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Research paper

Contrasting physiological traits of shade tolerance in *Pinus* and Podocarpaceae native to a tropical Vietnamese forest: insight from an aberrant flat-leaved pine

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The absence of pines from tropical forests is a puzzling biogeographical oddity potentially explained by traits of shade intolerance. *Pinus krempfii* (Lecomte), a flat-leaved pine endemic to the Central Highlands of Vietnam, provides a notable exception as it seems to compete successfully with shade-tolerant tropical species. Here, we test the hypothesis that successful conifer performance at the juvenile stage depends on physiological traits of shade tolerance by comparing the physiological characteristics of *P. krempfii* to coexisting species from two taxa: the genus *Pinus*, and a relatively abundant and shade-tolerant conifer family found in pantropical forests, the Podocarpaceae. We examined leaf photosynthetic, respiratory and biochemical traits. Additionally, we compiled attainable maximum photosynthesis, maximum RuBP carboxylation (V_{cmax}) and maximum electron transport (J_{max}) values for *Pinus* and Podocarpaceae species from the literature. In our literature compilation, *P. krempfii* was intermediate between *Pinus* and Podocarpaceae in its maximum photosynthesis and its V_{cmax} . *Pinus* exhibited a higher V_{cmax} than Podocarpaceae, resulting in a less steep slope in the linear relationship between J_{max} and V_{cmax} . These results suggest that *Pinus* may be more shade intolerant than Podocarpaceae, with *P. krempfii* falling between the two taxa. However, in contrast, Vietnamese conifers' leaf mass per areas and biochemical traits did not highlight the same intermediate nature of *P. krempfii*. Furthermore, regardless of leaf morphology or family assignment, all species demonstrated a common and extremely high carbon gain efficiency. Overall, our findings highlight the importance of shade-tolerant photosynthetic traits for conifer survival in tropical forests. However, they also demonstrate a diversity of shade tolerance strategies, all of which lead to the persistence of Vietnamese juvenile conifers in low-light tropical understories.

Keywords: carbon assimilation, *Pinus*, Podocarpaceae, shade tolerance, tropics.

Introduction

Pinaceae are found ubiquitously throughout the northern hemisphere (Farjon and Filer 2013). Yet, no natural Pinaceae have been found further south than 2°S despite the success of

Pinaceae as plantation species in the southern hemisphere (Richardson et al. 1994, Richardson and Higgins 1998, Procheş et al. 2012). The fossil records concur, demonstrating

abundant fossil evidence of Pinaceae throughout the northern hemisphere, but no evidence of Pinaceae in the southern hemisphere (Miller 1976, Taylor et al. 2009, Smith et al. 2016). In contrast, other conifer families are more broadly distributed. Cupressaceae are found globally, and Podocarpaceae cross the equator from the southern into the northern hemisphere despite their Gondwanan origins (Hill and Brodribb 1999, Morley 2011, Brodribb et al. 2012, Farjon and Filer 2013). Although Pinaceae are Laurasian in origin, many species, especially from the genus *Pinus*, have the ability to disperse widely and quickly (Huntley and Webb 1989, Richardson et al. 1994, Farjon and Filer 2013, Leslie et al. 2017). Furthermore, no geographical barrier definitively prevents pines from entering the southern hemisphere. Thus, it seems unlikely that either a confining geographical barrier or poor dispersal could be the only explanations for the northern confines of *Pinus* (Brodribb and Feild 2008, Brodribb et al. 2012).

An alternate hypothesis ascribes the inability of *Pinus* to expand southward to an 'ecological barrier' imposed by large swaths of equatorial, tropical forests (Brodribb and Feild 2008, Boyce et al. 2010, Brodribb et al. 2012). This hypothesis proposes that conifers are largely unable to compete with fast-growing tropical species for essential resources due to narrow tracheids and single-veined needles that result in small leaf areas and slow growth rates in conifer seedlings (Bond 1989, Becker 2000, Brodribb et al. 2007). Although needle-leaved *Pinus* are frequently successful in high-light conditions, their lack of a flat leaf in low-light tropical forest understories may make them poor competitors in the tropics where shady conditions are prevalent during regeneration. Wide, flat leaves are essential for capturing photosynthetically active radiation (PAR) when light availability is limited (Leverenz 1995, Brodribb and Hill 1997, Lusk 2002, Brodribb 2011). Many Podocarpaceae, in contrast to *Pinus*, have wider, flatter leaves and survive successfully in densely canopied tropical forests (Brodribb 2011). Only in the last 60 million years and after the rise of fast-growing angiosperms (Morley 2011, Quiroga et al. 2016), did podocarps colonize the tropics. The fossil records show a transition from an imbricate to a flatter leaf form as they adapted to shady, wet, tropical areas (Biffin et al. 2012). Thus, shade tolerance traits may be instrumental to Podocarpaceae survival, and *Pinus* exclusion from tropical forests. Comparisons between *Pinus* and Podocarpaceae foliar traits may provide further insight into shade tolerance as a driver of the contrasting success of these conifer families in tropical forests.

Wide leaves with large leaf areas are part of a suite of physiological and structural traits associated with shade tolerance. Physiologically, decreased light availability often correlates to lower rates of light-saturated net carbon assimilation (A_{\max}) as seen in many Podocarpaceae species (Lusk et al. 2003, Brodribb and Feild 2008). It also commonly leads to: lower maximum rates of electron transport (J_{\max}) and RuBP

carboxylation (V_{\max}); higher apparent quantum yields (Φ), in order to take greater advantage of light when it is available; lower light saturation points (LSPs), as high levels of irradiance are rarely experienced; lower light compensation points (LCPs); and lower respiration in the dark and the light (R_D and R_L , respectively), so that the plant can maintain a positive carbon balance (Loach 1967, Teskey and Shrestha 1985, Walters and Field 1987, Givnish 1988, Craine and Reich 2005, Sendall et al. 2016). Often, shade-tolerant species have superior carbon balance in low-light environments when compared with shade-intolerant species (Valladares and Niinemets 2008). Carbon balance has been examined using a variety of metrics including the ratio of R_D to gross photosynthesis (R_D/A_{gross}), carbon-use efficiency (CUE) ($1 - R_D/A_{\text{gross}}$) and carbon-gain efficiency (CGE) ($A_{\max}/(A_{\max} + R_L + \text{PR})$, where PR is photorespiration) (Valladares and Niinemets 2008, Heskell et al. 2013b, Pons and Poorter 2014). The inclusion of R_L and PR is rare in estimates of carbon flux; however, both are sensitive to changes in environmental conditions (e.g., irradiance), making their quantification important for accurate calculations of carbon balance. Structurally, leaf mass per area (LMA) also varies with shade tolerance (Poorter et al. 2009), although the direction of the relationship varies among functional groups (e.g., between deciduous and evergreen species; Lusk et al. 2008, Lusk 2019). Among evergreen conifer species, a universal LMA–shade tolerance relationship has not been identified (Leverenz et al. 2000, Wyka et al. 2012). Nevertheless, LMA is an important indicator of plant ecological strategies (Grime 1977, Wright et al. 2004), and may contribute to a strategy of shade tolerance, impacting the interpretation of our physiological data.

In this study, we compare foliar physiological, biochemical and structural traits of juveniles of the majority of conifer species from a tropical forest in the Central Highlands of Vietnam. This location is unusual compared with many tropical forests in that it contains a large number of conifer species, including two needle-leaved *Pinus* species, as well as *Pinus krempfii* (Lecomte), the only known flat-leaved pine in the world. Adult trees of this species have been previously shown to have structural characteristics similar to *Podocarpus neriifolius* (D. Don), including low maximum photosynthesis, wider leaves and sclerified tissues to transport water radially through the leaves (Brodribb and Feild 2008). However, there are no reports of these traits in juveniles of *P. krempfii* or other co-occurring conifers. The coexistence of needle-leaved *Pinus*, flat-leaved *P. krempfii* and flat-leaved Podocarpaceae provides a unique opportunity to compare and contrast these taxa and to consider whether *Pinus* absence and Podocarpaceae presence in the majority of tropical forests is related to physiological traits of shade tolerance in juveniles. We hypothesize that flat-leaved *P. krempfii* will demonstrate similar physiological characteristics to coexisting Podocarpaceae species and that these characteristics

will reveal a shade tolerance tendency lacking from needle-leaved *Pinus* also growing in the Central Highlands.

Materials and methods

Study site

This research was conducted in Bidoup Nui Ba National Park, near the city of Dalat in Lam Dong Province in the Central Highlands of Vietnam (12°08' 00.50" N latitude, 108° 32'00.65" E longitude). The Central Highlands are a part of the Annamite range along which the Laurasian and Gondwanan supercontinents converged (Gupta 2005, Thomas et al. 2007). The location receives seasonal precipitation from the Southeast Asian Summer Monsoon (SEAMS) from June through September and experiences an extended dry period from November through April (Wang and Ho 2002). The location is at approximately 1500 m above sea level, has a mean annual temperature of 18 °C and receives approximately 1800 mm of rainfall a year (Brodrribb and Feild 2008).

Compared with other tropical locations, montane tropical forests often contain greater numbers of conifer species (Enright and Jaffré 2011, Ushio et al. 2017). The Central Highlands of Vietnam are a prime example, containing a huge variety of conifer species and families: in total, 13 species of conifers from four of the eight conifer families. We examined seven of these species, hailing from two families: *Pinus kesiya* (Royle ex Gordon), *Pinus dalatensis* (Ferré), *P. krempfii* (Pinaceae); *P. nerifolius*, *Dacrydium elatum* ((Roxb.) Wall. ex Hook.), *Dacrycarpus imbricatus* ((Blume) de Laub.), *Nageia wallichiana* ((Presl.) O. Kuntze) (Podocarpaceae; Table 1). Several species from the Pinaceae are rare and/or endemic to the Central Highlands such as *P. dalatensis* and *P. krempfii* (Hiệp et al. 1996). *P. kesiya* is not found in the forest interior but in monoculture stands at forest edges. It is prevalent near human disturbed areas where fires are common (Goldammer and Penafiel 1990, Agee 1998). All podocarp species are found in the forest interior.

Measurements were taken on individuals that originated in a 20 ha forest plot in Bidoup Nui Ba National Park. Only *P. kesiya* was not found in this plot, but instead at the forest headquarters where *P. kesiya* grows in monoculture stands. Juveniles of at least 3 years old were transplanted from their understory forest locations into pots with their native forest soil, and grown in a shade house at the Bidoup Nui Ba Forest headquarters. During mid-day on 8 January 2016, light conditions measured inside the shade house with a Li-Cor Quantum sensor (Q24375, LiCor, Lincoln, NE, USA) were 70% lower than outside the shade house (400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ compared with 1350 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Plants were adapted to shade house conditions for at least 6 months before gas exchange measurements were taken. All measurements were taken in January and February.

Gas exchange measurements

Fully expanded, attached, top-of-canopy leaves were selected from at least six juvenile plants of each species for gas exchange measurements (between one and three curves per plant). Photosynthetic CO₂ response (A–C_i) curves and light response curves were collected using two portable photosynthesis systems (LI-6800, LiCor). Energy balance calculations of leaf temperature were used throughout in case the needle tissue was not in full contact with the chamber thermocouple.








For the A–C_i curves, the temperature of the cuvette was set to between 20 and 25 °C and the relative humidity was set to between 60 and 70%. The photosynthetic photon flux density (PPFD) was set to the PPFD required by each species to achieve saturating photosynthesis. The progression of CO₂ concentrations was as follows: 400, 300, 200, 100, 50, 25, 400, 400, 400, 400, 600, 800, 1000, 1200, 1500, 1700 ppm. Curves were analyzed to extract the values of V_{cmax} (the maximum carboxylation rate of ribulose 1,5 bisphosphate carboxylase oxygenase (RuBisCO) and J_{max} (the maximum electron transport rate) and corrected to 25 °C using the plantecophys package in R (Duursma 2015), which implements the photosynthesis model of Farquhar, von Caemmerer and Berry (Farquhar et al. 1980).

Light response curves were also measured using the same portable photosynthesis systems (LI-6800, LiCor) and the temperature of the cuvette and relative humidity were kept the same as the A–C_i curves for each species. The reference CO₂ concentration was set to 400 ppm and photosynthesis was measured at the following light intensities: 1500, 1200, 800, 200, 100, 90, 80, 70, 60, 50, 45, 40, 35, 30, 25, 20, 15, 10, 5, 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Samples were then left in the cuvette at zero irradiance for 15 min before measuring respiration in the dark (R_D). Maximum likelihood estimation (executed using the bblme package for R (Bolker and R Development Core Team 2020)) was used to fit the following model (describing a nonrectangular hyperbola) to the data:

$$A = \frac{\Phi * I + A_{\text{max}} - \sqrt{(\Phi * I + A_{\text{max}})^2 - 4 * \theta * \Phi * I * A_{\text{max}}}}{2 * \theta} - R_D$$

where θ is the curvature factor and I is the irradiance. Several parameters were extracted from the fit of the curves including maximum photosynthesis (A_{max}), apparent quantum yield (Φ), the LCP (the light level at which net carbon flux is zero) and the LSP (the light level at which 75% of maximum net carbon flux is obtained) (Singsaas et al. 2001). Measurements of respiration and photosynthesis were used to examine several parameters of carbon balance including the ratio of R_D to A_{gross} (the sum of A_{max} and R_D), the CUE, calculated according to the equation: $\text{CUE} = 1 - (R_D/A_{\text{gross}})$, and the CGE, calculated according to the equation: $\text{CGE} = A_{\text{max}} / (A_{\text{max}} + R_L + \text{PR})$ as presented in Heskell

Table 1. Characteristics of the conifers of the Central Highlands of Vietnam, compiled from the literature, including [Luu and Thomas \(2004\)](#), [Loc et al. \(2017\)](#), [Coomes and Bellingham \(2011\)](#) and [Farjon \(2017\)](#). All images show the juvenile leaf forms. Adult *P. krempfii* leaves become narrower and more needle-like; adult foliage of *D. elatum* and *D. imbricatus* becomes more imbricate.

	Species (Family)	Leaf morphology	Canopy dominance	Natural Regeneration	Distribution	Altitude
	<i>Pinus kesiya</i> (Pinaceae)	Needle	Pure stands	Pioneer species, heavy seed crops	Disturbed areas, acidic soils, fire resistant	1300- 2300m
	<i>Pinus dalatensis</i> (Pinaceae)	Needle	Canopy emergent	Shade intolerant, periodic fire or clearings	Moist evergreen subtropical forest, flat mountain tops	1500- 2200m
	<i>Pinus krempfii</i> (Pinaceae)	Flat	Canopy emergent	Abundant under adult trees (personal obs.)	Dense subtropical forests, moist soils, well-developed humus layer	1500- 1800m
	<i>Dacrydium elatum</i> (Podocarpaceae)	Needle / Imbricate	Canopy ~40m tall	Occasional	Scattered in moist rainforest, most abundant in disturbed, open conditions	700- 2000m
	<i>Dacrycarpus imbricatus</i> (Podocarpaceae)	Flat / Imbricate	Canopy ~50m tall	Occasional, shade tolerant seedlings, light demanding adults	Common in both primary and secondary rainforest	500- 1500m
	<i>Nageia wallichiana</i> (Podocarpaceae)	Flat	Barely canopy emergent ~54m tall	Occasional, saplings rare	Scattered in primary rainforest	700- 2100m
	<i>Podocarpus neriiifolius</i> (Podocarpaceae)	Flat	Canopy ~25m tall	Occasional, shade tolerant	Evergreen subtropical rainforest, fertile soils	600- 1500m

et al. (2013b). Note that PR represents photorespiration, the CO₂ released due to the oxygenation of RuBisCO, and it was calculated according to the equations of [von Caemmerer and Farquhar \(1981\)](#). See [Ayub et al. \(2011\)](#) for a description. Respiration in the light (R_L) was collected according to the methods described below.

Respiration in the light

A modified light curve (collected with the LI-6800, LiCor) with additional data points collected at every 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD below 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD was used to measure respiration in the light (R_L) following the methods of [Kok](#)

(1948), Sharp et al. (1984) and Heskell et al. (2013a). From these data, a breakpoint near the LCP marks a subtle change in slope between the upper and lower linear portions of the light response curve (Figure S1 available as Supplementary Data at *Tree Physiology* Online). Extending the fit of the line from the upper portion of the curve to the y-axis, Bessel Kok identified an alternate respiration value which he interpreted to represent respiration in the light (Kok 1948, Heskell et al. 2013a). The Kok method assumes that light is the sole driver of changes in carbon assimilation during the light response curve; however, low light levels often lead to an accumulation of CO₂ in the leaf which in turn alters the internal CO₂ concentration (c_i). Therefore, c_i was corrected to a constant value following Kirschbaum and Farquhar (1987) as described by Ayub et al. (2011). Kok curves were not obtained for *P. neriifolius*, and thus neither R_L nor PR estimates are available for this species.

Leaf trait measurements

All CO₂ flux measurements required leaf area correction in order to obtain accurate photosynthetic values. Leaves were photographed and the images used to measure projected leaf areas in ImageJ (Schnieder et al. 2012). The use of projected leaf area of needles may introduce some uncertainty (Lloyd et al. 2013), and should be kept in mind during the interpretation of these results. Leaves were then placed into coin envelopes, dried at 60 °C for a minimum of 48 h and weighed for leaf dry mass (g). Leaf areas and dry masses were used to calculate LMA (g m⁻²). Lastly, the dried leaf samples were prepared for carbon:nitrogen (C:N) ratio analysis. Leaves were ground into a fine powder using a ball mill (SPEX 8000 Mixer/Mill, Metuchen, NJ, USA), weighed into tin capsules and run through a carbon-nitrogen flash analyzer (CE Elantech, Lakewood, NJ, USA). From these data the C:N ratio, nitrogen per unit leaf area (N_{area}; mg N cm⁻²) and carbon per unit leaf area (C_{area}; mg C cm⁻²) were calculated.

Literature comparison

In order to ascertain more broadly whether *P. krempfii* exhibits physiological characteristics more similar to Podocarpaceae, several parameters measured from the species in Vietnam were compared with published data. The A_{max}, V_{cmax} and J_{max} values on projected area bases for species from the genus *Pinus* as well as for species from the Podocarpaceae were compiled from published studies (Gower et al. 1993, Wullschlegel 1993, Lewis et al. 1994, Thomas et al. 1994, Zhang and Marshall 1995, Porte and Loustau 1998, Turnbull et al. 1998, Bond et al. 1999, Jiang et al. 1999, Tissue et al. 1999, 2005, Griffin et al. 2000, Cernusak and Marshall 2001, Warren and Adams 2001, De Lucia et al. 2003, Lusk et al. 2003, Springer et al. 2005, Brodribb et al. 2007, Brodribb and Feild 2008, Han et al. 2008, Bown et al. 2009, Nagano et al. 2009, Guan and Wen 2011, Han 2011, Tosens et al. 2012, Hasper et al. 2017,

Li et al. 2019) and the TRY database (Kattge et al. 2020), including the following published studies: Wright et al. 2004, Choat et al. 2012, Atkin et al. 2015, Smith and Dukes 2017, Wang et al. 2018 (Table S1 available as Supplementary Data at *Tree Physiology* Online). A_{max} values were compared at ambient temperatures and saturating light, although these varied across species. V_{cmax} and J_{max} values were corrected to 25 °C using the equation:

$$k_{25} = \frac{k}{\exp [E_a (T_k - 298) / (298 * R T_k)]}$$

according to Medlyn et al. (2002) in which k₂₅ represents the parameter value at 25 °C (V_{cmax} or J_{max}), k is the parameter value at the measured temperature, T_k is the temperature in Kelvin and R is the universal gas constant (8.314 JK⁻¹ mol⁻¹). E_a is the function's exponential rate of increase, and was assumed to be 64.8 kJmol⁻¹ for V_{cmax} as determined in Badger and Collatz (1977), and implemented in Atkin et al. (2015), and 50 kJmol⁻¹ for J_{max} (Medlyn et al. 2002). We used the Arrhenius function for J_{max} as it has been found to perform as well as a peaked function in species of pines (Medlyn et al. 2002).

Statistical analyses

Before analysis, all data were assessed for and transformed in order to fulfill assumptions of normality. If transformation was necessary, the function transformTukey was used in the rcompanion package for R (Mangiafico 2020). Individual seedlings with more than one set of measurements were averaged. Interspecific comparisons were made using analysis of variance and Tukey post hoc tests with a significance level of 0.05.

Linear mixed effects models run using the lme4 package (Bates et al. 2015) were used to examine relationships between A_{max}, V_{cmax} and J_{max} as a function of either N_{area} or LMA, to compare the A_{max}, V_{cmax} and J_{max} values of *P. krempfii* with *Pinus* and Podocarpaceae literature values, and to examine the slope of the relationships between V_{cmax} and J_{max} by taxonomic group. In all cases, species was included as a random intercept. In order to examine the variation in slope between taxonomic groups in the relationship between V_{cmax} and J_{max}, an interaction effect was also included (log(J_{max}) ~ log(V_{cmax}) * group + (1|species)); the full model can be viewed in Methods S1 available as Supplementary Data at *Tree Physiology* Online). Fixed effect parameter estimates and their significance levels were determined using the lmerTest package (Kuznetsova et al. 2017) and the marginal coefficient of determination (the variance explained by the fixed effects; r_m²) and conditional coefficient of determination (the variance explained by the fixed and the random effects; r_c²) were determined using the MuMIn package (Bartoń 2020). Pairwise comparisons of the slopes between the taxonomic groups were conducted via Tukey post hoc in the emmeans package (Lenth 2020). All analyses were completed in R v. 3.6.3 (R Development Core Team 2020).

Table 2. Summaries of the one-way ANOVA model results for photosynthetic, respiratory and leaf trait characteristics for the conifer species in the Central Highlands of Vietnam.

	F-value	P > F	r ²	df
Photosynthesis				
A_{\max}	7.96	<0.001	0.46	6, 43
Φ	1.88	0.106	0.1	6, 43
LSP	11.12	<0.001	0.55	6, 43
LCP	9.31	<0.001	0.5	6, 43
V_{cmax}	16.2	<0.001	0.66	6, 41
J_{\max}	3.92	0.003	0.27	6, 41
Respiration				
R_{D}	4.89	0.001	0.32	6, 43
R_{L}	7.45	<0.001	0.55	5, 21
$R_{\text{D}}/A_{\text{gross}}$	4.93	0.001	0.32	6, 43
CUE	5.54	<0.001	0.36	6, 43
PR	9.1	<0.001	0.61	5, 21
CGE	2.2	0.092	0.19	5, 21
Leaf traits				
% carbon	10.83	<0.001	0.52	6, 48
% nitrogen	4.77	0.001	0.3	6, 48
C:N	6.25	<0.001	0.37	6, 48
N_{area}	3.42	0.007	0.21	6, 48
C_{area}	8.49	<0.001	0.45	6, 48
LMA	7.01	<0.001	0.40	6, 48

Abbreviations: A_{\max} , maximum photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$); Φ , apparent quantum yield; LSP, light saturation point ($\mu\text{mol m}^{-2} \text{s}^{-1}$); LCP, light compensation point ($\mu\text{mol m}^{-2} \text{s}^{-1}$); V_{cmax} , maximum carboxylation rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); J_{\max} , maximum electron transport ($\mu\text{mol m}^{-2} \text{s}^{-1}$); R_{D} , respiration in the dark ($\mu\text{mol m}^{-2} \text{s}^{-1}$); R_{L} , respiration in the light ($\mu\text{mol m}^{-2} \text{s}^{-1}$); A_{gross} , sum of A_{\max} and R_{D} ; CUE, carbon use efficiency; PR, photorespiration; CGE, carbon gain efficiency; C:N, ratio of carbon to nitrogen; N_{area} , nitrogen per leaf area (mg N cm^{-2}); C_{area} , carbon per leaf area (mg C cm^{-2}); LMA, leaf mass per area (g m^{-2}).

Results

Gas exchange and leaf traits

Among the conifers of the Central Highlands of Vietnam, *P. krempfii* and the Podocarpaceae had significantly lower area-based A_{\max} , V_{cmax} and LSP, and trended toward lower area-based J_{\max} than *P. kesiya* (the only *Pinus* not growing in the forest interior; $P < 0.05$; Figure 1a, c, e and f; Table 2). However, *P. dalatensis*, the only other needle-leaved pine from this area, often fell between *P. kesiya* and *P. krempfii*, showing no statistical difference from either species (Figure 1a, e and f). The only exception was that *P. dalatensis* reached saturation at significantly lower light levels than *P. kesiya*, converging on similar LSPs to *P. krempfii* and all Podocarpaceae except *P. neriifolius* (Figure 1c). Few significant differences were apparent in species' LCP, although it is notable that *D. elatum*, the only needle-leaved Podocarpaceae, had significantly higher LCP than the other Podocarpaceae. There were no significant differences across species in the apparent quantum yield ($P > 0.05$; Figure 1b, Table 2).

With regard to area-based respiratory characteristics such as respiration in the dark (R_{D}), respiration in the light (R_{L}) and photorespiration (PR), significant differences were apparent across species in the Central Highlands of Vietnam

(Table 2); however, these differences did not clearly separate between Pinaceae and Podocarpaceae (Figure 2a, b and e, respectively). While an overall trend of decreasing respiration was apparent from the Pinaceae to Podocarpaceae, *D. elatum*, the only needle-leaved Podocarpaceae, had R_{D} and R_{L} equal to the needle-leaved Pinaceae (Figure 2a and b). Overall, measures of these species' carbon balance showed few differences between species. The $R_{\text{D}}/A_{\text{gross}}$ ratio and the CUE showed no differences between species except for *D. elatum* and *N. wallichiana*, which had slightly higher $R_{\text{D}}/A_{\text{gross}}$ (Figure 2c), and slightly lower CUE (Figure 2d). All species had similar CGEs (Figure 2f, Table 2). Significant differences were apparent in % carbon, % nitrogen, C:N, N_{area} , C_{area} and LMA (Table 2); however, as with the respiratory characteristics, no patterns emerged with regard to species' familial assignments (Figure 3).

On a mass basis, *P. krempfii* had significantly lower A_{\max} than *P. kesiya*. *P. dalatensis*, the other Vietnamese needle-leaved conifer, had lower A_{\max} , similar to *P. krempfii* and most Podocarpaceae (Figure S2a, Table S2 available as Supplementary Data at *Tree Physiology* Online). Respiratory characteristics also exhibited few significant differences among species (Figure S2b, Table S2 available as Supplementary Data at *Tree Physiology* Online).

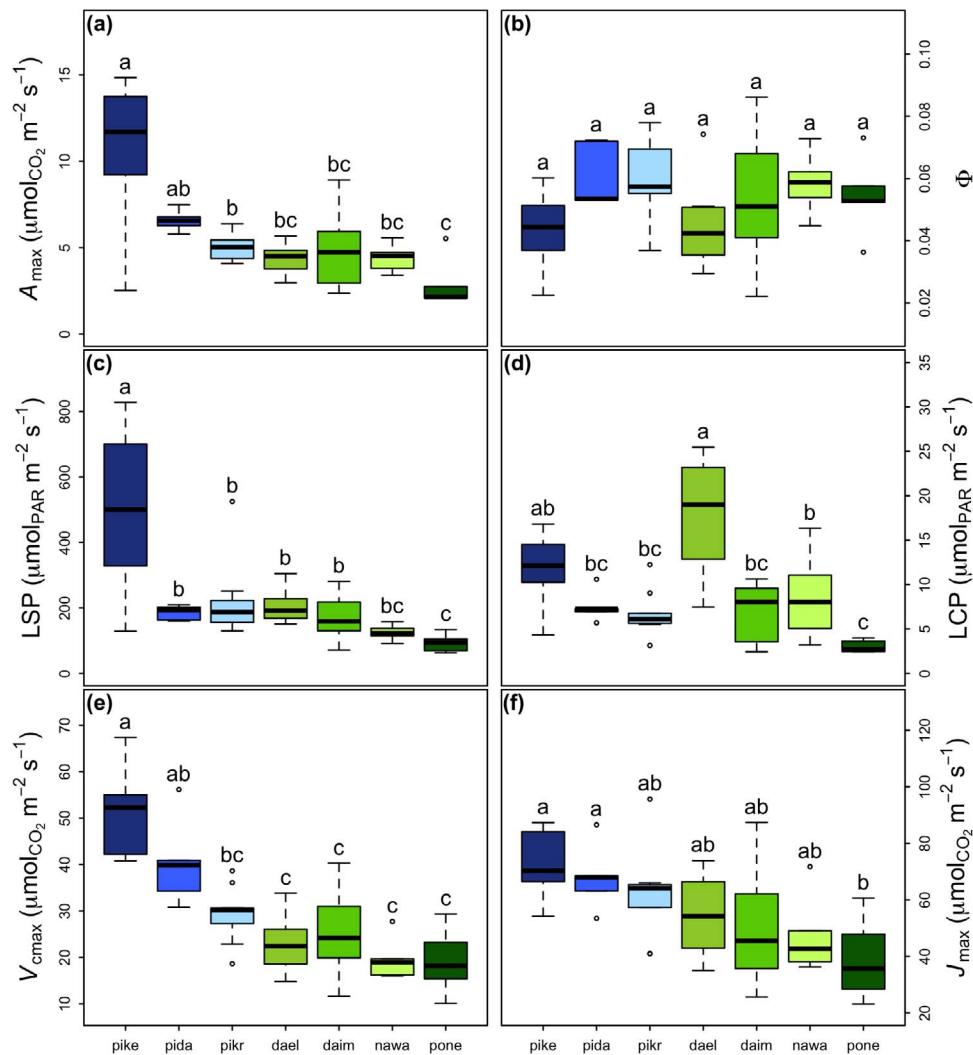


Figure 1. Leaf level photosynthetic characteristics on an area basis including maximum photosynthesis (A_{\max} ; a), apparent quantum yield (Φ ; b), LSP (c), LCP (d), maximum carboxylation rate (V_{cmax} ; e) and maximum electron transport (J_{max} ; f) of each of the conifer species including *P. neriifolius* (pone), *D. imbricatus* (daim), *D. elatum* (dael), *N. wallichiana* (nawa), *P. kesiya* (pike) and *P. dalatensis* (pida), compared with *P. krempfii* (pikr). Species from the Podocarpaceae are in shades of green, species from *Pinus* are in shades of blue and *P. krempfii* is in light blue. The boxplots represent the median as well as the first and third quartiles. Whiskers delimit the range for each group, with outliers falling outside $\times 1.5$ the interquartile range marked by points. Significant differences between groups are marked by different letters ($P < 0.05$).

Relationships between physiological parameters and leaf traits

On an area basis, relationships between physiological parameters and both leaf nitrogen content and LMA were examined. Significant relationships were found between all parameters ($P < 0.05$; Figure 4). For all relationships, the random effect of species explained a larger proportion of the variance in the full model than the fixed effects (Table 3), indicating species-specific differences in all these relationships. For the relationships between N_{area} and either A_{max} or V_{cmax} , excluding *P. kesiya* (a visual outlier) increased the variance explained by the fixed effects (Figure 4a and c, Table 3). For N_{area} vs J_{max} , however, the marginal coefficient of determination was similar regardless of *P. kesiya* exclusion (Figure 4e, Table 3).

Relationships between A_{max} , V_{cmax} and J_{max} and LMA were also examined both with and without *P. kesiya* (Figure 4b, d and f, Table 3). Once again, exclusion of *P. kesiya* increased the variance explained by the fixed effects in the relationships between A_{max} and V_{cmax} vs LMA (Figure 4b and d, Table 3), but had no effect on the relationship between J_{max} and LMA (Figure 4f, Table 3).

On a mass basis, a significant relationship was found between A_{max} and N_{mass} when all species were included but not when *P. kesiya* was excluded (Figure S3a; Table S3 available as Supplementary Data at *Tree Physiology Online*). As with area-based relationships, the variance explained by the fixed effects was much lower than that explained by the random effect of species (Table S3 available as Supplementary Data at *Tree Physiology*

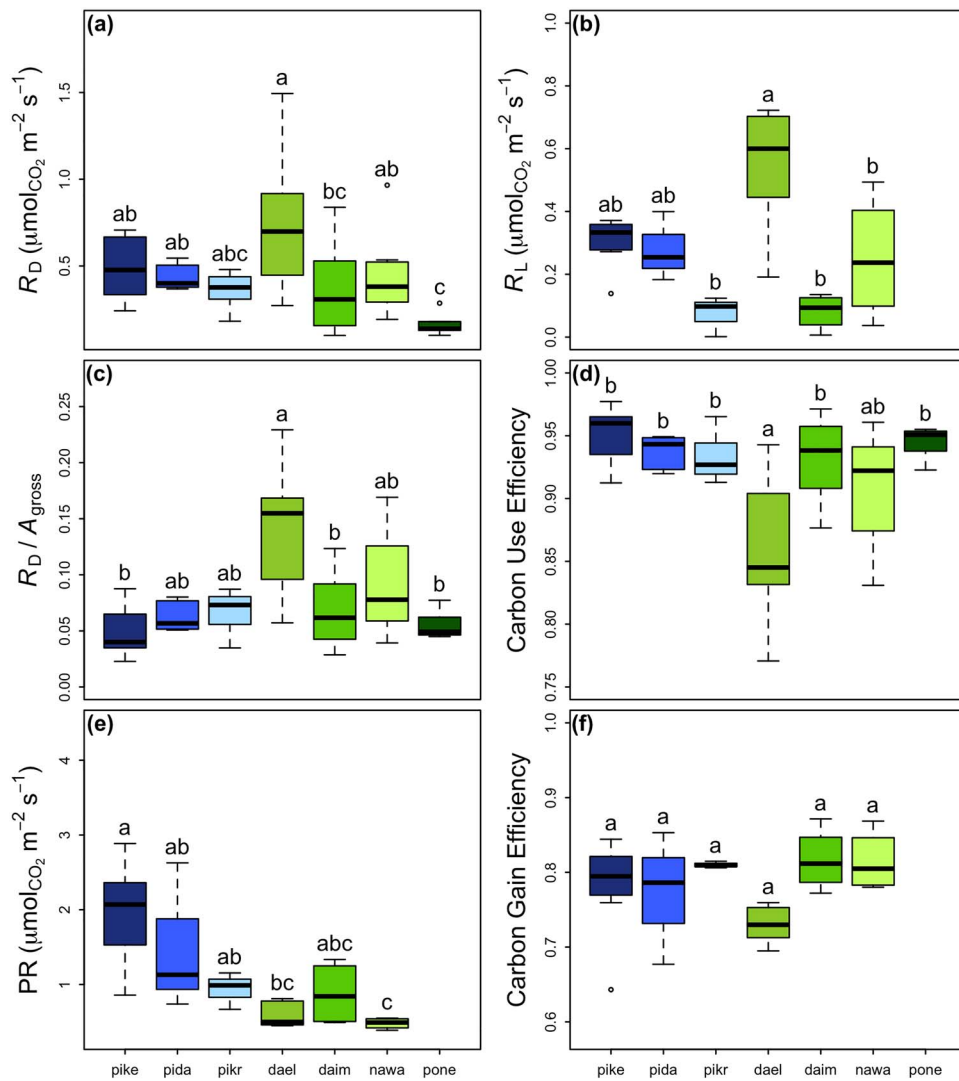


Figure 2. Foliar respiration in the dark (R_D ; a), respiration in the light (R_L ; b), ratio of R_D to gross photosynthesis (R_D/A_{gross} ; c), CUE (d), photorespiration (PR; e) and CGE (f) on an area basis of the conifer species compared with *P. krempfii*. Species are labeled and colored as in Figure 1. The boxplots represent the median as well as the first and third quartiles. Whiskers delimit the range for each group, with outliers falling outside $\times 1.5$ the interquartile range marked by points. Significant differences between groups are marked by different letters ($P < 0.05$).

Online). In contrast to area-based relationships, the relationship between mass-based A_{max} and LMA was not significant except when *P. kesiya* was excluded (Figure S3b available as Supplementary Data at *Tree Physiology* Online).

Comparisons with literature values of A_{max} , V_{cmax} and J_{max}

Comparisons of *P. krempfii* and the conifers from Vietnam with literature values of species from *Pinus* and the Podocarpaceae are important to ascertain whether shade-tolerant physiological characteristics determine broader *Pinus* exclusion from tropical forests and thus the southern hemisphere. *P. krempfii* had one of the lowest maximum photosynthetic (A_{max}) values of the pines, with only *P. edulis* and *P. serotina* demonstrating lower values (Figure 5). In contrast, *P. kesiya* had comparatively high A_{max} . As a group, *Pinus* had significantly higher A_{max} than Podocarpaceae,

with *P. krempfii* falling between the two groups ($P < 0.05$; Figure 5). Similar patterns were apparent in V_{cmax} and J_{max} (Figure 5). *P. krempfii* had one of the lower V_{cmax} and J_{max} values of the *Pinus* compiled from the literature. *P. kesiya* also had a relatively high V_{cmax} , although its J_{max} was closer to *P. krempfii*. As in A_{max} , *Pinus* had a significantly higher V_{cmax} than Podocarpaceae, with *P. krempfii* falling between the two groups ($P < 0.05$; Figure 5). In contrast, no significant differences were found between taxonomic groups (*Pinus*, *P. krempfii*, Podocarpaceae) for J_{max} ($P > 0.05$; Figure 5). These findings support past literature compilations in which A_{max} of *P. krempfii* was found to be in the range of flat-leaved Podocarpaceae (Brodrribb and Feild 2008), while providing additional, novel information on the mechanistic underpinnings of this pattern through the additional compilation of V_{cmax} and J_{max} values.

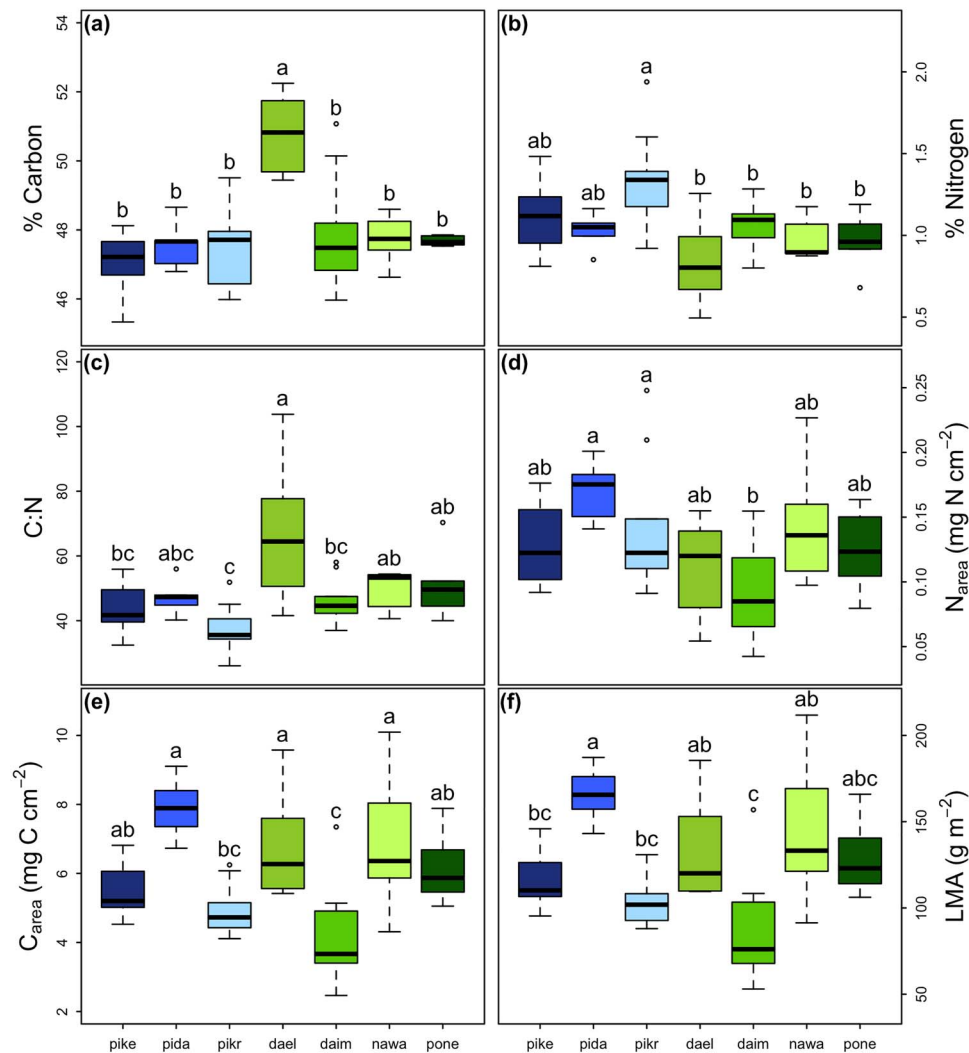


Figure 3. Foliar traits including % carbon (a), % nitrogen (b), C:N ratio (c), nitrogen per leaf area (N_{area} ; d), carbon per leaf area (C_{area} ; e) and LMA (f) for the conifers of the Central Highlands of Vietnam. Species are labeled and colored as in Figure 1. The boxplots represent the median as well as the first and third quartiles. Whiskers delimit the range for each group, with outliers falling outside $\times 1.5$ the interquartile range marked by points. Significant differences between groups are marked by different letters ($P < 0.05$).

When comparing the relationships between V_{cmax} and J_{max} as a function of taxonomic group (*Pinus*, *P. krempfii* or Podocarpaceae), species from *Pinus*, including *P. kesiya* and *P. dalatensis* from Vietnam, had a significantly less steep slope than the Podocarpaceae (Figure 6, Table 4). *P. krempfii*, despite its similar slope to Podocarpaceae, was not found to be significantly different from either *Pinus* or Podocarpaceae. Regardless of the differences between *Pinus* and Podocarpaceae, a strong relationship was found between V_{cmax} and J_{max} ($r_m^2 = 0.72$; $r_c^2 = 0.89$).

Discussion

Variation in shade tolerance strategies among the Vietnamese conifers

Several physiological traits support the convergence of *P. krempfii* toward flat-leaved Podocarpaceae and an adaptation

to shade. For example, *P. krempfii*, along with species from the Podocarpaceae, had lower maximum photosynthetic capacity (A_{max}), a trait common to shade-tolerant plants (Mulkey et al. 1993, Hikosaka and Terashima 1996, Niinemets et al. 1998). The two co-limiting processes controlling maximum photosynthetic capacity and representing the maximum rates of the light and dark reactions, J_{max} and V_{cmax} (Wullschlegel 1993), provide a more mechanistic interpretation of the decreasing A_{max} from needle-leaved *Pinus* to flat-leaved *P. krempfii* and Podocarpaceae. These two processes correlate strongly with each other. Yet, shade-intolerant species often preferentially allocate resources to the dark reactions, allowing for greater RuBisCO activity and carbon fixation, while shade-tolerant species may preferentially allocate resources to the light reactions of photosynthesis (Seemann et al. 1987, Evans 1989). This could account for the lack of significant differences among *P. krempfii*, other needle-leaved *Pinus* and

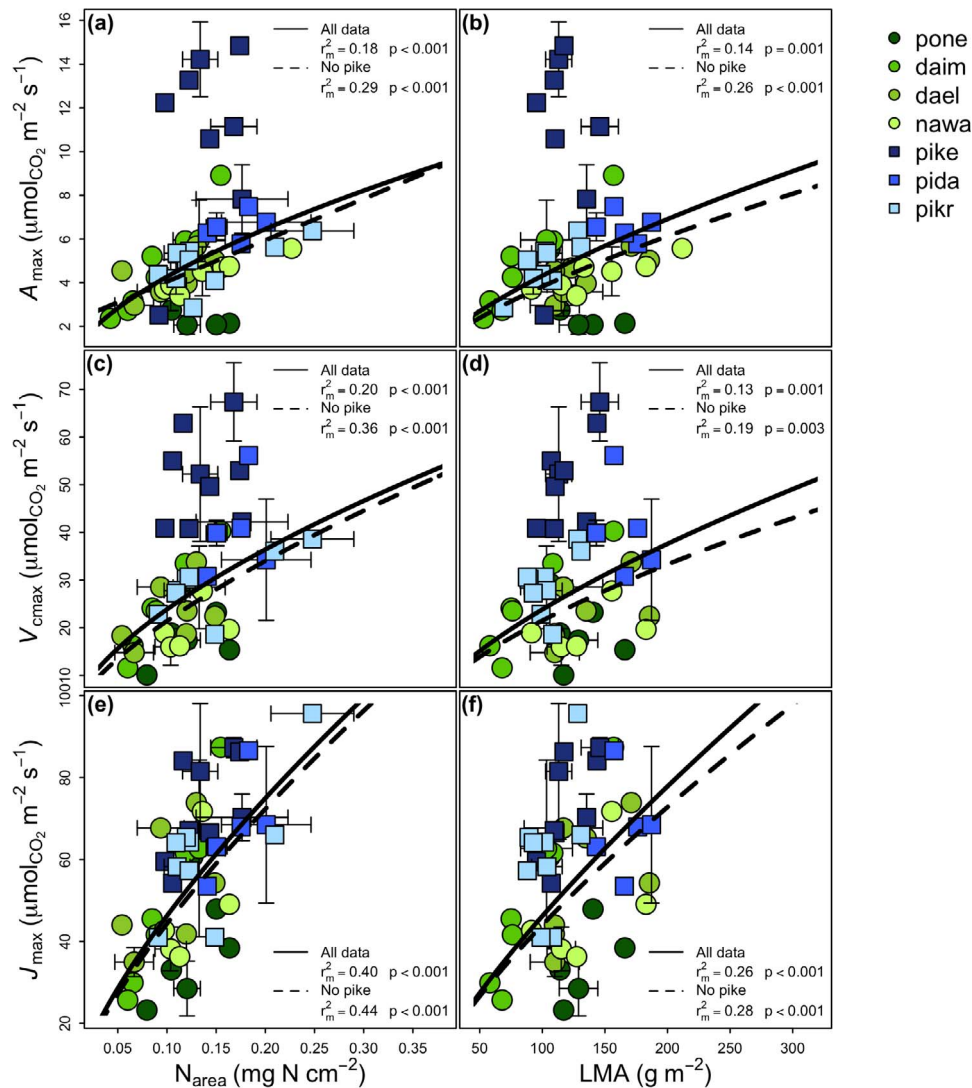


Figure 4. Linear mixed effects relationships between A_{max} , V_{cmax} or J_{max} and N_{area} (a, c, e), and A_{max} , V_{cmax} or J_{max} and LMA (b, d, f) for all conifer species measured in the Central Highlands of Vietnam. Individual species are labeled and colored as in Figure 1. The solid black line represents the linear relationship with all species included. The dashed black line shows the relationship if *P. kesiya* (pike) is excluded. Each point represents an individual seedling. If more than one measurement per seedling was taken, the values were averaged and error bars representing 1 SEM were included. The relationship was considered significant if $P < 0.05$. Marginal coefficients of determination (r_m^2) are also reported.

Podocarpaceae in J_{max} , but the more pronounced significant differences in V_{cmax} between *P. kesiya* (the only *Pinus* not growing in the forest interior) and both *P. krempfii* and the Podocarpaceae.

Differences in V_{cmax} and J_{max} between shade-tolerant and intolerant species also usually correlate with the investment of nitrogen in these processes. Thus, shade-intolerant species with higher V_{cmax} should invest more nitrogen into proteins necessary for the dark reactions of photosynthesis, primarily RuBisCO (Seemann et al. 1987, Evans 1989). In contrast, shade-tolerant species may invest greater nitrogen into chlorophyll and the light harvesting complexes necessary for the production of ATP and NADPH in the light reactions (Seemann et al. 1987, Evans 1989). Including *P. kesiya* in the

relationship between N and both A_{max} and V_{cmax} substantially reduced the coefficients of determination, suggesting that *P. kesiya*, following a pioneering, high-light strategy, may invest greater N toward RuBisCO. In contrast, no differences were seen in the N vs J_{max} relationship between *P. kesiya* and other Vietnamese conifers, indicating that shade-tolerant *P. krempfii* and Podocarpaceae may invest a greater proportion of available N toward light-harvesting thylakoid proteins in order to maintain similar rates of electron transport to *P. kesiya*. Larger leaf areas may further assist shade-tolerant Podocarpaceae and *P. krempfii* to maintain high J_{max} , highlighting, again, the importance of light interception and the multiple mechanisms through which shade-tolerant species can enhance light capture (Lusk and Reich 2000).

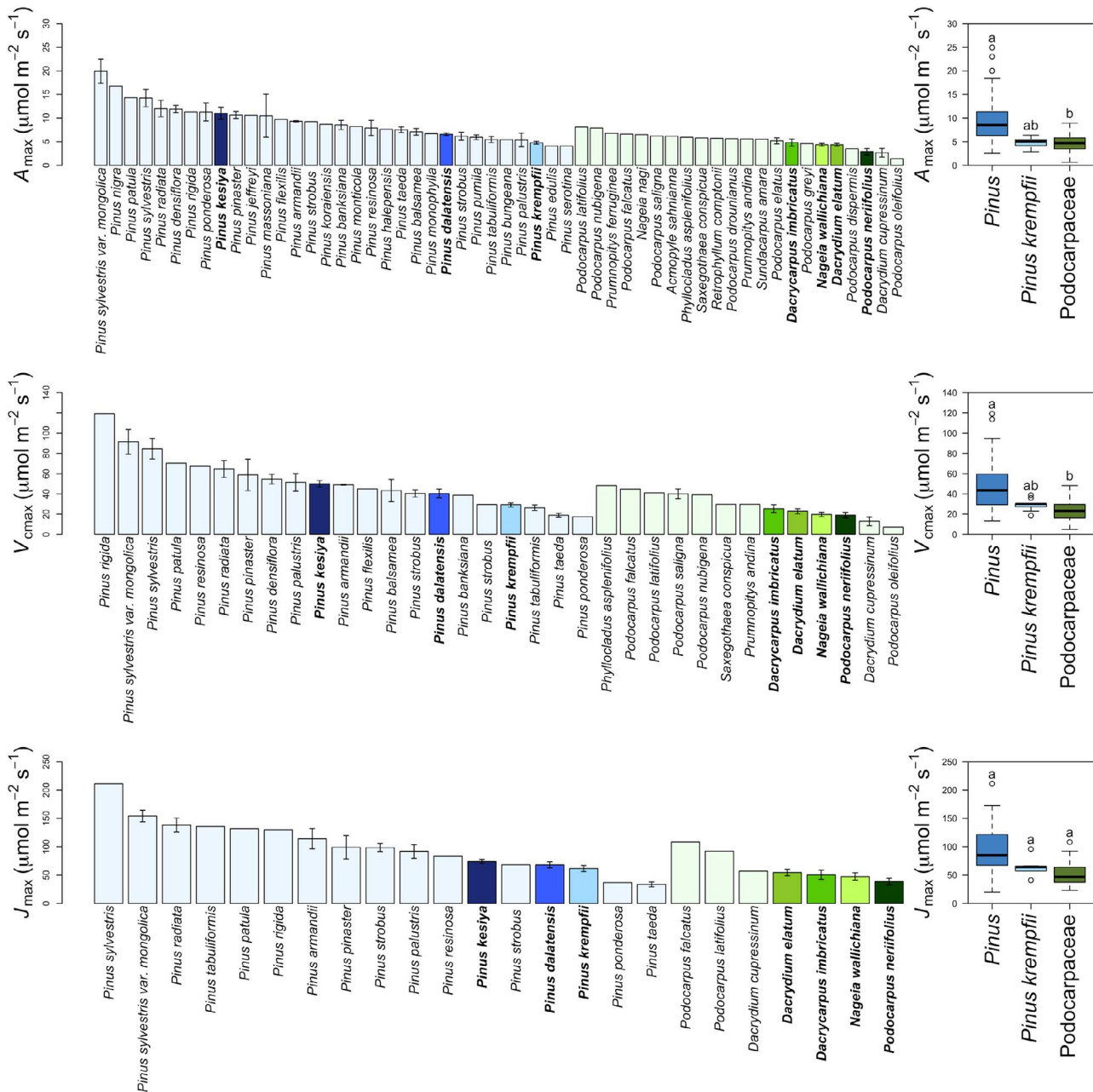


Figure 5. Bar graphs of maximum photosynthesis (A_{max}), maximum RuBP carboxylation (V_{cmax}) and maximum electron transport rate (J_{max}) values taken under optimal conditions for species from *Pinus* and for flat-leaved species from the Podocarpaceae. The bar graphs show mean values for each species ± 1 standard error, if more than one record for the species existed in the literature. Bolded species are those that were measured in Vietnam with species' colors matching Figure 1. The boxplots show data in the bar graphs grouped taxonomically by *Pinus* (blue), *P. krempfii* (light blue) and Podocarpaceae (green). The boxplots represent the median as well as the first and third quartiles. Whiskers delimit the range for each group, with outliers falling outside $\times 1.5$ the interquartile range marked by points. Significant differences between groups are marked by different letters ($P < 0.05$).

The suite of photosynthetic characteristics quantified here provides additional mechanistic details on previous work in which *P. krempfii* was shown to converge toward shade-tolerant traits of Podocarpaceae (Brodrribb and Feild 2008). Our findings further expand on past work by quantifying, often for the first time, the photosynthetic characteristics of a majority of the conifers growing in this unique forest with high conifer diversity.

P. dalatensis, a relatively rare needle-leaved conifer growing in the forest interior, may hold additional clues. It shows a similar LSP to *P. krempfii* and coexisting Podocarpaceae, but possesses other physiological traits that fall between *P. kesiya*, and flat-leaved *P. krempfii* and Podocarpaceae. It is possible that *P. dalatensis*, like many higher-light species, can demonstrate greater plasticity in response to light availability (Poorter et al.

Table 3. Marginal and conditional coefficients of determination (r_m^2 ; r_c^2 , respectively) for linear mixed effects models of physiological and leaf traits for all the conifers of the Central Highlands of Vietnam (all data), and excluding *P. kesiya* (pike).

	N _{area}		LMA	
	r_m^2	r_c^2	r_m^2	r_c^2
<i>A</i> _{max}				
All data	0.18	0.63	0.14	0.67
No pike	0.29	0.57	0.26	0.70
<i>V</i> _{cmax}				
All data	0.20	0.72	0.13	0.73
No pike	0.36	0.61	0.19	0.58
<i>J</i> _{max}				
All data	0.40	0.65	0.26	0.63
No pike	0.44	0.63	0.28	0.57

Abbreviations: *A*_{max}, maximum photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$); *V*_{cmax}, maximum carboxylation rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); *J*_{max}, maximum electron transport ($\mu\text{mol m}^{-2} \text{s}^{-1}$); N_{area}, nitrogen per leaf area (mg N cm^{-2}); LMA, leaf mass per area (g m^{-2}).

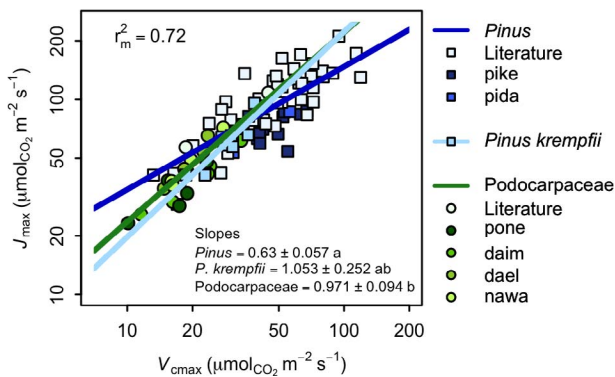


Figure 6. Linear relationships between *V*_{cmax} and *J*_{max} for species from the genus *Pinus*, compared with *P. krempfii* and species from the Podocarpaceae determined from a single linear mixed effects model in which *J*_{max} was modeled as a function of *V*_{cmax} and the interaction between taxonomic group (*Pinus*, *P. krempfii* or Podocarpaceae). Colored points for each group are those values that were measured in Vietnam. Unshaded points were those collected from the literature. A random intercept for species was included to account for any multiple measures per species. The marginal coefficient of variation (r_m^2) is reported. Slope estimates, ± 1 standard error are also presented for each group. Slopes considered significantly different from other groups are marked by different letters ($P < 0.05$).

2009). This may account for the continued presence of *P. dalatensis* in the forest interior, albeit in small stands, as juveniles may be able to persist until released by canopy disturbance events that then permit rapid growth (Loc et al. 2017). Regardless, the similarity between *P. dalatensis* and *P. krempfii* in many foliar traits suggests a continuum between shade-intolerant *Pinus* and shade-tolerant Podocarpaceae in which *P. krempfii* trends toward more shade-tolerant and Podocarp-like traits.

Adaptation of *P. krempfii* to shaded environments has resulted in convergence toward similar photosynthetic traits to shade-tolerant podocarps. However, we found no significant differences in Φ across species and few discernible differences in LCP,

respiratory characteristics, LMA, or carbon balance between needle-leaved pines, *P. krempfii* and Podocarpaceae species. It is not uncommon for conifer leaf traits such as *R*_D to demonstrate lower variability than, for example, broad-leaved angiosperms across differing light environments (Lusk and Reich 2000). Given the generally low *R*_D values in all Vietnamese conifers, it may be difficult to detect significant interspecific differences between these species. Even so, both *P. kesiya* and *D. elatum* had slightly lower Φ and higher LCP, *R*_D and *R*_L than flat-leaved Podocarpaceae with *P. krempfii* falling between needle- and flat-leaved species. These trends conform to expected patterns of shade tolerance, namely increasing Φ and downregulation of foliar *R*_D and LCP in order to improve leaf carbon balance in shade-tolerant species in low-light environments (Loach 1967, Givnish 1988, Craine and Reich 2005). These findings again emphasize the intermediate nature of *P. krempfii*, as well as the high-light tendencies of *P. kesiya*.

Among the Podocarpaceae, there is further evidence of leaf morphology being linked to a physiological strategy of shade tolerance. *D. elatum* appears to adopt a high-light strategy, in contrast to coexisting flat-leaved Podocarpaceae. Its needle-like leaf morphology and physiological characteristics are often comparable to high-light *P. kesiya*. Existing literature describing the ecology of this species is limited, although it has been suggested that regeneration is only occasional (Luu and Thomas 2004) and that this imbricate-leaved species may thrive in more open, disturbed conditions (Coomes and Bellingham 2011). A broader examination of podocarp species across both temperate and tropical environments suggests that flat-leaved podocarps may be more shade-tolerant, and imbricate podocarps less so (Coomes and Bellingham 2011, Biffin et al. 2012). However, *D. elatum* is heteroblastic, having both a juvenile and an adult leaf morphology, a characteristic common also to *D. imbricatus* and *P. krempfii*, where the adult form is either imbricate or more needle-like, respectively, than the juvenile leaf form (Luu and Thomas 2004, Loc et al. 2017). These variable leaf forms may moderate light absorption in canopy or canopy-emergent adults and aid in light capture in understory juveniles, providing greater overall ecological flexibility.

Leaf morphology can impart greater shade tolerance and may be an adaptive trait used by coniferous species to grow and survive in this tropical highland forest. However, LMA values of the species measured here do not show consistent differences between pines and podocarps, rather, differences are species-specific. Past conifer studies similarly show no significant relationship between shade tolerance and LMA (Leverenz et al. 2000, Wyka et al. 2012). LMA is a complex variable driven by a combination of leaf thickness and density, although leaf density alone has been found to be the most important driver of interspecific differences in LMA (Poorter et al. 2009). A variety of factors can influence leaf density including investment in structural components, nonstructural materials (e.g., proteins or

Table 4. Fixed effects parameter estimates and significance for the linear mixed effects model of J_{\max} and V_{cmax} with an interaction term for taxonomic group and a random intercept for species. The full model can be seen in the [Methods S1](#) available as Supplementary Data at *Tree Physiology Online*.

Parameter name	Value	SE	df	t	P
b0	2.0905	0.2259	89.0126	9.255	<0.001***
b1	0.6304	0.0568	91.8780	11.104	<0.001***
b2	-1.5312	0.8971	80.7348	-1.707	0.09168
b3	-1.1645	0.3848	91.8634	-3.026	0.00322**
b4	0.4222	0.2582	70.3738	1.635	0.10658
b5	0.3407	0.1102	88.7264	3.092	0.00266**

Note: Significant P-values are marked by asterisks (P < 0.001 (***), 0.001 < P < 0.01 (**)).

nonstructural carbohydrates) or both (Poorter et al. 2009). It is not known to what extent these different components contribute to the LMA of these conifers. Nevertheless, given the longer leaf lifespans of many conifers, the importance of tissue strength for herbivory resistance in shaded understories, as well as variability in structural components such as resin ducts and transfusion tissues, it is likely that both leaf structure and function play important roles in the LMAs of these species.

Relationships between morphological and physiological adaptations may further illuminate the ecological strategies employed by coniferous species in this tropical montane forest. Globally, across species and functional types, LMA has a strong negative relationship with mass-based A_{\max} , but a weakly positive relationship with area-based A_{\max} (Wright et al. 2004). However, we show no significant relationship between LMA and mass-based A_{\max} except when the high-light specialist, *P. kesiya*, is excluded. While this relationship is consistent with the leaf economics spectrum (LES) in which slow-return species have higher LMA, lower mass-based photosynthesis and longer leaf life span, the determination coefficient for the fixed effect is very small. The insignificant mass-based relationship across all species may result from large interspecific variation in conifer leaf traits in the shade (Wyka et al. 2012) and the fact that LES mass-based patterns are driven by differences between functional groups, and not by differences within a single functional group (Wright et al. 2004). In contrast, significant relationships between LMA and area-based photosynthetic traits adhere to intraspecific patterns of increasing mass with increasing light availability seen commonly in many broad-leaved deciduous species (Lusk et al. 2008, Valladares and Niinemets 2008). While the determination coefficients are still relatively small and often within range of other conifer studies (Wyka et al. 2012), the larger fixed effects' determination coefficients in the area-based relationships may reflect the large number of broad-leaved conifers for which leaf area and light capture are critical.

Finally, by combining the suite of measured physiological traits, we can calculate the potential leaf carbon balance and assess its adaptive significance for the coniferous species native to this forest. We find that despite larger differences in A_{\max} between shade-intolerant *P. kesiya* and shade-tolerant Podocarpaceae and *P. krempfii*, and smaller differences in

respiration, all species demonstrated similar carbon balance. Many studies propose that shade-tolerant species have better carbon balance in low-light conditions than shade-intolerant species, a hypothesis tentatively supported by decreases in foliar R_D and increases in leaf area accumulation in shade-tolerant species (Noguchi et al. 1996, 2005, Lusk 2002, Craine and Reich 2005). Although we find no significant differences in the carbon balance of our shade-tolerant and shade-intolerant species, similar to other studies (e.g., Pons and Poorter 2014), all the Vietnamese conifers demonstrated extremely high CGEs and much lower R_D/A_{gross} than the angiosperm species examined by Pons and Poorter (2014). Superior CGEs may be imperative for conifer persistence at the juvenile stage in this Vietnamese tropical forest.

Pinus krempfii as an intermediate between shade-intolerant *Pinus* and shade-tolerant Podocarpaceae: evidence from a literature compilation of physiological traits

The unusual leaf morphology of *P. krempfii*, together with the wealth of information collected on the physiology of this species, provides a unique opportunity to explore mechanistically the contrasting biogeographic success of the two largest conifer families – Pinaceae and Podocarpaceae – in tropical forests. Combining data on photosynthetic capacity of Vietnamese conifers, including *P. krempfii*, with an extensive literature survey of *Pinus* and Podocarpaceae species reveals significantly higher photosynthetic capacities in *Pinus* than in Podocarpaceae, with *P. krempfii* an intermediate between the two taxa. The literature survey vastly strengthens the findings from Brodrribb and Feild (2008) and our own study of Vietnamese conifers that low photosynthetic rates are characteristic of coniferous survival in tropical forests. Furthermore, we show that these low photosynthetic rates are mechanistically realized through the lower RuBisCO carboxylation capacity in Podocarpaceae. This is likely a result of allocating proportionally more N to light capture (Seemann et al. 1987), leading to the observed steeper slope in the relationship of J_{\max} to V_{cmax} in Podocarpaceae compared with *Pinus*. Combined with leaf anatomical differences (Brodrribb and Feild 2008), and the importance of leaf area for light interception (Brodrribb and Hill 1997), these photosynthetic traits indicate opposing shade

tolerance strategies in these two taxa. The intermediate nature of *P. krempfii* between *Pinus* and Podocarpaceae suggests that a single photosynthetic and morphological strategy, already in use by flat-leaved Podocarpaceae, can permit conifer survival in low-light tropical environments. While the aberrant flat-leaved *P. krempfii* provides evidence that it is possible for structural and photosynthetic evolution to take place in *Pinus* as a taxa, it is evident that, so far, such adjustments are rare. Unless *Pinus* can alter both its foliar morphology and its physiology, it is unlikely to expand its geographical range naturally beyond its Laurasian origins.

Conclusions

We examined the physiological and morphological traits of the majority of conifer species in a unique tropical forest from two biogeographically contrasting taxa: *Pinus* and Podocarpaceae, in order to assess whether a lack of shade tolerance traits in *Pinus* contributes to their exclusion from the majority of tropical forests. Comparisons among the species from the Central Highlands of Vietnam showed evidence of low photosynthetic rates, low carboxylation capacity and a trend toward low respiratory rates and LCP in flat-leaved Podocarpaceae. *P. krempfii*, the only-known flat-leaved *Pinus*, often fell between flat-leaved Podocarpaceae and needle-leaved *Pinus*, suggesting its convergence toward a shade-tolerant podocarp-like survival strategy. However, few differences were observed among species' LMA, likely as a result of the large number of leaf components contributing to LMA. Despite variation in the foliar traits among these conifers, all species converged on an extremely high CGE, suggesting that, regardless of shade tolerance, CGE is key for conifer survival in this unique study region. Lastly, combining our Vietnam data with a literature survey, we demonstrate that there are inherent phylogenetic differences in photosynthetic traits between *Pinus* and Podocarpaceae, with low photosynthetic rates in Podocarpaceae due to lower RuBisCO carboxylation. Ultimately these findings are consistent with a lack of shade tolerance in *Pinus* and imply that without physiological and morphological adaptation, as occurs in *P. krempfii*, *Pinus* will be unsuccessful in colonizing tropical locations.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* online.

Data and materials availability

All foliar trait data, as well as raw gas exchange data, are archived with Dryad Digital Repository: <https://doi.org/10.5061/dryad.1g1jwstss> (Schmiede et al. 2020).

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Conflict of interest

None to declare.

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Authors' contributions

S.C.S., K.L.G. and B.M.B. designed the research and were assisted in data collection by T.Q.C. and L.C.N. S.C.S., K.L.G., B.M.B. and D.S. performed the data analysis and wrote the manuscript.

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