



Research paper

Why are tropical conifers disadvantaged in fertile soils? Comparison of *Podocarpus guatemalensis* with an angiosperm pioneer, *Ficus insipida*

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Conifers are, for the most part, competitively excluded from tropical rainforests by angiosperms. Where they do occur, conifers often occupy sites that are relatively infertile. To gain insight into the physiological mechanisms by which angiosperms outcompete conifers in more productive sites, we grew seedlings of a tropical conifer (*Podocarpus guatemalensis* Standley) and an angiosperm pioneer (*Ficus insipida* Willd.) with and without added nutrients, supplied in the form of a slow-release fertilizer. At the conclusion of the experiment, the dry mass of *P. guatemalensis* seedlings in fertilized soil was approximately twofold larger than that of seedlings in unfertilized soil; on the other hand, the dry mass of *F. insipida* seedlings in fertilized soil was ~20-fold larger than seedlings in unfertilized soil. The higher relative growth rate of *F. insipida* was associated with a larger leaf area ratio and a higher photosynthetic rate per unit leaf area. Higher overall photosynthetic rates in *F. insipida* were associated with an approximately fivefold larger stomatal conductance than in *P. guatemalensis*. We surmise that a higher whole-plant hydraulic conductance in the vessel bearing angiosperm *F. insipida* enabled higher leaf area ratio and higher stomatal conductance per unit leaf area than in the tracheid bearing *P. guatemalensis*, which enabled *F. insipida* to capitalize on increased photosynthetic capacity driven by higher nitrogen availability in fertilized soil.

Keywords: carbon isotope ratio, conifer, nitrogen, photosynthesis, relative growth rate, water-use efficiency.

Introduction

Conifers have been largely excluded from tropical rainforests since the angiosperm radiation during the Cretaceous (Regal 1977, Morley 2000, Fragnière et al. 2015). It has been suggested that, in general, angiosperms are able to outcompete conifers in productive habitats through faster growth rates, especially in early life stages (Bond 1989, Midgley and Bond 1991, Brodribb and Feild 2010, Brodribb et al. 2012). This superior competitive ability has been linked to innovations in leaf vascular architecture and sapwood porosity. However, conifers do still occur in habitats where plant growth is constrained by

low nutrient availability and/or by cooler temperatures. Although conifers are not well represented in tropical forests overall, the family Podocarpaceae is an exception, reaching its highest diversity in low latitudes of the Southern Hemisphere (Biffin et al. 2011, Morley 2011), particularly in Southeast Asia and the neotropics (Dalling et al. 2011, Enright and Jaffré 2011, Punyasena et al. 2011). Some podocarp taxa display prominent leaf flattening, which presumably allows them to capture light more efficiently in the dark understories of tropical forests than could be done with imbricate or needle leaves (Brodribb 2011, Lusk 2011). Because some podocarps do co-occur with

angiosperm trees in tropical forests (Cernusak et al. 2011), comparing the two may be especially informative in terms of understanding the underlying mechanisms through which angiosperms outcompete conifers in the more productive sites.

Podocarpus guatemalensis Standley is a neotropical podocarp with flattened leaves able to attain a maximum height of ~30 m. It has a relatively widespread distribution in Central America and northern South America, where it occurs at elevations ranging from near sea level to more than 2000 m above sea level (Dalling et al. 2011, Ornelas et al. 2019). In Panama, *P. guatemalensis* occurs on a number of islands along both the Pacific and Atlantic coasts and occasionally in wet, lowland forests of the mainland. On the Coiba Island, off the Pacific Coast of Panama, *P. guatemalensis* appears to be confined to habitats associated with infertile soils (Dalling et al. 2011), suggesting that its ability to compete successfully with co-occurring angiosperms for site occupancy is favored under such conditions.

Two important determinants of the relative growth rate of a plant are the leaf area ratio (i.e., the photosynthetic surface area per total plant dry mass) and the photosynthetic rate per unit leaf area (Blackman 1919, Evans 1972, Masle and Farquhar 1988, Poorter and Remkes 1990). Multiplying these two terms together gives the amount of CO₂ assimilated by the plant per unit plant mass per unit time. Photosynthesis is also an important part of the unit leaf rate, the amount of plant dry matter accumulated per unit leaf area per unit time. Leaf area ratio has the same units as the more familiar specific leaf area but differs in that it takes as the quotient the dry mass of the entire plant rather than the dry mass of leaves (Beadle 1985). Increasing nutrient availability, especially nitrogen, can be expected to result in increases in one or both of leaf area ratio and photosynthetic rate. With more nitrogen available, a plant can produce more leaf material, which is nitrogen rich compared with stems and roots (Aerts and Chapin 2000). Furthermore, because photosynthetic enzymes, especially Rubisco, are nitrogen rich, the plant may also be able to build more photosynthetic machinery and thereby photosynthesize at higher rates per unit leaf area (Garrish et al. 2010).

In addition to photosynthetic rate increasing due to a larger biochemical demand for CO₂, it can also increase if the resistance to CO₂ diffusion into the leaf is reduced, which occurs when stomatal conductance is higher (Farquhar and Sharkey 1982, Cernusak 2020). In previous experiments, we observed that *P. guatemalensis* had lower intercellular CO₂ concentrations and lower stomatal conductance than angiosperm tree species it was compared to, which corresponded to higher water-use efficiency but also lower photosynthetic rates (Cernusak et al. 2008, Dalling et al. 2016). One reason why broad-leaved conifers might have lower stomatal conductance and associated lower intercellular CO₂ concentrations and photosynthetic rates is because the hydraulic conductivity

of conifer wood is less than that of angiosperms, due to conifers having tracheids and angiosperms having vessels (Sperry et al. 2006). In conifers, tracheids both conduct water and provide mechanical support, whereas in angiosperms the role of mechanical support has been parsed out, freeing the vessels to have larger diameters and therefore conduct water more efficiently. In conifers with flattened leaves, relatively inefficient tracheids might not be able to deliver water to the evaporative sites within leaves with the efficiency needed to allow high stomatal conductance and therefore high intercellular CO₂ concentrations typically associated with high photosynthetic rates (Brodribb and Feild 2000, Brodribb and Feild 2010).

The ratio of intercellular to ambient CO₂ concentrations (c_i/c_a) can be assessed through instantaneous gas exchange measurements to provide insight into the balance between supply of CO₂ by stomatal conductance and demand for CO₂ by photosynthesis. In addition, the stable carbon isotope ratio ($\delta^{13}\text{C}$) of leaf dry matter can provide a time-integrated measure of c_i/c_a for the period of time over which the carbon contained in the leaf tissue was assimilated (Farquhar et al. 1982, Farquhar and Richards 1984, Cernusak 2020). Furthermore, the stable nitrogen isotope ratio ($\delta^{15}\text{N}$) of plant tissues can also provide insight into the dynamics of nitrogen uptake from the soil and metabolism within the plant (Evans 2001, Kahmen et al. 2008, Cernusak et al. 2009).

In this study, we aimed to explore the processes through which the tropical conifer *P. guatemalensis* and a fast-growing pioneer angiosperm, *Ficus insipida* Willd., adjust their relative growth rates in response to increased nitrogen availability, to gain insight into the habitat association of *P. guatemalensis* with low fertility soils. We also aimed to test whether the lower intercellular CO₂ concentrations that we observed previously in *P. guatemalensis* compared with angiosperms are a general feature of the species, consistent with a more pronounced diffusive limitation on photosynthesis than angiosperms typically experience.

Materials and methods

The experiment took place at the Smithsonian Tropical Research Institute, Santa Cruz Experimental Field Facility, Gamboa, Panama (9°07'N, 79°42'W; 28 m above sea level). Recently germinated seedlings of *P. guatemalensis* (Podocarpaceae) were collected from lowland forest at Playa Hermosa, Coiba Island, Panama. These were grown under shadehouse conditions for a few months to allow them to recover following the transplant. Seeds of *F. insipida* (Moraceae) were collected from mature trees growing in the Panama Canal watershed and germinated in trays containing a commercial potting mix. Observations of the species under natural conditions have shown *F. insipida* to be shade intolerant (Albrecht et al.

2017) and *P. guatemalensis* to be moderately shade tolerant (Corea-Arias et al. 2016).

In December 2010, 20 seedlings of each species were transplanted individually into 19 l pots. The soil in the pots comprised a 1:1 by volume mix of soil and rice husks, with the rice husks added to improve drainage and soil structure and to contribute toward a soil environment in which nitrogen was relatively unavailable (Dalling et al. 2013). At the start of the experiment, three seedlings of each species were harvested to determine initial dry mass. Mean values were 3.7 g for *P. guatemalensis* and 0.02 g for *F. insipida*. Initial leaf area was 262 cm² for *P. guatemalensis* and 5.0 cm² for *F. insipida*. In addition to the pots with seedlings, eight control pots were also prepared that did not contain seedlings. For all pots, the water content was brought to field capacity prior to transplant of seedlings by allowing saturated pots to freely drain overnight. The pots were situated beneath a rain shelter with a glass roof but were otherwise in open-air conditions little shaded by adjacent forest. Half of the pots for each species were randomly allocated to receive 12 g of Osmocote Plus controlled-release fertilizer (Scotts-Sierra, Maryville, OH, USA). The fertilizer contained by weight 15% nitrogen (N), 9% phosphorus (P), and 12% potassium (K) and had an estimated release time of 5–6 months at a temperature of 32 °C according to the manufacturer.

At the initiation of the experiment, drain holes at the bottom of the pots were sealed, and the soil surface was covered with gravel to minimize soil evaporation. The pots were then weighed at weekly intervals to the nearest 5 g with a 64 kg capacity balance (Sartorius QS64B, Thomas, Swedesboro, NJ, USA). After the weight was recorded, water was added to the pot to return it to its weight at field capacity. As the experiment progressed, pots with larger plants were weighed at shorter intervals to prevent excessive water loss. Plant transpiration over the course of the experiment was calculated as the difference between cumulative pot water loss and the mean water loss of the control pots. After 3.5 months, the plants were harvested and leaf area determined with an LI-3100 Leaf-Area Meter (Li-Cor Inc., Lincoln, NE, USA). Dry mass of leaves, stems, and roots was measured after oven drying at 70 °C. The whole-plant water-use efficiency was calculated as the plant dry mass increment over the experiment divided by the cumulative transpiration. Mean relative growth rate and mean unit leaf rate were calculated according to standard methods (Beadle 1985).

Leaf gas exchange was measured on three leaves per plant in the week preceding plant harvest with an Li-6400 portable photosynthesis system (Li-Cor Inc.). Leaves were illuminated with an artificial light source (6400-02B LED, Li-Cor Inc.) with 1500 μmol photons m⁻² s⁻¹. Measurements were made between 9 a.m. and midday local time. The mean leaf temperature during measurements was 32.0 ± 0.7 °C (mean ± 1 SD), and the mean leaf-to-air vapor pressure difference was 1.6 ± 0.4 kPa.

Leaf dry mass was ground to a fine powder for chemical and isotopic analyses using a Cyclotec 1093 mill with a 0.5 mm screen (FOSS, Eden Prairie, MN, USA). Foliar phosphorus was determined by ashing at 550 °C, followed by dissolution in 1 M H₂SO₄, with phosphate detection by automated molybdate colorimetry using a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO, USA). Foliar dry matter was analyzed for its δ¹³C, and total carbon and nitrogen concentrations, with an elemental analyzer (CE Instruments, Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta V; Thermo Fisher Scientific, Bremen, Germany). The δ¹³C was expressed relative to the PeeDee Belemnite international standard.

At the time of plant harvest, soil was sampled from each pot, and nitrate and ammonium were extracted in 2 M KCl. KCl-extractable concentrations were determined by automated colorimetry using a Lachat Quikchem 8500 (Hach Ltd). Available phosphorus was determined by extraction in Bray solution (Bray and Kurtz 1945) followed by automated colorimetry.

Variation in measured parameters among species and treatments was analyzed using two-way analysis of variance, followed by pairwise comparisons with Tukey tests. Bivariate relationships were assessed using Pearson correlations or least-squares linear regressions. Statistical analyses were performed in R (R core Team 2019).

Results

In Table 1, we provide a summary of variation among species and treatments in growth and physiological parameters. We observed significant variation in final dry mass between fertilized and unfertilized plants and between the two species, *F. insipida* and *P. guatemalensis* (Figure 1A). When combined with initial dry masses, this translated to significantly higher relative growth rates in fertilized plants compared with unfertilized plants and in *F. insipida* compared with *P. guatemalensis* (Figure 1B). The proportional increase in relative growth rate with fertilization was similar between the two species. It increased from 46.0 to 75.6 mg g⁻¹ day in *F. insipida* (an increase of 1.6-fold) and from 8.5 to 14.5 mg g⁻¹ day in *P. guatemalensis* (an increase of 1.7-fold). However, the much higher inherent relative growth rate in *F. insipida* resulted in much larger gains in biomass in fertilized compared with unfertilized plants.

The leaf area ratio of fertilized plants of both species was reduced compared with the unfertilized plants (Table 1). However, the decreases in leaf area ratio with nutrient addition appeared related to variation in plant size, and the treatment effect may have come about simply because fertilized plants were larger than unfertilized plants (Figure 2). Leaf nitrogen concentration in fertilized plants of both species was significantly higher compared with unfertilized plants (Table 1), with fertilized *F. insipida* showing the highest mean value

Table 1. Growth and physiological parameters quantified in the experiment. Values are means \pm SEM. Means within a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way analysis of variance and the Tukey post hoc test. S \times F = species \times fertilizer interaction effect.

Variable	<i>Ficus insipida</i>		<i>Podocarpus guatemalensis</i>		P value		
	Unfertilized (n = 8)	Fertilized (n = 10)	Unfertilized (n = 10)	Fertilized (n = 10)	Species	Fertilizer	S \times F
Final dry mass (g)	2.23 \pm 0.19 ^c	49.20 \pm 4.41 ^a	9.28 \pm 0.84 ^{bc}	17.30 \pm 1.44 ^b	<0.001	<0.001	<0.001
Cumulative transpiration (kg)	2.36 \pm 0.20 ^b	15.00 \pm 1.28 ^a	2.11 \pm 0.29 ^b	2.85 \pm 0.20 ^b	<0.001	<0.001	<0.001
Whole-plant water-use efficiency (g dry mass kg ⁻¹ H ₂ O)	1.02 \pm 0.16 ^c	3.27 \pm 0.07 ^b	2.96 \pm 0.30 ^b	4.79 \pm 0.35 ^a	<0.001	<0.001	0.432
Mean relative growth rate (mg g ⁻¹ day ⁻¹)	46.0 \pm 1.0 ^b	75.6 \pm 1.1 ^a	8.5 \pm 0.8 ^d	14.5 \pm 0.8 ^c	<0.001	<0.001	<0.001
Mean unit leaf rate (g m ⁻² day ⁻¹)	3.72 \pm 0.13 ^b	8.41 \pm 0.38 ^a	1.37 \pm 0.15 ^d	2.66 \pm 0.21 ^c	<0.001	<0.001	<0.001
Specific leaf area (m ² kg ⁻¹)	23.2 \pm 0.4 ^a	18.2 \pm 0.5 ^b	9.9 \pm 0.3 ^c	8.4 \pm 0.4 ^c	<0.001	<0.001	<0.001
Leaf area ratio (m ² g ⁻¹)	9.9 \pm 0.3 ^a	7.6 \pm 0.3 ^b	5.7 \pm 0.2 ^c	4.8 \pm 0.2 ^c	<0.001	<0.001	0.02
Leaf mass fraction (g g ⁻¹)	0.43 \pm 0.01 ^b	0.42 \pm 0.01 ^b	0.58 \pm 0.01 ^a	0.56 \pm 0.01 ^a	<0.001	0.114	0.612
Root mass fraction (g g ⁻¹)	0.40 \pm 0.01 ^a	0.30 \pm 0.01 ^b	0.24 \pm 0.01 ^c	0.19 \pm 0.01 ^d	<0.001	<0.001	0.022
Photosynthesis (μ mol CO ₂ m ⁻² s ⁻¹)	14.7 \pm 0.6 ^b	19.3 \pm 0.8 ^a	6.7 \pm 0.5 ^c	7.6 \pm 0.5 ^c	<0.001	<0.001	0.004
Stomatal conductance (mol H ₂ O m ⁻² s ⁻¹)	0.440 \pm 0.039 ^a	0.410 \pm 0.045 ^a	0.079 \pm 0.006 ^b	0.091 \pm 0.005 ^b	<0.001	0.788	0.49
c _i /c _a (μ mol mol ⁻¹)	0.81 \pm 0.02 ^a	0.73 \pm 0.03 ^b	0.61 \pm 0.01 ^c	0.62 \pm 0.02 ^c	<0.001	0.066	0.029
Leaf N concentration (%)	2.3 \pm 0.1 ^b	3.2 \pm 0.1 ^a	1.4 \pm 0.04 ^d	1.9 \pm 0.1 ^c	<0.001	<0.001	0.003
Leaf P concentration (%)	0.37 \pm 0.01 ^a	0.22 \pm 0.01 ^b	0.39 \pm 0.02 ^a	0.09 \pm 0.01 ^c	0.005	<0.001	<0.001
Leaf N/P (g N g ⁻¹ P)	6.23 \pm 0.25 ^c	14.71 \pm 0.22 ^b	3.73 \pm 0.15 ^c	22.24 \pm 1.72 ^a	0.035	<0.001	<0.001
Photosynthetic N-use efficiency (μ mol CO ₂ mol ⁻¹ N s ⁻¹)	209 \pm 9 ^a	154 \pm 6 ^b	65 \pm 5 ^c	48 \pm 4 ^c	<0.001	<0.001	0.004
Leaf $\delta^{13}\text{C}$ (‰)	-30.3 \pm 0.3 ^d	-28.6 \pm 0.2 ^c	-27.2 \pm 0.3 ^b	-25.7 \pm 0.1 ^a	<0.001	<0.001	0.757
Leaf $\delta^{15}\text{N}$ (‰)	-1.55 \pm 0.10 ^c	4.84 \pm 0.15 ^a	0.05 \pm 0.15 ^b	0.70 \pm 0.26 ^b	<0.001	<0.001	<0.001

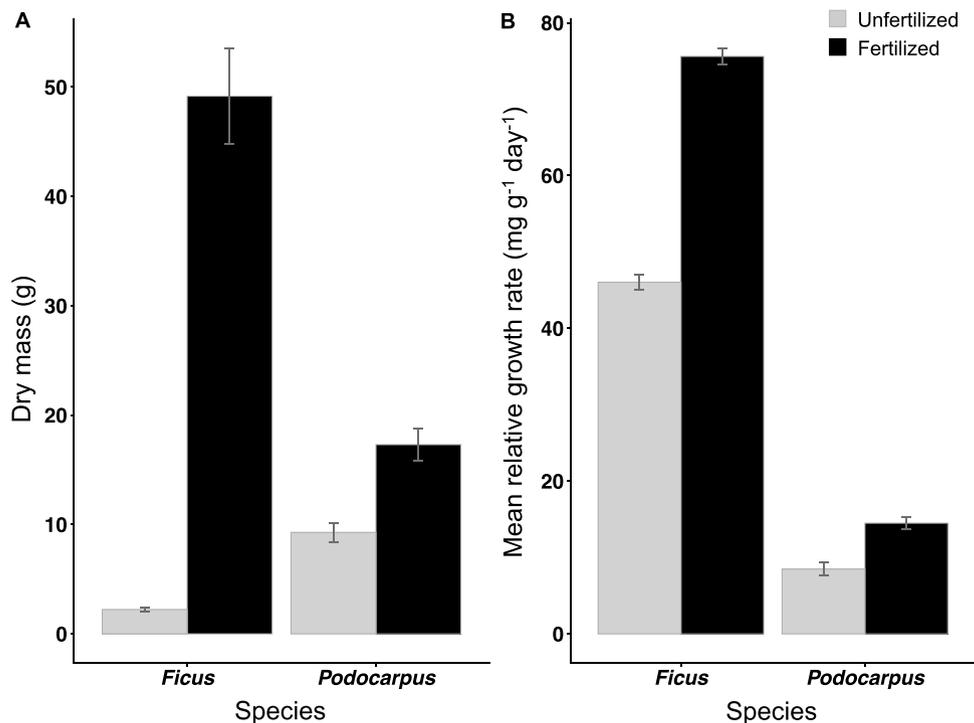


Figure 1. Final dry mass at the conclusion of the experiment (A) and mean relative growth rate (B) for *F. insipida* and *P. guatemalensis*. Error bars show ± 1 SEM.

(3.2%) and unfertilized *P. guatemalensis* the lowest (1.4%). Variation in photosynthesis between species and treatments tracked with variation in leaf nitrogen concentration (Figure 3); fertilized individuals of *F. insipida* displayed the highest mean value (19.3 μ mol CO₂ m⁻² s⁻¹) and unfertilized individuals of

P. guatemalensis the lowest (6.7 μ mol CO₂ m⁻² s⁻¹). The mean unit leaf rate differed both between species and between treatments, tracking variation in photosynthetic rates (Table 1). Unit leaf rates and photosynthetic rates were reasonably closely correlated ($r = 0.86$, $P < 0.001$, $n = 36$).

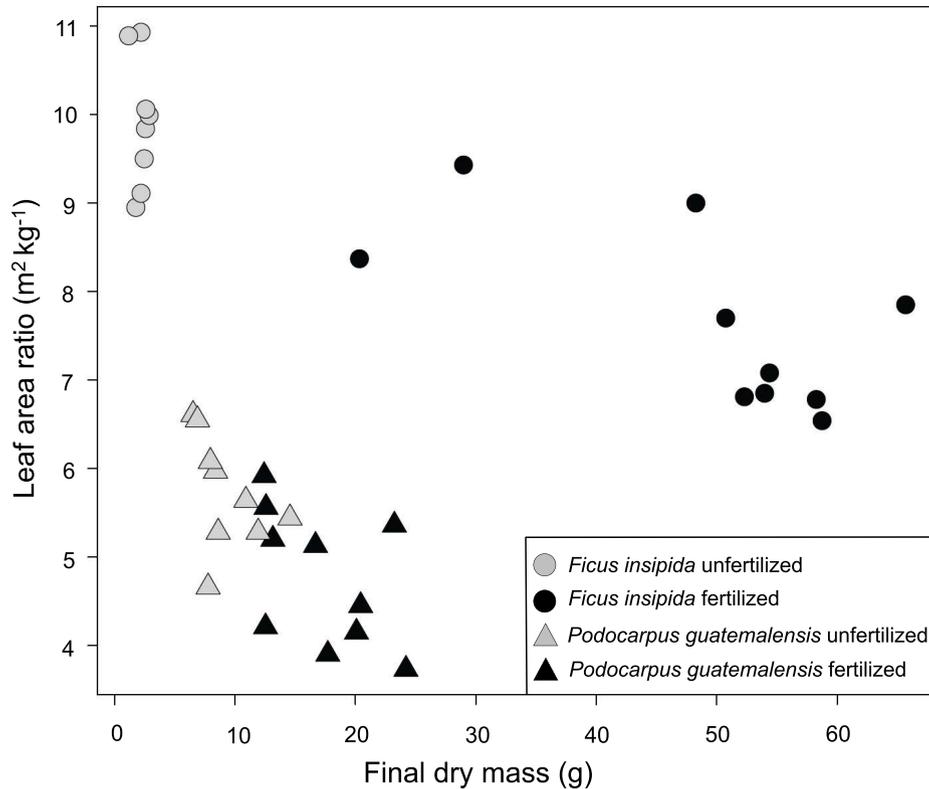


Figure 2. Leaf area ratio (leaf area per total plant dry mass) plotted against plant dry mass at the conclusion of the experiment for *F. insipida* and *P. guatemalensis*.

Stomatal conductance to H_2O was significantly different between species (Table 1), with *F. insipida* showing a mean value about fivefold higher than that for *P. guatemalensis* (0.42 versus $0.09 \text{ mol m}^{-2} \text{ s}^{-1}$, respectively). Generally speaking, photosynthesis showed a curvilinear relationship with stomatal conductance across the full dataset, with fertilized plants of *F. insipida* reaching a higher asymptote at high stomatal conductance than unfertilized plants (Figure 4). *Podocarpus guatemalensis* occupied the space in the linear portion of the curvilinear relationship, such that an increase in stomatal conductance could be expected to result in a proportional increase in photosynthesis (Figure 4). Across the full dataset, relative growth rate was positively correlated with photosynthesis, the latter expressed per unit leaf area (Figure 5), with photosynthesis explaining 89% of variation in relative growth rate ($R^2 = 0.89$, $P < 0.001$, $n = 38$).

Whole-plant water-use efficiency provided an experiment-long integration of the relationship between growth and plant water use (Figure 6A), which mirrored in part the relationships between photosynthesis and stomatal conductance captured in the instantaneous gas exchange measurements. Whole-plant water-use efficiency was significantly higher with nutrient addition in both species, with fertilized individuals producing more dry mass per unit of water transpired (Table 1). Between

species, *P. guatemalensis* had higher whole-plant water-use efficiency with mean values of 4.8 and $3.0 \text{ g dry mass kg}^{-1} \text{ H}_2\text{O}$ for fertilized and unfertilized individuals, respectively. Unfertilized individuals of *F. insipida* displayed the lowest whole-plant water-use efficiency with a mean value of $1.0 \text{ g dry mass kg}^{-1} \text{ H}_2\text{O}$ transpired.

The $\delta^{13}\text{C}$ of leaf tissue further supported the patterns for instantaneous gas exchange and whole-plant water-use efficiency (Figure 6B). Fertilized individuals of both species showed less negative $\delta^{13}\text{C}$ than unfertilized individuals. Overall, *P. guatemalensis* had less negative $\delta^{13}\text{C}$ than *F. insipida*, consistent with its position on the linear part of the curvilinear relationship between photosynthesis and stomatal conductance shown in Figure 4.

A summary of soil parameters measured at the conclusion of the experiment is given in Table 2. Soil ammonium concentration did not differ significantly among species or treatments. On the other hand, soil nitrate concentration was significantly elevated in fertilized compared with unfertilized pots, and there was a significant interaction with species such that fertilized *P. guatemalensis* pots had higher soil nitrate concentration than fertilized *F. insipida* pots (Table 2). Available phosphorus, assayed with the Bray method, was slightly lower in fertilized pots than in unfertilized pots, with a significant pairwise

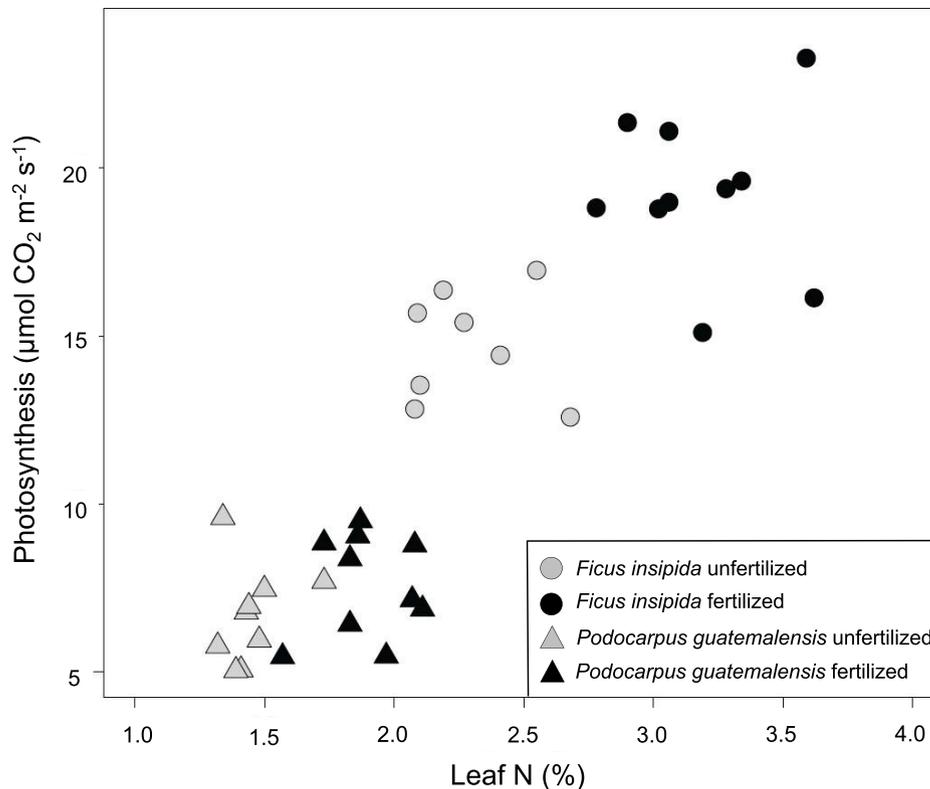


Figure 3. Photosynthesis plotted against leaf nitrogen concentration for *F. insipida* and *P. guatemalensis*.

difference between unfertilized *P. guatemalensis*, showing the highest value (21.6 mg P kg⁻¹ dry soil), and fertilized *F. insipida*, showing the lowest (16.1 mg P kg⁻¹ dry soil). The soil pH decreased slightly but significantly in fertilized compared with unfertilized pots for assays both in water and in calcium chloride solution (Table 2).

There was a significant shift in foliar nitrogen/phosphorus (N/P) of both species with the addition of the slow release fertilizer, such that plants in pots receiving fertilizer had higher N/P than those in unfertilized pots (Table 1). The shift for *P. guatemalensis* was especially striking, with the mean value increasing from 3.7 to 22.2 g N g⁻¹ P. Foliar $\delta^{15}\text{N}$ showed a somewhat different pattern, increasing by more than 6‰ in *F. insipida* with fertilizer addition, but remaining unchanged in *P. guatemalensis* (Table 1).

Discussion

Our aim in this research was to better understand the physiological mechanisms by which angiosperm tropical trees are able to outcompete conifers under conditions of relatively high soil fertility. We hypothesized that inherently higher stomatal conductance in an angiosperm pioneer species would allow it to capitalize on increased nitrogen availability, with higher stomatal conductance assumed to result from higher whole-plant

hydraulic conductance associated with known angiosperm–conifer trait differences, such as greater sapwood porosity and higher leaf vein density in the angiosperm. It is worth noting, however, that there is no coniferous analogue of a tropical angiosperm pioneer in terms of ecological strategy. Therefore, our comparison also necessarily comprised a comparison of a species that employs a more exploitative strategy with one that employs a more conservative strategy. While we have chosen to focus our interpretation on characteristics known to differ between angiosperms and conifers due to their deep phylogenetic divergence, the different ecological strategies imply that there may also be relevant trait differences associated more generally with pioneers compared with later successional species. These could include, for example, shade tolerance mechanisms, such as saturation of the photosynthetic light response at lower light intensity.

We grew seedlings of the angiosperm pioneer and conifer under controlled conditions with fertilizer added to half the pots. Although the fertilizer contained both nitrogen and phosphorus, along with additional nutrients, the overwhelming effect on the rhizosphere nutrient availability was to increase soil nitrate with little additional effect on soil ammonium or soil phosphorus availability (Table 2). The addition of rice husks promoted microbial immobilization of nitrogen in unfertilized pots, helping to constrain nitrogen availability to levels similar to field soils

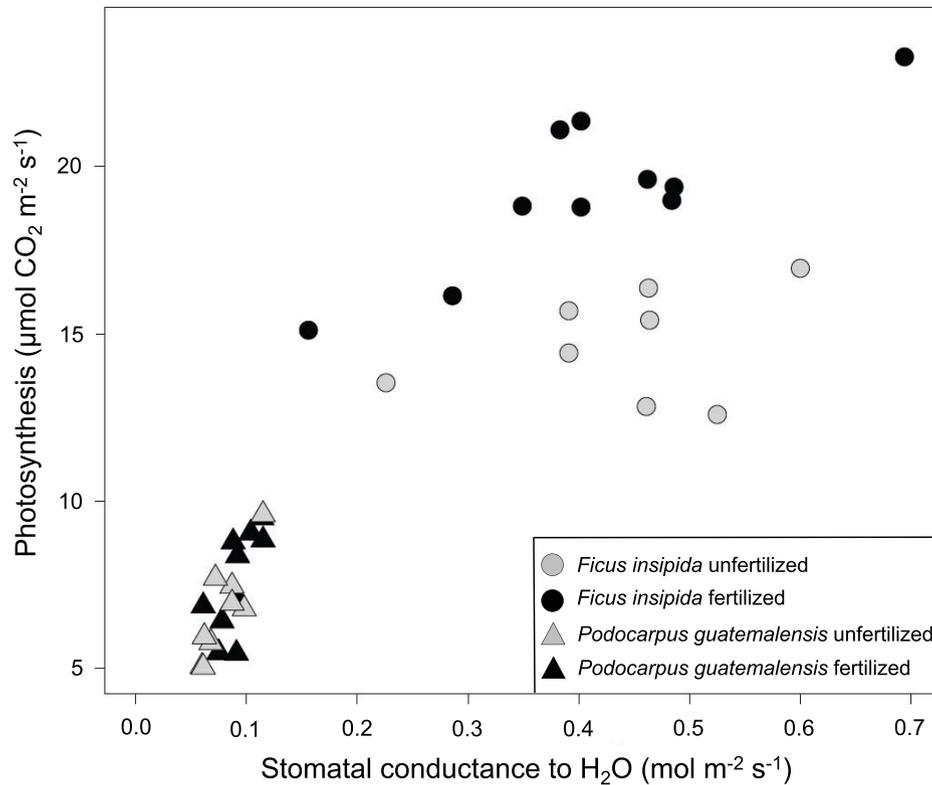


Figure 4. Photosynthesis plotted against stomatal conductance for *F. insipida* and *P. guatemalensis*. Gas exchange measurements were made ~1 week before the conclusion of the experiment.

Table 2. Soil chemical parameters measured at the conclusion of the experiment. Values are means \pm SEM. Means within a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way analysis of variance and the Tukey post hoc test. S \times F = species \times fertilizer interaction effect.

Variable	<i>Ficus insipida</i>		<i>Podocarpus guatemalensis</i>		P value		
	Unfertilized (n = 10)	Fertilized (n = 10)	Unfertilized (n = 10)	Fertilized (n = 10)	Species	Fertilizer	S \times F
NH ₄ (mg N kg ⁻¹ dry soil)	0.52 \pm 0.11 ^{ab}	1.43 \pm 0.24 ^{ab}	0.37 \pm 0.09 ^b	3.72 \pm 1.72 ^a	0.226	0.019	0.169
NO ₃ (mg N kg ⁻¹ dry soil)	0.9 \pm 0.1 ^b	40.0 \pm 11.1 ^b	0.8 \pm 0.1 ^b	148.0 \pm 21.0 ^a	<0.001	<0.001	<0.001
Bray P (mg P kg ⁻¹ dry soil)	19.7 \pm 1.3 ^{ab}	16.1 \pm 1.0 ^b	21.5 \pm 1.6 ^a	18.8 \pm 1.6 ^{ab}	0.107	0.026	0.759
pH (measured in water)	5.6 \pm 0.1 ^a	5.3 \pm 0.1 ^b	5.6 \pm 0.1 ^a	5.1 \pm 0.04 ^b	0.182	<0.001	0.249
pH (measured in CaCl ₂)	4.9 \pm 0.02 ^a	4.8 \pm 0.03 ^b	4.9 \pm 0.02 ^a	4.7 \pm 0.03 ^b	0.212	<0.001	0.781

(Dalling et al. 2013). The increase in foliar N/P of both species with fertilizer addition suggests that the nutritional constraint on plant growth likely shifted from nitrogen limitation in unfertilized pots to nitrogen and phosphorus co-limitation in fertilized pots (Garrish et al. 2010). The increase in foliar N/P with fertilizer addition was similar to previous observations using a similar experimental design (Cernusak et al. 2007, 2010).

Both species took advantage of the increase in nitrogen availability with fertilizer addition to fuel faster growth. Although the proportional stimulation of relative growth rate was comparable between the two species, the inherently larger relative growth rate in the angiosperm *F. insipida* meant that the increase in plant

size by the end of the experiment was much greater than for the conifer, *P. guatemalensis* (Figure 1). Leaf area ratio did not appear to drive increases in relative growth rate in response to fertilizer addition, although it can explain part of the inherent difference in relative growth rate between the two species (Figure 2). On the other hand, both photosynthetic rates and unit leaf rates increased in response to fertilizer addition and therefore likely drove growth rate responses to fertilization. These also contributed, along with leaf area ratio, to the inherent difference in relative growth rates between the two species (Figure 5). The between species variation in photosynthesis was associated with fivefold higher stomatal conductance in *F.*

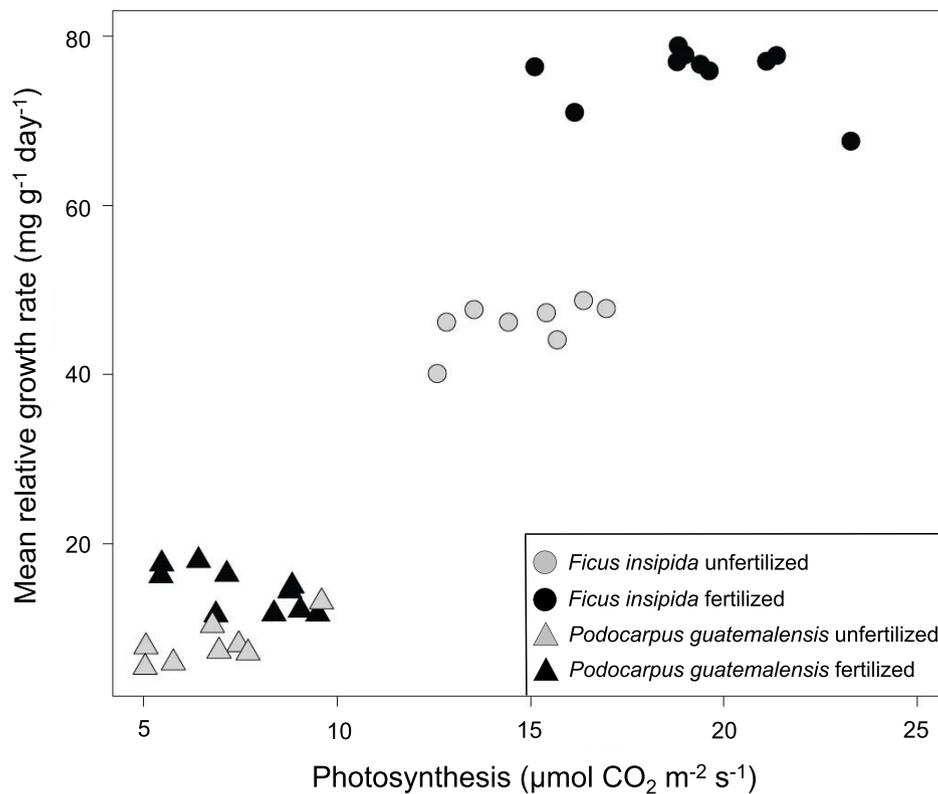


Figure 5. Mean relative growth rate plotted against photosynthesis for *F. insipida* and *P. guatemalensis*. Mean relative growth rate was averaged over the course of the experiment and photosynthesis was measured near the end of the experiment.

insipida compared with *P. guatemalensis* (Table 1). This, in turn, likely related to the more efficient vascular transport of liquid water from roots to evaporative sites in leaves, known to be a general feature of angiosperm trees compared with conifers (Bond 1989, McElwain et al. 2005, Brodribb et al. 2005a, Brodribb and Feild 2010, Feild et al. 2011).

Plotting leaf area ratio against total plant mass, as in Figure 2, revealed two notable trends. First, leaf area ratio for a given plant mass was about twice as large for *F. insipida* as for *P. guatemalensis*. Second, leaf area ratio declined with increasing plant mass in both species. Leaf area ratio is the product of leaf mass fraction (i.e., the fraction of total plant dry mass that is leaves) and specific leaf area. In the case of *F. insipida* and *P. guatemalensis*, the higher leaf area ratio in *F. insipida* could be entirely accounted for by a more than twofold higher specific leaf area (Table 1). Leaf mass fraction, on the other hand, was higher in *P. guatemalensis* than in *F. insipida*, with mean values of 0.57 and 0.42, respectively, with no significant effect of fertilizer in either species. Higher specific leaf area has been previously shown to be associated with higher relative growth rates when compared among species, especially in seedlings (Lambers and Poorter 1992, Veneklaas and Poorter 1998, Shipley 2006). However in larger trees, the association between specific leaf area and growth rates appears to break down (Gower et al.

1993, Paine et al. 2015, Gray et al. 2019, Wright et al. 2019). It has been suggested that this can be linked to the decreasing importance of leaf mass as a proportion of whole-plant mass as plants grow larger, such that building leaves with lower construction costs no longer confers a strong growth advantage (Gibert et al. 2016, Falster et al. 2018). Thus, the inherent growth advantage that angiosperms enjoy over conifers as a result of higher specific leaf area, as exemplified in our results, is likely strongest in the seedling stage, supporting the slow seedling hypothesis of angiosperm dominance over conifers (Bond 1989).

The other contributors to higher relative growth rate in *F. insipida* compared with *P. guatemalensis* that we quantified were the photosynthetic rate per unit leaf area (Figure 5) and the unit leaf rate (Table 1). Unlike leaf area ratio, these were enhanced by fertilization, with *F. insipida* showing a proportionally larger stimulation than *P. guatemalensis* (Table 1; Figures 3 and 4). *Ficus insipida* had higher foliar nitrogen concentrations than *P. guatemalensis* in both fertilized and unfertilized plants (Table 1; Figure 3). A higher nitrogen concentration is typically associated with a higher photosynthetic rate (Field and Mooney 1986), due to the high nitrogen cost of photosynthetic enzymes. Thus, the primary mechanism by which fertilization influenced relative growth rate of both species appears to have been through an

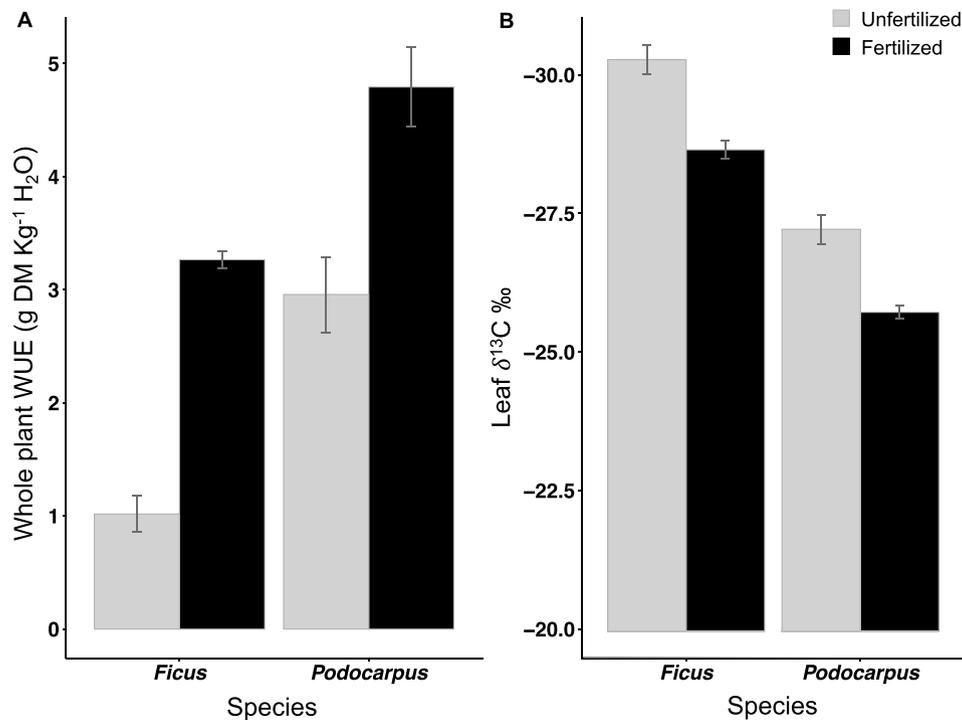


Figure 6. Whole-plant water-use efficiency (A) and leaf $\delta^{13}\text{C}$ (B) for *F. insipida* and *P. guatemalensis*. Whole-plant water-use efficiency was calculated as the dry mass increment over the course of the experiment divided by cumulative plant water use. The $\delta^{13}\text{C}$ was measured in leaf tissue collected at the conclusion of the experiment. Error bars show ± 1 SEM.

increase in photosynthetic capacity. Interestingly, the positive association between photosynthetic rate and growth rates also appears to persist in larger trees (Gray et al. 2019, Wright et al. 2019), and therefore its role in driving growth rate variation does not diminish with increasing plant size in the way that the role of specific leaf area does (Gibert et al. 2016, Falster et al. 2018).

Another reason that photosynthetic rates were higher in *F. insipida* than in *P. guatemalensis*, in addition to higher photosynthetic capacity associated with higher leaf nitrogen concentration, is that stomatal conductance was fivefold higher (Table 1; Figure 4). This allowed higher intercellular CO_2 concentrations, shown in Table 1 as the ratio of intercellular to ambient CO_2 concentrations, c_i/c_a . Photosynthesis typically increases as a saturating function of intercellular CO_2 concentration (Farquhar et al. 1980). Therefore, the photosynthetic rate of a leaf can be increased both by increasing photosynthetic capacity and by increasing the intercellular CO_2 concentration by opening stomata (Farquhar and Sharkey 1982). However, opening stomata leads to an increasing evaporation rate from the leaf, and this must be balanced by a hydraulic system able to supply water to the sites of evaporation at rates sufficient to replace water loss. This appears to be one of the key innovations that angiosperms made in their evolutionary history; in the simplest terms, they freed the water conductive elements in their stems from needing to also provide structural support, thereby allowing larger

conduit diameters (Tyree and Zimmermann 2002, Brodribb et al. 2005a, Sperry et al. 2006), and they increased the density of veins in their leaves (Brodribb et al. 2005b, Boyce et al. 2009, Brodribb and Feild 2010, Feild et al. 2011).

The higher c_i/c_a in *F. insipida* compared with *P. guatemalensis* also plays out in terms of water-use efficiency. We observed more negative leaf $\delta^{13}\text{C}$ values in *F. insipida* than in *P. guatemalensis*, consistent with higher time-integrated c_i/c_a and lower leaf-level water-use efficiency (Farquhar et al. 1982, Cernusak et al. 2013). We also observed lower whole-plant water-use efficiency in *F. insipida* than in *P. guatemalensis*, which is a manifestation at the whole-plant level of the leaf-level difference in c_i/c_a (Cernusak 2020). This difference between a broad-leaved podocarp and angiosperm tree species in water-use efficiency, either leaf level or whole-plant level, is similar to previous observations (Cernusak et al. 2008, Orchard et al. 2010, Dalling et al. 2016, Cernusak 2020). It is tempting to speculate that the higher water-use efficiency in *P. guatemalensis* is adaptive, allowing it to compete successfully in sites where soil water holding capacity is low, such as in sandy soils, or where precipitation rates are relatively low for tropical rainforests. One could further speculate that this reflects the evolutionary origins of the Podocarpaceae in tropical forests of the Cretaceous before angiosperms radiated, increasing evapotranspiration and accelerating the tropical water cycle (Boyce et al. 2009, 2010, Biffin et al. 2012, Boyce and Lee 2017).

This could be consistent with modern day distributions of some podocarp taxa in sandy coastal soils (Enright and Jaffré 2011), with some species even extending into semiarid regions of southern Australia (Ladd and Enright 2011). However, an alternative perspective is that intercellular and chloroplastic CO₂ concentrations in broad-leaved podocarps are constrained by low stomatal conductance and low mesophyll conductance, respectively, and that this relegates them to a high water-use efficiency/low productivity strategy, whether it is beneficial or not. One important consequence of this perspective is that podocarps could be expected to respond strongly to elevated CO₂ because their photosynthetic rates are sharply constrained by low chloroplastic CO₂ concentrations. We have indeed demonstrated this for *P. guatemalensis* (Dalling et al. 2016).

The role of an increase in nitrogen availability in stimulating water-use efficiency has been shown previously for *F. insipida* (Cernusak et al. 2007, Garrish et al. 2010) and is an expected consequence of an increase in photosynthetic capacity in leaves that have higher nitrogen concentrations (Field et al. 1983). Interestingly, we did not observe a difference in instantaneous c_i/c_a in *P. guatemalensis* in fertilized compared with unfertilized plants, although leaf $\delta^{13}\text{C}$ values were less negative and whole-plant water-use efficiency was higher (Table 1). This could reflect the better signal to noise ratio in the time-integrated measures (leaf $\delta^{13}\text{C}$ and whole-plant water-use efficiency) compared with instantaneous gas exchange measurements (Cernusak and Marshall 2001). Leaf $\delta^{15}\text{N}$ showed a response to fertilizer addition in *F. insipida* but not in *P. guatemalensis*. This could be interpreted to suggest that *F. insipida* is more flexible in its nitrogen uptake strategy, shifting its preference for nitrogen source or its discrimination during nitrogen uptake and/or metabolism (Cernusak et al. 2009, Kahmen et al. 2008).

Leaf $\delta^{13}\text{C}$ and whole-plant water-use efficiency suggested that both species took advantage of the higher nitrogen availability in fertilized pots to increase the amount of photosynthesis and dry matter production for a given amount of water loss to the atmosphere. In terms of mechanisms that would see angiosperm species such as *F. insipida* outcompete conifers such as *P. guatemalensis* in high fertility sites more so than in low fertility sites, it appears that the absolute increase in relative growth rate is the decisive factor rather than the proportional increase in water-use efficiency or proportional increase in relative growth rate. In our experiment, this was driven by larger gains in photosynthesis in *F. insipida* compared with *P. guatemalensis* in response to fertilizer addition. The ability of *F. insipida* to ramp up photosynthesis was further supported by higher stomatal conductance, allowing sufficient CO₂ to diffuse into the leaf interior to support high CO₂ assimilation rates.

A useful framework for thinking about how maximum stomatal conductance might influence a plant species' ability to respond to a change in nitrogen availability is through the concept of

photosynthetic nitrogen-use efficiency (Field et al. 1983). This is typically quantified as the instantaneous photosynthetic rate divided by leaf nitrogen content, giving it units, for example, of $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ N s}^{-1}$. Generally speaking, a leaf with a higher maximum stomatal conductance will have a higher intercellular CO₂ concentration. Because photosynthetic rate increases as a saturating function of the intercellular CO₂ concentration, this will typically be associated with a higher photosynthetic rate per unit leaf nitrogen. Thus, *F. insipida*, with a more efficient vascular system and a higher maximum stomatal conductance than *P. guatemalensis*, also had a higher photosynthetic nitrogen-use efficiency (Table 1). This means that any additional nitrogen supplied to the plant have a higher payoff in terms of carbon gain, which provides another perspective through which to view the larger increase in growth of *F. insipida* in response to fertilizer addition compared with *P. guatemalensis*.

Based on an elevated CO₂ experiment, we previously concluded that *P. guatemalensis*, and perhaps other low-latitude conifers, are likely to have experienced a significant enhancement of competitive ability as atmospheric CO₂ concentration has risen over the past century (Dalling et al. 2016). The results of the experiment presented here suggest that a second dimension of global change may also be relevant, which is that both globally and in tropical forests, anthropogenic activity has accelerated the nitrogen cycle, likely leading to increases in nitrogen availability as atmospheric CO₂ also increased (Gruber and Galloway 2008, Hietz et al. 2011). Although our experiment only included one tropical conifer species and one angiosperm tree species, the results strongly indicated that the angiosperm was better equipped to take advantage of an increase in nitrogen availability due to its higher photosynthetic nitrogen-use efficiency. Clearly experiments with more species are needed to establish the generality of this pattern. However, based on the evidence provided here, it would appear necessary to temper our previous conclusion that tropical conifers will likely benefit more from rising atmospheric CO₂ than co-occurring angiosperm trees, as the overall impact will also depend on how nitrogen cycling changes in tropical forests.

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Conflict of interest

None declared.

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