initial leaf area (2.81 cm²). These pots later served as controls for transpiration measurements.

The seven nutrient solution treatments contained varying amounts of N and P (Table 1), based on the treatments described by Wong *et al.* (1985). A micronutrient solution was also applied. Base solutions were prepared using distilled water. For application to plants, base solutions were diluted with rainwater collected from the roof of the rain shelter. Rain collected on Barro Colorado Island, ~20 km from the experimental site, during 2008 had [N] of $\leq 10 \mu\text{M}$ and [P] of $\leq 0.3 \mu\text{M}$ (BL Turner and A Zimmermann, unpublished). The N was supplied to the nutrient solutions as nitrate and P as phosphate. The amounts of other chemicals in the solutions were equalized across the treatments, to avoid any confounding effects (Table 1). Each pot received 1.0 l of nutrient solution, including 1 ml of micronutrient solution, three times per week; on Monday, Wednesday, and Friday.

After harvest, the leaf area of each plant was determined with an LI-3100 Leaf-Area Meter (Li-Cor Inc., Lincoln, NE, USA). Plants were oven-dried at 70 °C. Dry mass of leaves, stems, and roots was determined separately for each plant. The final plant dry mass of control plants, irrigated with only rainwater, was 0.19 ± 0.15 g (mean ± SD), indicating that nutrient availability in the absence of added nutrient solution was very low.

Gravimetric transpiration measurements

Prior to harvest, the pots were weighed for a 5 d period, between 9 and 13 November 2007, in order to estimate transpiration rates on a whole-plant basis. The pots were weighed with a balance that had a capacity of 60 kg and resolution of 2 g (Sartorius EA60EDE-1, Thomas, Swedesboro, NJ, USA) at dawn and dusk on each day. The pots were watered to field capacity each day after the dusk measurements, with either rainwater or the nutrient solution, depending on the day. To account for evaporation from the soil surface, water loss from the control pots without plants was measured and this value was then subtracted from the water loss of pots with plants to calculate plant transpiration. Mean transpiration rates were expressed on a leaf area basis by dividing the mean water loss per plant by the leaf area determined at plant harvest.

Table 1. Chemical concentrations (mM) of nutrient solutions (S-1 to S-7) fed to seedlings of *Ficus insipida*

The N:P mass ratio of each solution is also given in the last row of the table.

Chemical (mM)	Constant P, variable N				Constant N, variable P			
	S-1	S-2	S-3	S-4	S-2	S-5	S-6	S-7
NO ₃ ⁻	24	12	4	0.6	12	12	12	12
H ₂ PO ₄ ⁻	1.33	1.33	1.33	1.33	1.33	0.53	0.133	0.04
K ⁺	4	4	4	4	4	4	4	4
Mg ²⁺	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Ca ²⁺	4	4	4	4	4	4	4	4
Na ⁺	13.33	1.33	1.33	1.33	1.33	0.53	0.133	0.04
SO ₄ ²⁻	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Cl ⁻			8	11.4				
N:P mass ratio (g g ⁻¹)	8.1	4.1	1.4	0.2	4.1	10.2	40.7	135.5

Leaf gas exchange measurements

Gas exchange of the youngest, fully expanded leaf of each plant was measured between 09:00 and 11:00 local time on 29 and 30 October 2007 with a Li-6400 portable photosynthesis system (Li-Cor Inc.). Measurements were made under irradiance of $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ supplied by an artificial light source (6200-02B Li-Cor Inc.). Mean leaf temperature during gas exchange measurements was $30.6 \pm 0.4 \text{ }^\circ\text{C}$ (mean \pm SD), and the leaf-to-air vapour pressure difference was $0.7 \pm 0.1 \text{ kPa}$.

Isotopic and elemental analyses

Leaf, stem, and root dry matter were ground to a fine, homogeneous powder for elemental and isotopic analyses. The $\delta^{13}\text{C}$ of leaf, stem, and root dry matter was measured at the Soil Analysis Laboratory at the Smithsonian Tropical Research Institute with an isotope ratio mass spectrometer (Delta V Advantage, Thermo, Bremen, Germany) coupled by a continuous flow interface to an elemental analyser (Flash HT, Thermo). In addition to isotopic analysis, the C and N elemental concentrations were determined from peak areas obtained from mass spectrometric measurements. Whole-plant $\delta^{13}\text{C}$ was calculated by mass balance using the dry mass of each plant organ (leaves, stems, and roots), the C mass fraction, and the $\delta^{13}\text{C}$ composition.

To measure the P concentration of leaf, stem, and root dry matter, $\sim 200 \text{ mg}$ of oven-dried plant material was digested under pressure in PTFE vessels at $180 \text{ }^\circ\text{C}$ in concentrated HNO_3 for 6 h. P was detected in the digestion solutions by inductively coupled plasma optical emission spectrometry (Optima 2100, Perkin Elmer, Shelton, CT, USA), with samples evaluated against standards prepared in the same matrix.

Statistical analyses

Analysis of variance was used to assess variation in elemental composition and physiological processes in response to nutrient solution treatments. Where significant variation was detected, pairwise comparisons between treatment means were carried out by Tukey's method. Where necessary, data were log-transformed or inverse-transformed prior to analysis to meet assumptions of normality and homogeneity of variance. Relationships between continuous variables were assessed using least-squares linear regression. To determine the regulatory coefficient, H, from log-log plots of plant biomass N:P ratios against nutrient solution N:P ratios, geometric mean regression was used. In this case, the primary interest was in interpreting the values of the regression coefficients, and both parameters were measured with error, thereby indicating the use of geometric mean regression (Sokal and Rohlf, 1995).

Results

Variation in final plant dry mass, leaf area ratio, and root:shoot ratio as a function of nutrient solution [N] and [P] is shown in Fig. 1. The left panels in Fig. 1 (A, C, and E) show responses as a function of variation in nutrient solution [N] with nutrient solution [P] held constant at 1.33 mM. The right panels in Fig. 1 (B, D, and F) show responses to variation in nutrient solution [P] with nutrient solution [N] held constant at 12 mM. Final plant dry mass increased as a saturating function of nutrient solution [N] when nutrient solution [P] was held constant (Fig. 1A). On the other hand, final plant dry mass increased approximately linearly as a function of nutrient solution [P] when nutrient solution [N] was held constant (Fig. 1B). Leaf area ratio, defined as final plant leaf area divided by final plant

dry mass, increased with increasing nutrient solution [N] at constant nutrient solution [P] (Fig. 1C), but showed no response to increasing nutrient solution [P] at constant nutrient solution [N] (Fig. 1D). The root:shoot ratio showed a similar pattern, with a strong response to variation in nutrient solution [N] at constant nutrient solution [P] (Fig. 1E), but no response to variation in nutrient solution [P] at constant nutrient solution [N] (Fig. 1F).

Variation in N and P content per plant and in plant N:P ratio as a function of nutrient solution [N] and [P] is shown in Fig. 2. The plant N and P content and plant N:P ratio appeared to increase as saturating functions of nutrient solution [N] at constant nutrient solution [P], as seen in Fig. 2A, C, and E, respectively. On the other hand, responses to variation in nutrient solution [P] at constant nutrient solution [N] were approximately linear over the range of nutrient solution [P] employed in the study (Fig. 2B, D, and F).

Variation in the [N] of plant biomass as a function of the nutrient solution [N] and [P] is shown in Fig. 3. The [N] of leaves, stems, and roots increased with increasing [N] of the nutrient solution when [P] was held constant, as seen in Fig. 3A, C, and D, respectively. The [N] of plant biomass showed little response to variation in nutrient solution [P] when nutrient solution [N] was held constant (Fig. 3B, D, and F). However, the stem [N] did increase significantly at the lowest nutrient solution [P], as seen in Fig. 3D.

In contrast to these results, plant biomass [P] showed a strong response to variation in nutrient solution [N] when nutrient solution [P] was held constant (Fig. 4A, C, and E). For leaves and stems, the response was non-linear, such that [P] of leaf and stem biomass were lowest at intermediate nutrient solution [N] (Fig. 4A and C, respectively). Leaf, stem, and root [P] also increased in response to increasing nutrient solution [P] when nutrient solution [N] was held constant, as seen in Fig. 4B, D, and F. Across all treatments, the highest [P] of plant biomass occurred when the [P] of the nutrient solution was at its highest value (1.33 mM) and the [N] of the nutrient solution was at its lowest value (0.6 mM).

Comparison of the N:P ratio of plant biomass with that of the nutrient solution is shown in Fig. 5. The plot of leaf N:P against nutrient solution N:P suggests that on a logarithmic scale the relationship between the two was approximately linear over most of the range of nutrient solution N:P ratios employed in the study. At the lowest nutrient solution N:P ratio, however, the relationship departed from linearity (Fig. 5A). For the N:P ratio of stem biomass, on the other hand, the relationship to the nutrient solution N:P ratio on a logarithmic scale was linear over the full range of values (Fig. 5B). The relationship between root N:P and nutrient solution N:P followed a similar pattern to that for stem N:P, but with slight departures from linearity at the highest and lowest nutrient solution N:P ratios (Fig. 5C). Given these patterns, geometric mean regressions were fitted to the log-log plots of plant biomass N:P against nutrient solution N:P excluding the lowest nutrient solution N:P treatment. With this treatment excluded, the nutrient

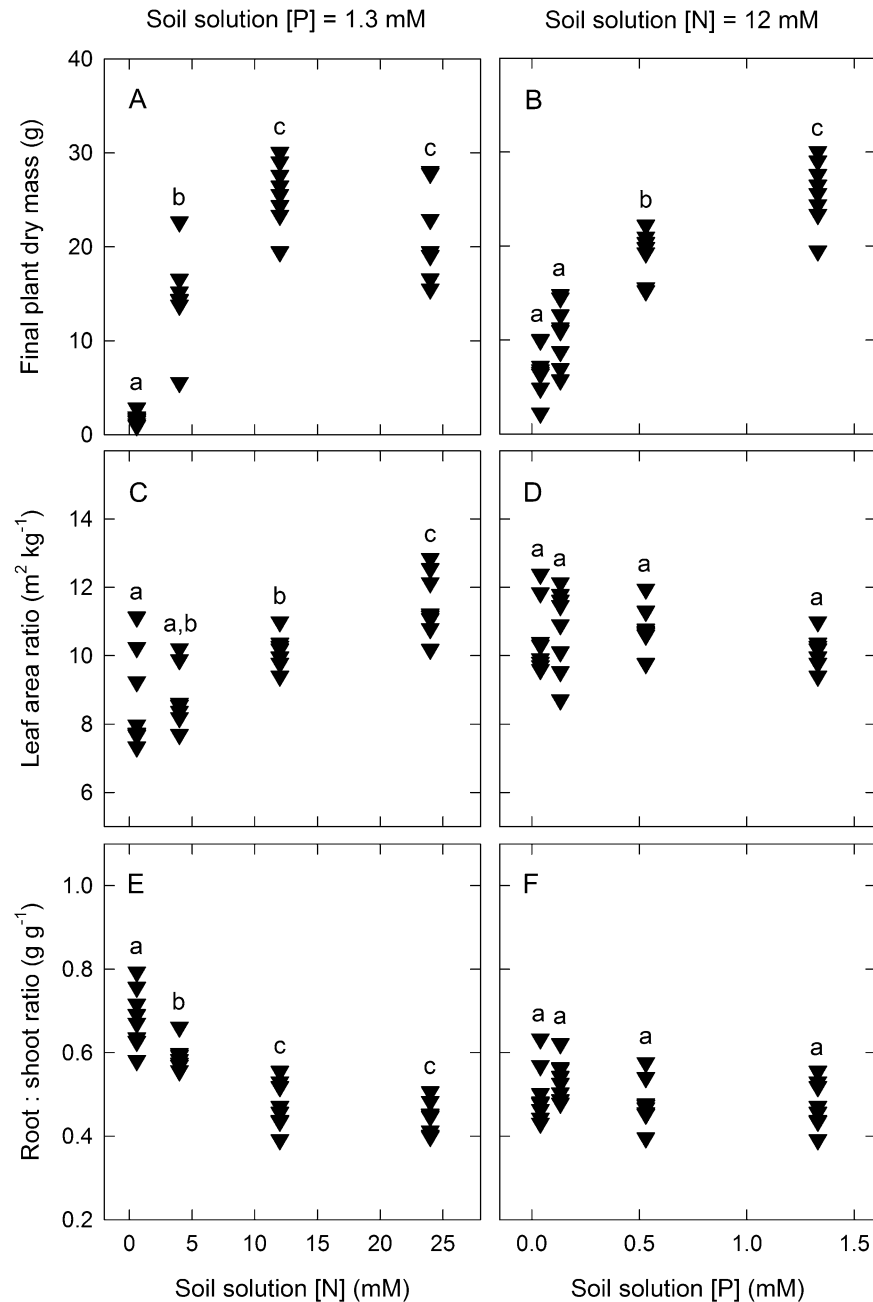


Fig. 1. Final plant dry mass (A and B), leaf area ratio (C and D), and root:shoot ratio (E and F) of *Ficus insipida* seedlings plotted against nutrient solution nitrogen and phosphorus concentrations. The left-hand panels (A, C, and E) show variation as a function of nutrient solution nitrogen concentration with nutrient solution phosphorus concentration held constant at 1.33 mM. The right-hand panels (B, D, and F) show variation as a function of nutrient solution phosphorus concentration with nutrient solution nitrogen concentration held constant at 12 mM. Different lower case letters within a panel indicate significantly different means at $P < 0.05$.

solution N:P ratios ranged from 1 to 135. Across this range of nutrient solution N:P, the estimated H , describing the extent of homeostatic control over plant biomass N:P, was 6.3 for leaves, 2.5 for stems, and 2.8 for roots. Thus, regulatory control over N:P stoichiometry was considerably stronger in leaves than in stems or roots. For whole-plant N:P, the relationship to nutrient solution N:P was approximately linear over the full range of nutrient solution N:P (Fig. 5D). The H calculated for whole-plant N:P was 3.1.

Leaf N:P ratios were higher than stem and root N:P ratios over nearly the full range of values achieved in the

study (Fig. 6A and B, respectively). However, at the very lowest values, leaf N:P ratios appeared to converge with those of stems and roots. Thus, relationships between N:P ratios of leaves and those of stems and roots were non-linear (Fig. 6A, B). The N:P ratios of stems and roots were generally similar to each other (Fig. 6C), although the relationship also appeared to depart from strict linearity.

The largest plant dry mass at the conclusion of the experiment was achieved in the nutrient solution treatment that had an N:P ratio of 4.1. Nutrient solution treatments with higher or lower N:P ratios resulted in smaller plants

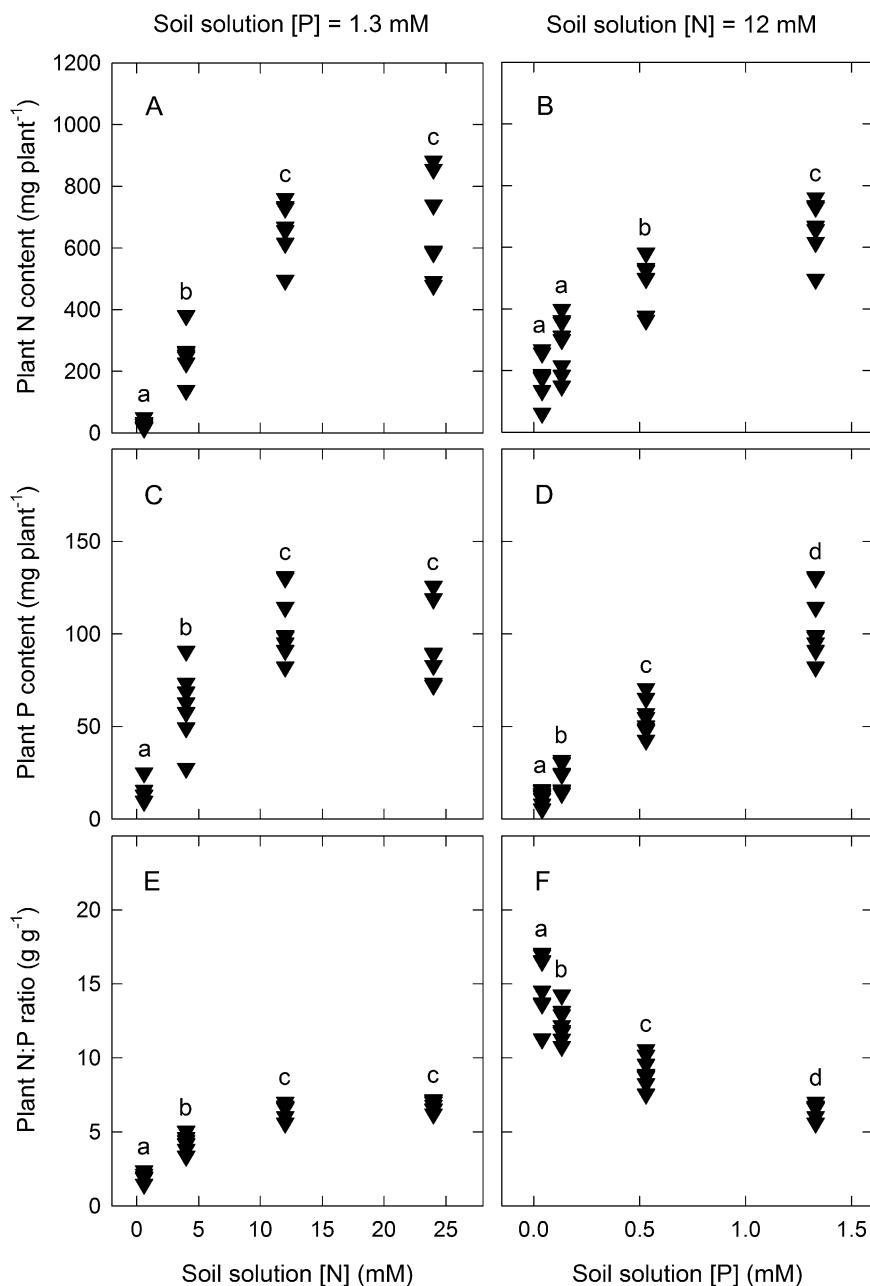


Fig. 2. Nitrogen content per plant (A and B), phosphorus content per plant (C and D), and plant nitrogen to phosphorus ratio (E and F) of *Ficus insipida* seedlings as a function of variation in the nitrogen and phosphorus concentration of nutrient solutions fed to the plants. The left-hand panels (A, C, and E) show variation as a function of nutrient solution nitrogen concentration with nutrient solution phosphorus concentration held constant at 1.33 mM. The right-hand panels (B, D, and F) show variation as a function of nutrient solution phosphorus concentration with nutrient solution nitrogen concentration held constant at 12 mM. Different lower case letters within a panel indicate significantly different means at $P < 0.05$.

(Fig. 7A). When plotted against leaf N:P ratio, final plant dry mass increased with increasing leaf N:P ratio up to a value of ~ 11 , and then decreased as the leaf N:P ratio increased further (Fig. 7B). This suggests that the optimal leaf N:P ratio for *F. insipida* is ~ 11 . When plotted against whole-plant N:P ratio, final plant dry mass increased up to a value of ~ 6 , and then decreased as the whole-plant N:P ratio increased further (Fig. 7C). Therefore, the optimal whole-plant N:P ratio was about half the optimal leaf N:P ratio.

The diurnal whole-plant transpiration rate, measured gravimetrically and expressed on a leaf area basis, varied significantly in response to variation in the nutrient solution [N] when nutrient solution [P] was held constant. Transpiration was highest at the lowest [N], and decreased as nutrient solution [N] increased (Fig. 8A). On the other hand, gravimetric transpiration showed no response to variation in nutrient solution [P] when nutrient solution [N] was held constant (Fig. 8B).

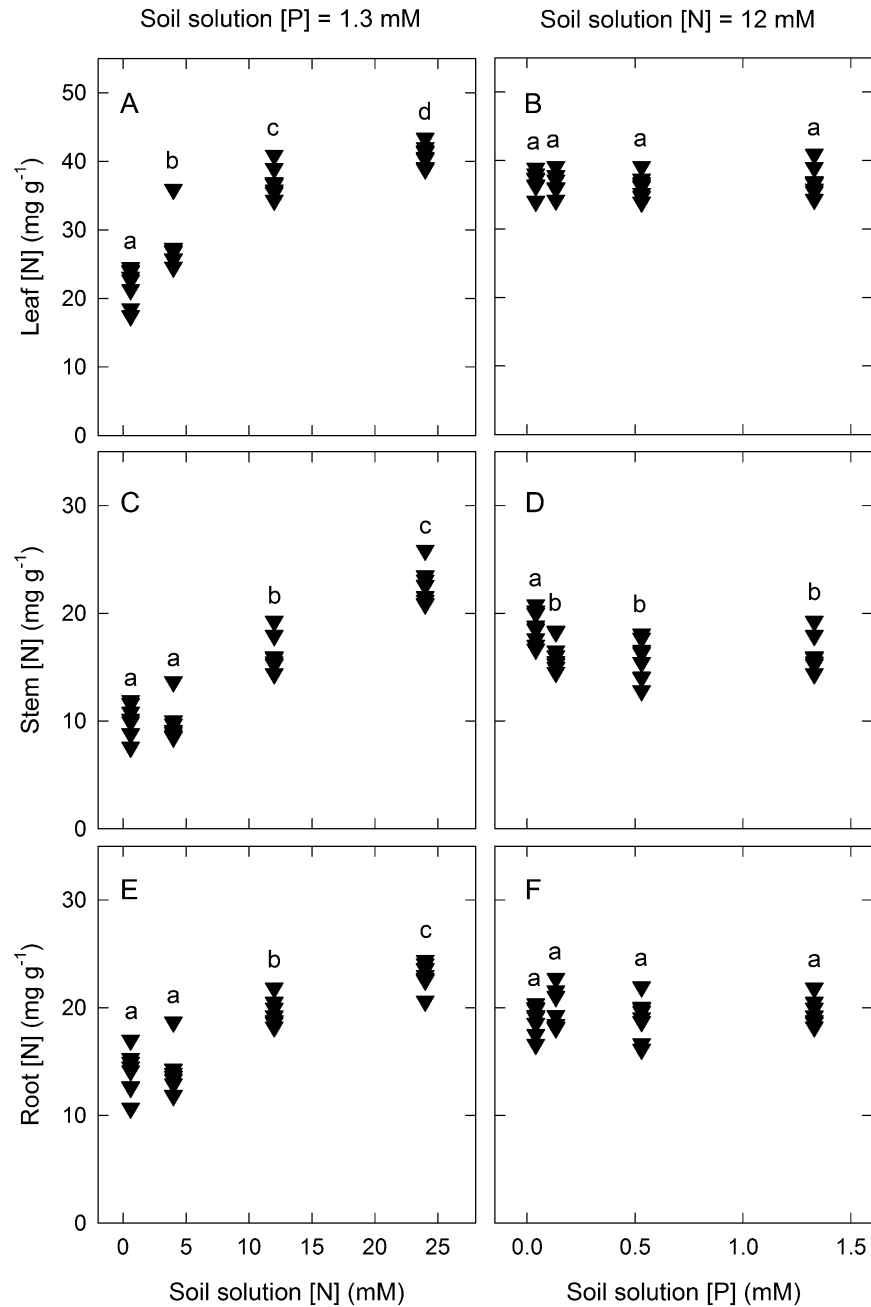


Fig. 3. The nitrogen concentration of leaves (A and B), stems (C and D), and roots (E and F) of *Ficus insipida* seedlings as a function of variation in the nitrogen and phosphorus concentration of nutrient solutions fed to the plants. The left-hand panels (A, C, and E) show variation as a function of nutrient solution nitrogen concentration with nutrient solution phosphorus concentration held constant at 1.33 mM. The right-hand panels (B, D, and F) show variation as a function of nutrient solution phosphorus concentration with nutrient solution nitrogen concentration held constant at 12 mM. Different lower case letters within a panel indicate significantly different means at $P < 0.05$.

Photosynthesis, expressed on an area basis, was lower at the lowest nutrient solution [N] than at other nutrient solution [N] (Fig. 9A). Area-based photosynthesis did not vary in response to variation in nutrient solution [P] (Fig. 9B). Stomatal conductance increased as nutrient solution [N] increased from 0.6 mM to 4 mM, then decreased as nutrient solution [N] increased further (Fig. 9C). Stomatal conductance did not vary in response to nutrient solution [P] (Fig. 9D). The ratio of internal to ambient CO_2

mole fractions, c_i/c_a , decreased in response to increasing nutrient solution [N] with nutrient solution [P] held constant (Fig. 9E). However, c_i/c_a showed no response to variation in nutrient solution [P] with nutrient solution [N] held constant (Fig. 9F).

Variation in whole-plant $\delta^{13}\text{C}$ confirmed the trends observed across nutrient solution treatments for c_i/c_a . Whole-plant $\delta^{13}\text{C}$ increased with increasing nutrient solution [N] with nutrient solution [P] held constant (Fig. 10A),

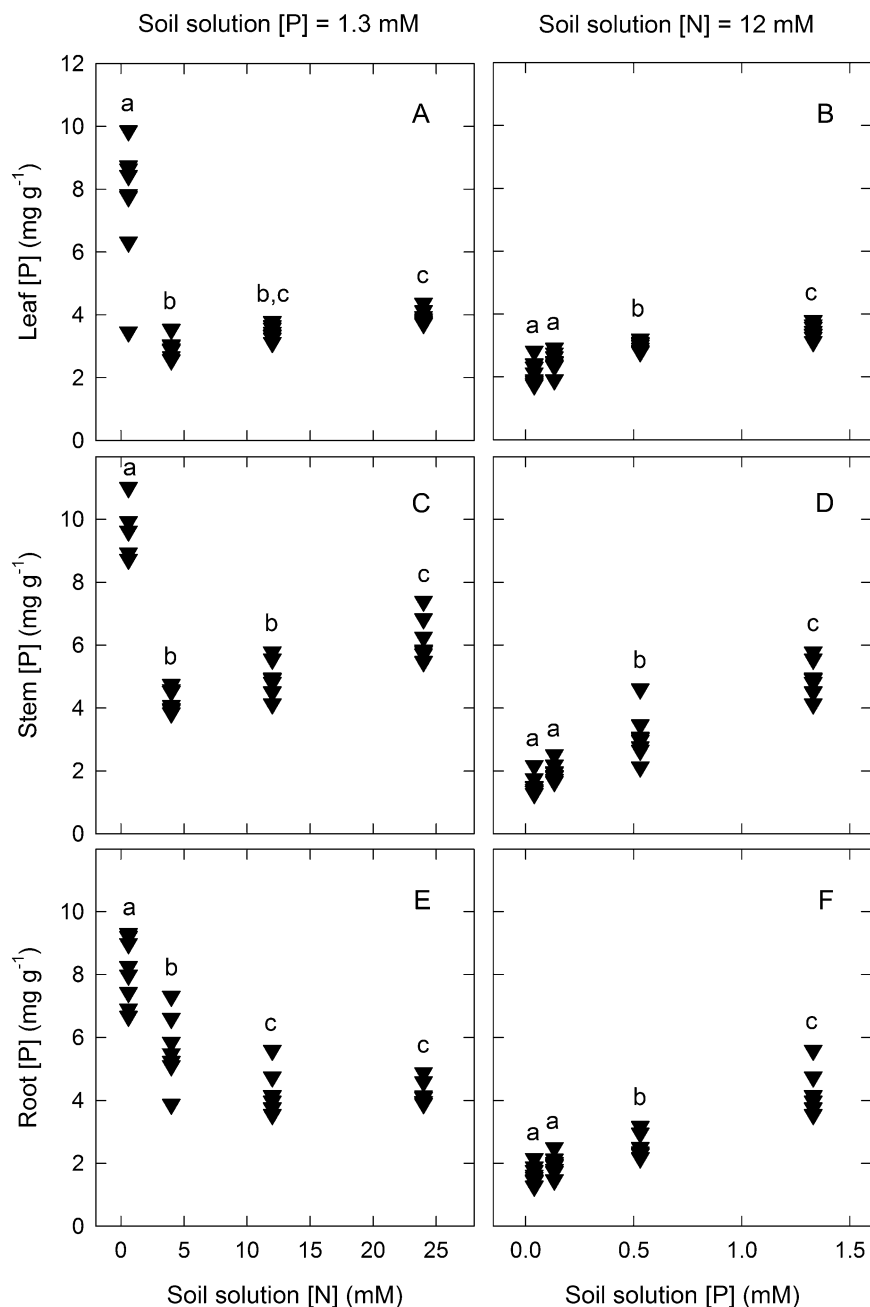


Fig. 4. The phosphorus concentration of leaves (A and B), stems (C and D), and roots (E and F) of *Ficus insipida* seedlings as a function of variation in the nitrogen and phosphorus concentration of nutrient solutions fed to the plants. The left-hand panels (A, C, and E) show variation as a function of nutrient solution nitrogen concentration with nutrient solution phosphorus concentration held constant at 1.33 mM. The right-hand panels (B, D, and F) show variation as a function of nutrient solution phosphorus concentration with nutrient solution nitrogen concentration held constant at 12 mM. Different lower case letters within a panel indicate significantly different means at $P < 0.05$.

and showed no response to variation in nutrient solution [P] with nutrient solution [N] held constant (Fig. 10B).

For plants grown at variable nutrient solution [N], photosynthesis expressed on a mass basis increased linearly with increasing leaf [N] (Fig. 11A). The regression equation relating the two was $A_m = 9.0N_m + 255$ ($R^2 = 0.68$, $P < 0.001$, $n = 31$), where A_m is mass-based photosynthesis and N_m is mass-based leaf [N]. For plants grown at variable soil

solution [P], photosynthesis also increased linearly with increasing leaf [P] (Fig. 11B), but the relationship was weaker than for leaf [N]. The relationship relating A_m to leaf [P] on a mass basis (P_m) was $A_m = 33P_m + 500$ ($R^2 = 0.14$, $P = 0.03$, $n = 32$). A plot of mass-based photosynthesis against leaf N:P ratio showed that photosynthesis increased up to a leaf N:P of ~ 12 , and then decreased as leaf N:P increased further (Fig. 11C).

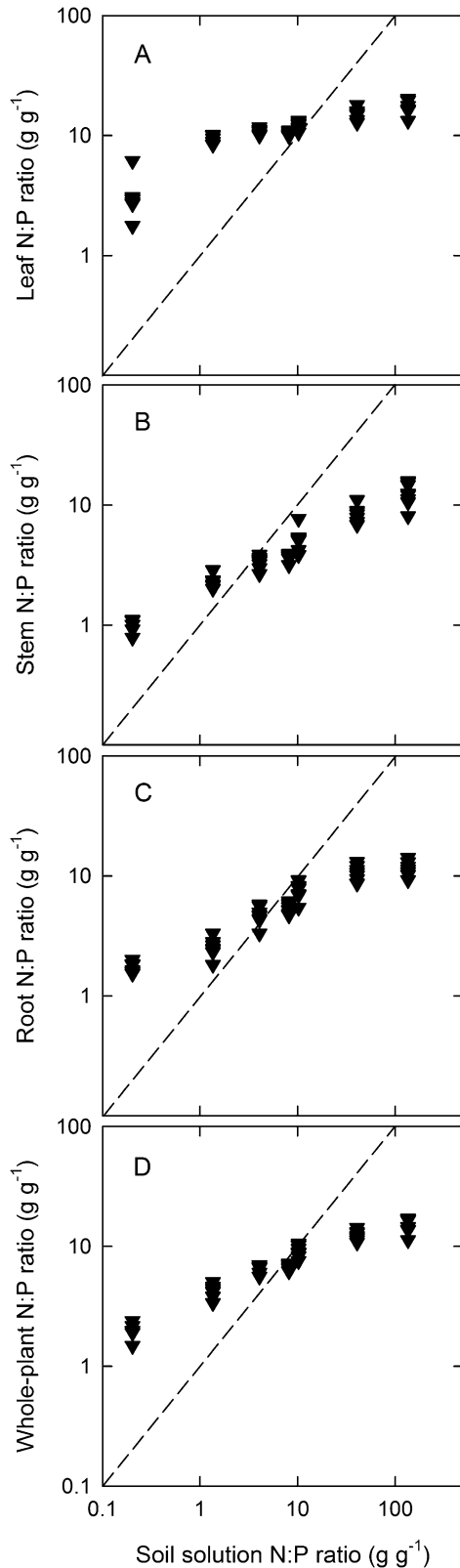


Fig. 5. The nitrogen to phosphorus ratio of leaves (A), stems (B), roots (C), and whole plants (D) of *Ficus insipida* plotted as functions of the nitrogen to phosphorus ratio of the nutrient solutions fed to the plants. Both the x-axis and y-axis are shown on logarithmic scales. The dashed lines represent one-to-one lines.

Discussion

Plant biomass N:P ratios for the tropical pioneer tree *F. insipida* varied as a function of the N:P ratio of the nutrient solutions fed to the plants. Estimates of H, the inverse slope of the log–log relationship between plant N:P and nutrient solution N:P, demonstrated the extent of regulatory control over plant N:P expressed in this species. For nutrient solution N:P ratios ranging from 1 to 135, H for leaf dry matter was 6.3, whereas values for stems and roots were 2.5 and 2.8, respectively. For whole-plant biomass, H for *F. insipida* was 3.1. These values can be compared with previously observed values for plant biomass ranging from 1.7 to 4.6 (Güsewell, 2004). Therefore, the degree of homeostatic control over the N:P ratio at the whole-plant level in *F. insipida* was generally within the range of values observed for other vascular plant species. The N:P ratio of plant biomass reflected variation in the N:P ratio of the soil solution, but the range of N:P observed in plant biomass was damped in comparison with the range of nutrient solution N:P experimentally supplied to the plants.

Leaf dry matter showed a higher value for H than stem or root dry matter (Fig. 5). This observation could have important implications for diagnosing relative availabilities of N and P in ecosystems based on measurements of plant biomass N:P ratios. The higher H in leaves indicated that the strength of regulatory control over N:P stoichiometry was stronger in leaves than in stems or roots. This is consistent with the idea that excess N or P taken up from the soil solution could be more effectively stored in stems and roots than in leaves when the supply of one of these nutrients limits growth but the supply of the other does not (Dyckmans et al., 2000; Cernusak et al., 2010). This would be a favourable strategy for perennial plants because leaf life spans are probably much shorter than those of stems and coarse roots. Thus, excess N or P stored in leaves would be more vulnerable to loss from the plant by leaf abscission or herbivory than excess N or P stored in stems or coarse roots. Based on these observations in *F. insipida*, it is concluded that the N:P ratio of leaf dry matter will probably be a less sensitive indicator of variation in the N:P ratio of the soil solution than that of stem or root dry matter.

The present study was conducted on seedlings, the largest of which had a final plant dry mass of ~30 g (Fig. 1A, B). The stems of these plants were clearly not woody structures in the same sense as the boles of mature trees. Therefore, it is unknown to what extent results from this study describing organ-specific patterns in N:P stoichiometry can be extrapolated to large trees growing in forests. It is possible that the stems of the seedlings studied here could be more analogous to small diameter branches or twigs than to the wood in the main stems of large trees in the way that they regulate their biomass N:P ratios. It is noteworthy, however, that the [P] of bole wood appears to show a high sensitivity to soil fertility (Lloyd et al., 2001), presumably reflecting variation in the [P] of the soil solution. Thus, it is

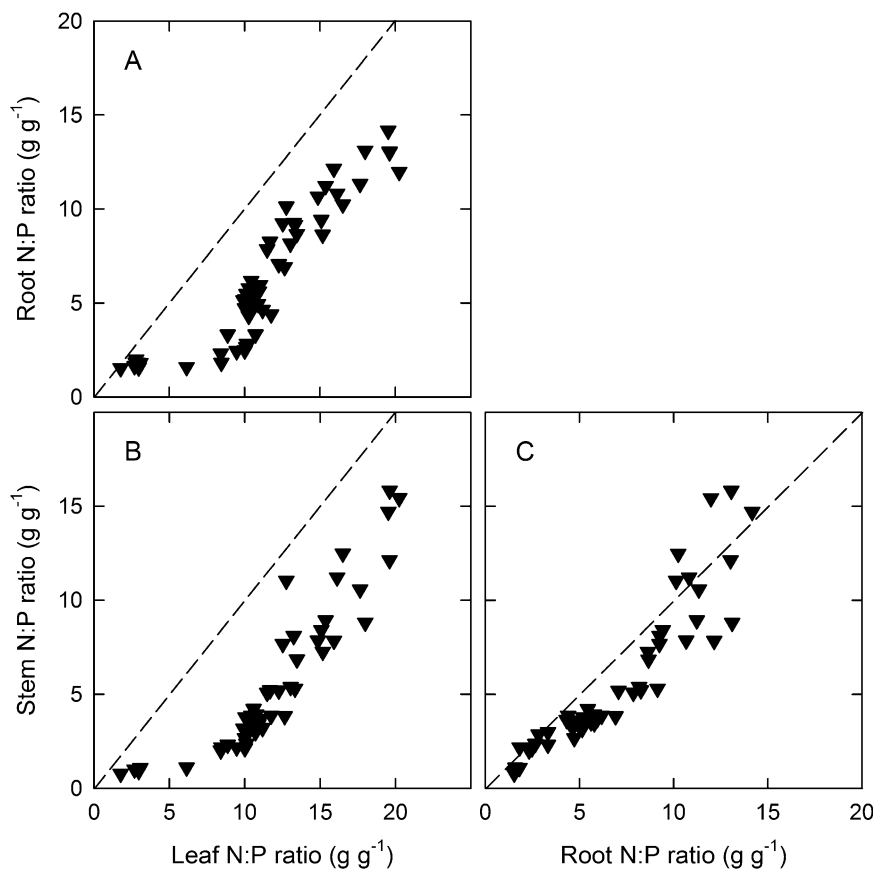


Fig. 6. The nitrogen to phosphorus ratio of roots plotted against that of leaves (A), the nitrogen to phosphorus ratio of stems plotted against that of leaves (B), and the nitrogen to phosphorus ratio of stems plotted against that of roots (C) for seedlings of *Ficus insipida*. Dashed lines represent one-to-one lines.

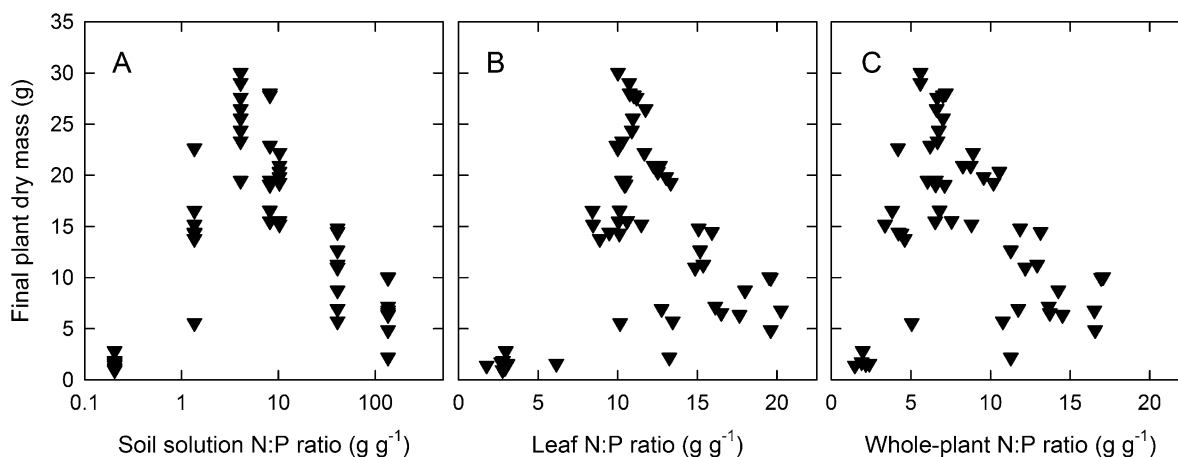


Fig. 7. Final plant dry mass of *Ficus insipida* seedlings plotted as a function of the nutrient solution nitrogen to phosphorus ratio (A), leaf nitrogen to phosphorus ratio (B), and whole-plant nitrogen to phosphorus ratio (C).

also possible that the N:P ratio of the bole wood in mature trees will show a higher sensitivity to the N:P ratio of the soil solution than leaves. Further investigation is required to test this hypothesis.

The optimal leaf N:P ratio for *F. insipida* observed in the present study was ~ 11 (Fig. 7B), whereas the optimal whole-plant N:P ratio was ~ 6 (Fig. 7C). These were the

values with which the largest final plant dry mass was achieved in the experiment. The optimal leaf N:P ratio determined in this way was similar to the optimal value determined from measurements of leaf photosynthesis. The highest rates of mass-based photosynthesis were achieved at a leaf N:P ratio of ~ 12 (Fig. 11C). These values are less than N:P ratios ranging from 14 to 16 that have been

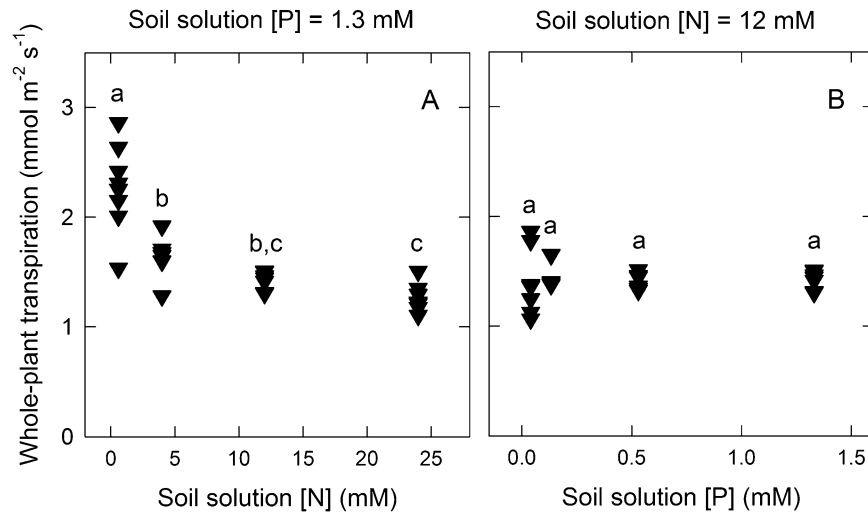


Fig. 8. Daytime transpiration of *Ficus insipida* seedlings determined gravimetrically plotted against nutrient solution nitrogen concentration with phosphorus concentration held constant (A) and nutrient solution phosphorus concentration with nitrogen concentration held constant (B). Different lower case letters within a panel indicate significantly different means at $P < 0.05$.

suggested for plants with balanced supplies of N and P (Koerselman and Meuleman, 1996; Aerts and Chapin, 2000; Tessier and Raynal, 2003; Güsewell, 2004). *Ficus insipida* is a fast-growing pioneer tree. Plants with high relative growth rates tend to have lower biomass N:P ratios than plants with low relative growth rates (Ågren, 2004; Güsewell, 2004; Niklas et al., 2005; Niklas, 2006; Matzek and Vitousek, 2009; Cernusak et al., 2010). This could explain why *F. insipida* achieved the highest growth and photosynthesis at a lower leaf N:P ratio than that expected from studies of some other vascular plant species.

The leaf N:P ratio was higher than the N:P ratio of either stems or roots across the full range of biomass N:P ratios observed in the present study, whereas stems and roots were generally similar (Fig. 6). It was previously observed that N:P ratios of leaves were higher than those of stems and roots in herbaceous plants, but not in woody plants (Kerckhoff et al., 2006). It seems likely that there would be significant ontogenetic variation in these trends for woody perennial plants. Thus, it is possible that because we used seedlings in our study, interorgan variation in biomass N:P ratios was more similar to that previously observed for herbaceous species than for woody species.

The [P] of plant biomass showed a strong response to variation in nutrient solution [N] with the [P] of the nutrient solution held constant (Fig. 4A, C, and E). It is suggested that the [P] of plant biomass was modulated by changes in transpiration rate under variable [N] of the nutrient solution. Transpiration rate varied in response to nutrient solution [N] (Fig. 8A). Expressing transpiration rates per unit dry mass, to make them compatible with [P] per unit dry mass, resulted in a significant correlation between whole-plant [P] and transpiration. The relationship was $[P] = 0.59E - 3.85$ ($R^2 = 0.54$, $P < 0.001$, $n = 25$), where [P] is whole-plant [P] (mg P g⁻¹ dry mass), and E is average transpiration rate measured gravimetrically over 3 d, expressed per unit plant dry mass ($\mu\text{mol H}_2\text{O g}^{-1}$ dry

mass s⁻¹). This relationship is for plants grown at constant nutrient solution [P].

Solutes can be transported to root surfaces either by diffusion through the soil solution or by mass flow of the soil solution (Barber, 1995; Tinker and Nye, 2000; McDonald et al., 2002; Cramer et al., 2008). Mass flow of the soil solution occurs as a consequence of plant transpiration. As a result, some fraction of nutrient transport to root surfaces occurs by mass flow. Under steady-state conditions, an increased rate of water uptake by roots will result in a higher solute concentration at the root surface than would occur at a lower rate of water uptake (Nye and Tinker, 1977). This could lead to an increased rate of solute transport across the membranes of root cells due to 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). It is suggested here that transpiration-induced variation in the mass flow of P to root surfaces in *F. insipida* caused the correlation between plant [P] and transpiration rate at constant nutrient solution [P]. This was probably facilitated by the relatively high [P] of the nutrient solution, which was 1.33 mM. In contrast, inorganic [P] in natural soils is generally $< 10 \mu\text{M}$ (Bielecki, 1973; Barber, 1995; Schachtman et al., 1998). Thus, although mass flow appeared to play an important role in modulating plant [P] in the present experiment, the extent to which mass flow influences P acquisition in *F. insipida* plants growing in natural systems is unknown.

Plant transpiration was higher at low nutrient solution [N] than at high nutrient solution [N] (Fig. 8A), consistent with previous observations in *F. insipida* and other species (Guehl et al., 1995; Livingston et al., 1999; Cernusak et al., 2007b; Cramer et al., 2008). Plant transpiration was measured gravimetrically, and expressed per unit leaf area. Variation in plant transpiration rate in response to nutrient solution [N] resulted from variation in both canopy architecture and stomatal conductance. Self-shading was

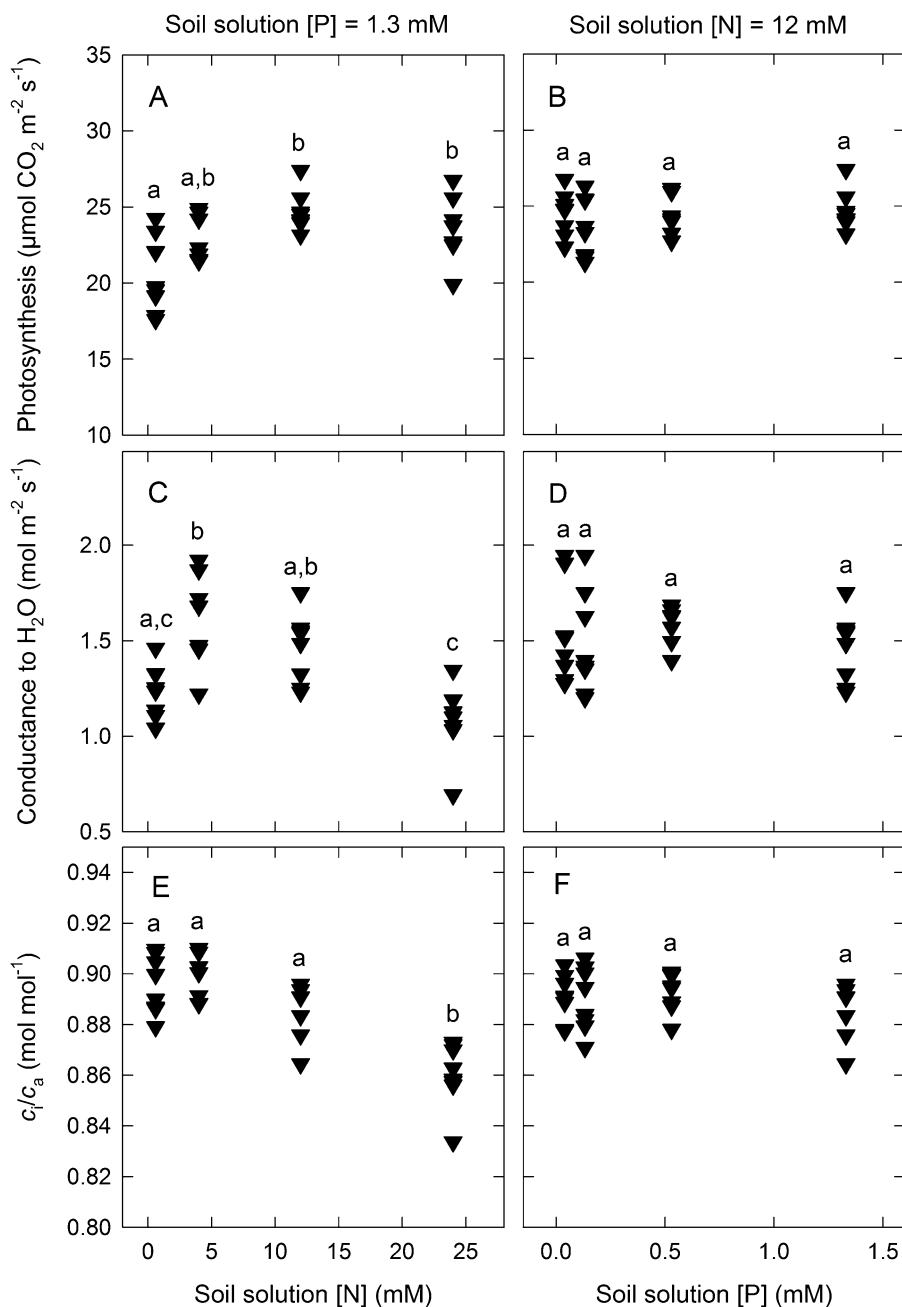


Fig. 9. Photosynthesis (A and B), stomatal conductance (C and D), and the ratio of intercellular to ambient CO₂ mole fractions, c_i/c_a (E and F), plotted as functions of nutrient solution nitrogen and phosphorus concentration for seedlings of *Ficus insipida*. The left-hand panels (A, C, and E) show variation as a function of nutrient solution nitrogen concentration with nutrient solution phosphorus concentration held constant at 1.33 mM. The right-hand panels (B, D, and F) show variation as a function of nutrient solution phosphorus concentration with nutrient solution nitrogen concentration held constant at 12 mM. Different lower case letters within a panel indicate significantly different means at $P < 0.05$.

greater at high than at low [N], due to greater canopy development and leaf area ratio at high than at low [N] (Fig. 1A, C). Thus, the average intercepted irradiance per unit leaf area was lower in high [N] than in low [N] plants. In addition, stomatal conductance decreased as nutrient solution [N] increased, except for at the lowest nutrient solution [N] (Fig. 9C). These results support the suggestion that plants express some degree of regulation over transpirational water losses in order to control the

delivery of N to root surfaces by mass flow (Cramer *et al.*, 2009).

An important distinction can be drawn between the interaction observed in the present experiment between N and transpiration and the interaction between P and transpiration. In *F. insipida*, the transpiration rate varied in response to N availability (Fig. 8A). On the other hand, the transpiration rate did not vary in response to P availability (Fig. 8B). Plant [P] increased with increasing transpiration

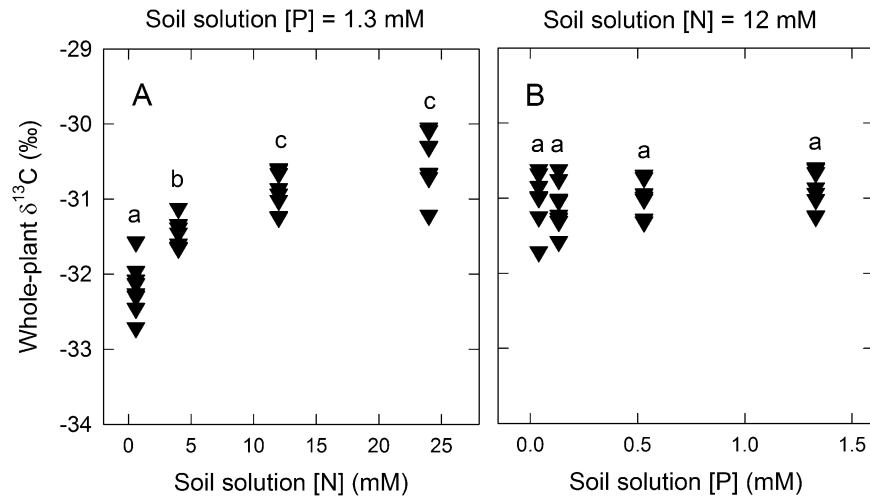


Fig. 10. Whole-plant carbon isotope ratio ($\delta^{13}\text{C}$) of *Ficus insipida* seedlings plotted against nutrient solution nitrogen concentration with phosphorus concentration held constant (A) and nutrient solution phosphorus concentration with nitrogen concentration held constant (B). Different lower case letters within a panel indicate significantly different means at $P < 0.05$.

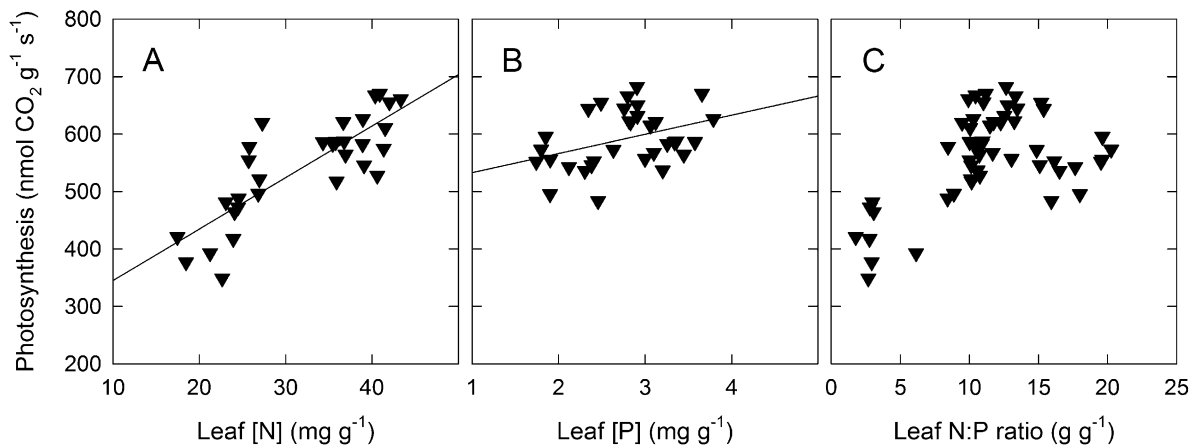


Fig. 11. Mass-based photosynthesis plotted against leaf nitrogen concentration (A), leaf phosphorus concentration (B), and leaf nitrogen to phosphorus ratio (C). Data in A are for plants grown at variable nitrogen concentration and constant phosphorus concentration, and data in B are for plants grown at variable phosphorus concentration and constant nitrogen concentration. Data in C are for both sets of plants. Solid lines in A and B are least-squares linear regressions. Both are significant at $P < 0.05$.

rate at constant nutrient solution [P]. It is assumed that plant [N] would also increase with increasing transpiration rate at constant nutrient solution [N], but this cannot be tested in the present data set because plant transpiration did not vary among treatments at constant nutrient solution [N] (Fig. 8B). Thus, the results suggest that variation in N availability in the soil solution caused variation in transpiration, whereas accumulation of P in plant biomass was modulated by transpiration, but variation in P availability in the soil solution did not cause variation in transpiration.

Biomass allocation responded to variation in N availability, but did not respond to variation in P availability. This was true for both leaf area ratio and root:shoot ratio (Fig. 1). Similarly, leaf gas exchange characteristics varied in response to N availability, but did not respond to variation in P availability (Fig. 9). These results suggest

that variation in soil solution [N] triggered stronger physiological and morphological responses in *F. insipida* than did variation in soil solution [P] over the concentration ranges for the two nutrients employed in this study.

The concentration ranges of both N and P employed in this study induced pronounced variations in plant growth, as indicated by variation in final plant dry mass in response to either [N] or [P] when the other was held constant (Fig. 1A, B). Thus, growth clearly responded to variation in P availability, whereas biomass allocation and leaf gas exchange did not. The mean final plant dry mass at the highest [N] was less than that at the second highest [N] (Fig. 1A), suggesting the possibility of a toxicity response at the highest [N]. However, the two treatments were not significantly different, suggesting that the difference in means occurred by chance, rather than as a result of the

treatments. In contrast to the saturating response of final plant dry mass to [N], final plant dry mass continued to increase at the highest nutrient solution [P] (Fig. 1B), suggesting that P availability limited growth up to the highest nutrient solution [P] applied to the plants.

Plant water-use efficiency is known to increase with increasing [N] in plant biomass, all else being equal (Guehl *et al.*, 1995; Livingston *et al.*, 1999; Raven *et al.*, 2004; Ripullone *et al.*, 2004; Cernusak *et al.*, 2007a, b, 2010). Some observations also suggest that water-use efficiency increases with increasing P availability, or at least that it increases with relief of P deficiency (Raven *et al.*, 2004). Water-use efficiency at a given air vapour pressure deficit (VPD) varies primarily as a function of the ratio of leaf internal to ambient CO₂ mole fractions, c_i/c_a , during photosynthesis (Farquhar and Richards, 1984; Farquhar *et al.*, 1989b; Hubick and Farquhar, 1989; Cernusak *et al.*, 2007b, 2008, 2009a, b). Plant $\delta^{13}\text{C}$ provides a time-integrated estimate of c_i/c_a (Farquhar *et al.*, 1982, 1989a; Brugnoli and Farquhar, 2000). Measurements of c_i/c_a and $\delta^{13}\text{C}$ in *F. insipida* indicated that water-use efficiency at a given VPD varies as a function of N availability in this species, but does not vary as a function of P availability (Figs 9, 10) over the concentration ranges of the two nutrients employed in this study.

Conclusions

The N:P ratio of plant biomass in *F. insipida* reflected variation in the N:P ratio of the soil solution, but biomass N:P spanned a much smaller range than soil solution N:P due to regulatory control by the plant. The extent of homeostatic regulation over biomass N:P was stronger in leaves than in stems or roots. Therefore, it is concluded that stem or root dry matter is probably a more sensitive indicator of environmental variation in the relative availability of N and P than leaf dry matter. Plant [P] varied as function of transpiration rate at constant nutrient solution [P], possibly due to transpiration-induced variation in the mass flow of P to root surfaces. The transpiration rate responded to nutrient solution [N], but not to nutrient solution [P], demonstrating nutritional control over transpirational water loss by N, but not P. Measurements of c_i/c_a and $\delta^{13}\text{C}$ indicated variation in water-use efficiency as a function of N availability, but not as a function of P availability.

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