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Research paper

Relationship between stomatal density, size and speed of opening in Sumatran rainforest species

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Recent studies have suggested that an association between size and speed of stomatal opening of stomata within taxa is likely to play a role in photosynthesis and transpiration. In this study we investigate whether this correlation applies for seedlings of 11 rainforest species from different taxa, and whether differences in stomatal and gas exchange parameters were related to initial growth under field and controlled conditions. The experiment was conducted on seedlings of nine late successional species and two early successional species, placed in full sunlight or 70% shade. We assessed density, size, length and width of guard cells, coupled with gas exchange parameters in the transition from darkness to light, recording minimum stomatal conductance during daytime darkness ($g_{s\text{-}dark}$), operating maximum stomatal conductance ($g_{s\text{-}op}$), speed of stomatal opening and the time to reach 50% conductance ($T\text{-}_{50\%}$). All stomata and gas exchange parameters were different between species. Shade significantly affected size and density, and all gas exchange parameters except $g_{s\text{-}op}$ were different between light situations. Stomatal size correlated negatively with speed of opening and positively with $T\text{-}_{50\%}$, confirming that smaller stomata open faster than large stomata. The two early successional species were very different in stomatal size and density, and in response to light. Anatomic parameters and physiological traits were not related to height growth, but $g_{s\text{-}dark}$, $g_{s\text{-}op}$ and speed of stomatal opening were associated with biomass growth in a subselection of six late successional species.

Keywords: gas exchange, Indonesia, stomatal anatomy, stomatal conductance.

Introduction

Water is a prerequisite for plant growth, and an important mechanism controlling water balance and leaf gas exchange is stomatal opening and closure (Düring 2015, Voelker et al. 2016). The thickness of the boundary layer (Schuepp 1993), the position on the abaxial and adaxial sides, and the size and density of stomata on the leaf surface sets the limits for the rates of gas exchange between free air surrounding the leaf and its humid interior. The stomatal conductance is further regulated through opening and closing, influenced by various environmental conditions (Rahnama et al. 2010, Cano et al. 2013, Fanourakis et al. 2016, Varela et al. 2016). Usually, exposure to light leads to opening of stomata for CO_2 uptake, although the conductivities are affected by the light spectrum (Kim et al. 2004). This leads to a diurnal pattern of opening where stomata of C3 plants typically have high stomatal conductance during the day and low during the night.

The maximum number of stomata per leaf area is restricted by the size of the stomata (Franks and Beerling 2009), but there is a considerable variation in the stomatal density and size between species (Zarinkamar 2007), leading to differences in rates of gas exchange. Plants with large and few stomata tend to have higher water-use efficiency, but also reduced rates of photosynthesis, compared with plants that have many but smaller stomata (Drake et al. 2013). For example, a study of rainforest species in Bolivia showed that both stomatal conductance

and photosynthesis correlated positively with the growth rate (Poorter and Bongers 2006), suggesting a close link between stomatal conductance and biomass production. However, while effects of stomatal size and density on gas exchange can be modeled (Franks and Farquhar 2001, Boer et al. 2016), much less is known about their effects on stomatal dynamics. A slow opening and closure of stomata could lead to sub-optimization of gas exchange and loss of water through transpiration as well as lost opportunities for photosynthesis (Farquhar and Sharkey 1982, Drake et al. 2013, Lawson and Blatt 2014). This was demonstrated in a study of *Banksia* species, showing that smaller stomata opened more rapidly than large stomata (Drake et al. 2013). However, whether this applies broadly across all species was questioned by Elliott-Kingston et al. (2016), who found that speed of stomatal closure was not related to stomatal length across a range of taxa including ferns, cycads, gymnosperms, grasses and dicots. Although stomatal length may not be an appropriate proxy for size of stomata across species, there is a need to further strengthen the empirical base about relationships between stomatal dimensions and dynamics as emphasized by Raven (2014).

Rainforests have a complex structure from the understory to the top of the canopy, resulting in a diverse range of environments. The photosynthetically active radiation (PAR) may be less than 10% compared with open areas (Chazdon and Fetcher 1984), while the humidity generally increases from the top of the canopy to the forest floor (Kenzo et al. 2012). Furthermore, the light environment may be very variable as flecks of light move with the diurnal course of the sun and as the movement of branches by wind causes alteration of shade and sun (Allen and Pearcy 2000). It can be speculated that in plants adapted to conditions of high humidity and variable light in the understory, stomata would tend to be open because fast assimilation of CO_2 would be more important than loss of water. On the other hand, under open conditions in the top canopy or in the gaps, species adapted to high light and more variable humidity conditions would need to react faster to changes in humidity, thus possessing smaller and more dynamic stomata. Besides, increasing stomatal densities from the bottom to the top of the tree may indicate an acclimation to a changed light and humidity climate (Ashton et al. 1998, Fanourakis et al. 2013, Ichie et al. 2016). However, knowledge about stomatal dynamics of species adapted to different successional situations and light climate is very limited. In this paper we investigate 11 rainforest species representing different successional situations to investigate this hypothesis. Specifically, we attempt to answer the following questions: (i) Is the speed of stomatal opening correlated with the size of stomata across a broad range of species? (ii) Will acclimation to light result in differing stomatal anatomy and physiology, leading to faster opening of stomata? (iii) Are differences in stomatal parameters related to growth of the trees?

Materials and methods

Plant material and study site

The trial comprised of 11 tree species (Table 1). Nine of the species were selected from species grown in the nursery of Harapan Rainforest, Sumatra, intended to be used as framework species in restoration projects in the forest. Although the autecology of many of the species is unknown, they were believed to represent species from the later successional stages in rainforest regeneration. These seedlings were collected in 2013 and 2014 as wildlings with heights about 20 to 30 cm in the surrounding lowland rainforest, located at 2°08′S and 103°23′E and with an elevation of 30–120 m above sea leavel (m.a.s.l.). The annual precipitation is estimated at 2456 mm with an annual average temperature of 26.9 °C (Worldclim estimate, 1950–2000) (Hijmans et al. 2005). Plants were raised in small polybags (15 cm height and 7 cm diameter) filled with a mixture of topsoil and organic fertilizer. The fertilizer contained sawdust, rice husks, manure and compost. On 2 November 2015, 108 plants belonging to the nine species were moved to the experimental site in Padang, located at Department of Biology, University of Andalas Padang, Indonesia (0°54′34′S 100°27′41′E at ~250 m.a.s.l.). An additional two species (*Mallotus paniculatus* and *Macaranga triloba*) were collected in the botanical garden of University of Andalas on 3 November 2015. These species are known locally as fast-growing, early successional pioneer species and are widely distributed across secondary forest in Harapan Rainforest. The plants were collected together with the topsoil. All species were then carefully potted into large polybags (22 cm height and 15 cm diameter) filled with a mixture of topsoil and organic fertilizer. The fertilizer contained husk, manure and other organic materials, and the topsoil was collected from natural forest surrounding the experimental area with small roots and seeds removed. After site preparation, the plants were placed in their position on 8 November 2015. The natural vegetation at the trial site is tropical lowland rainforest, and total rainfall, average temperature and relative humidity during the experiment, from November 2015 to May 2016, was 152 mm, 27 °C and 85%, respectively (no data were recorded in December).

Experimental design

Two light treatments were applied by placing the plants either under full sunlight or under a shading net (reducing the photosynthetic radiation by 70%), and each treatment contained six plants of the 11 species. Hereafter, the two treatments will be referred to as ‘shade’ and ‘sun’. Plants were placed randomly 15 cm apart in the two treatments. The plants were watered daily. Measurements took place in the Herbarium of University of Andalas from 31 March 31 to 18 May 2016.

Table 1. Stomatal morphology of 11 rainforest species as affected by light treatments, and results from analysis of variance of the stomatal parameters as a function of species and light treatments. Numbers indicate mean ± standard deviation. Significant effects of light within species (*t*-tests) are represented by asterisks: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, (*) $P < 0.10$ and 'ns' non-significant. Species names and authors correspond to www.theplantlist.org

Species	Family	Length (μm)	Width (μm)	Stomatal size (μm^2)		Density (mm^{-2})	
				Shade	Sun	Shade	Sun
Early successional species							
Mallotus paniculatus (Lam.) Müll.Arg.	Euphorbiaceae	18 ± 1.3	12 ± 0.9	236 ± 31 (*)	204 ± 16	62 ± 18	52 ± 30
Macaranga triloba (Thunb.) Müll.Arg.[1]	Euphorbiaceae	24 ± 1.5	13 ± 1.1	302 ± 46	334 ± 19	131 ± 45	161 ± 32
Late successional species							
Artocarpus elasticus Reinw. ex Blume	Moraceae	18 ± 1.3	14 ± 1.5	245 ± 34	242 ± 46	371 ± 128	389 ± 108
Palaquium gutta (Hook.) Baill.	Sapotaceae	24 ± 1.1	14 ± 0.8	335 ± 30	354 ± 24	155 ± 17	161 ± 28
Eusideroxylon zwageri Teijsm. & Binn.	Lauraceae	18 ± 1.3	18 ± 0.9	319 ± 31	326 ± 41	167 ± 18	202 ± 73
Hopea mengarawan Miq.	Dipterocarpaceae	20 ± 2	16 ± 1.5	309 ± 56 (*)	366 ± 44	230 ± 27	232 ± 43
Aquilaria malaccensis Lam.	Thymelaeaceae	21 ± 2.2	16 ± 1.6	364 ± 49*	279 ± 48	224 ± 41	232 ± 46
Dyera costulata (Miq.) Hook.f.	Apocynaceae	21 ± 2.7	13 ± 1.7	299 ± 38	262 ± 74	363 ± 98 (*)	486 ± 41
Sterculia oblongata R.Br.	Sterculiaceae	22 ± 1.7	14 ± 1.6	296 ± 56	328 ± 54	137 ± 39	142 ± 36
Shorea leprosula Miq.	Dipterocarpaceae	20 ± 2.3	13 ± 1.7	267 ± 70	255 ± 43	195 ± 56	318 ± 126
Irvingia malayana Oliv. ex A.W.Benn.	Irvingiaceae	17 ± 0.9	18 ± 1.2	314 ± 31	316 ± 28	204 ± 48*	275 ± 42
F-values and significance levels from analysis of variance of (df)							
Species (10)		22.7***	30.2***	9.72***		25.7***	
Light (1)		0.07 ns	0.02 ns	0.01 ns		8.78**	
Species × light (10)		1.79 (*)	1.87 (*)	1.97*		1.29 ns	
Log transformation		No	No	No		Yes	

[1]The species is amphistomatous, and data are for the abaxial side of the leaf.

Data collection

Gas exchange parameters (photosynthesis, transpiration and stomatal conductance) were measured for all plants using a portable photosynthesis system (Ciras 1, PP-systems, Hitchin, Hertfordshire, SG5 1RT, UK) equipped with a broad leaf autocuvette chamber covering a leaf disk of 2.5 cm diameter. Temperature in the cuvette was set to 30 °C and CO_2 concentration was maintained at 400 ppm. The vapor pressure deficit was kept at ~2.4 kPa, which was a typical daytime value in the experimental station. Plants were placed in a dark room for one night before measurements and were moved quickly in almost darkness to the cuvette chamber to avoid stomatal opening by external light. The youngest fully developed leaf was selected. All measured leaves had developed while they were in the sun and shade treatments. Leaves were kept in the dark cuvette chamber for ~30 min before the light (PAR of 800 μmol $m^{-2}s^{-1}$) was turned on. Data were recorded automatically every 60 s to describe gas exchange in the transition from darkness to light. The setup was monitored every 15 min and the light was switched off when the stomatal conductance reached a steady and high level, typically after 20–60 min of light. The leaf area covered by the cuvette chamber was marked and used for sampling of stomatal size and density. Due to the fragility of our old equipment and the need to achieve the desired number of replicates of each species, measurements were conducted species by species except where errors in the datasets or equipment failure made the reassessment of certain plants necessary.

Parameters describing the development of stomatal conductance were calculated based on the records of gas exchange over time (Drake et al. 2013). The average of four records immediately before the light was turned on constituted dark conductance ($g_{s\text{-}dark}$), while the average of four sequential values at the highest level attained in the light constituted the operating maximum stomatal conductance ($g_{s\text{-}op}$). The increasing stomatal conductance with time (after the light was turned on) was modeled by a third degree polynomial regression, and the slope of the steepest part indicated the maximal speed of stomatal opening (speed of opening). The change in stomatal conductance was expressed per second, and the resulting unit was micromole m^{-2} s^{-2}. The $g_{s\text{-}50\%}$ was calculated by the following formula: $g_{s\text{-}50\%} = (g_{s\text{-}op} + g_{s\text{-}dark})/2$, and the time to reach $g_{s\text{-}50\%}$ from the light was turned on was determined ($T\text{-}_{50\%}$).

Assessments of stomata were conducted on both leaf surfaces of the selected and marked leaves by using nail polish imprints. The sampling took place on the 25 and 26 May 2016 after all measurements of stomatal conductance were completed. Sizes of stomata were measured under a light microscope (Carl Zeiss MicroImaging GmbH 37,081, Göttingen, Germany, equipped with an AxioCam ERc 5 s, MKG 1175 camera and the software AxioVisio 4.8.2 for measuring) at magnification 400×. On each plant, the length and width of guard cells of five randomly selected stomata were measured under a field of view of 272 μm × 204 μm or 55 488 $μm^2$, and their size was approximated as the product of length and width ($μm^2$). Furthermore, stomatal densities were recorded on three plants of each species and light treatment, based on counts at magnification 100× representing a field of view of 0.875 mm^2. All measurements were conducted at the Department of Biology in Padang Indonesia.

Finally, to be able to correlate stomatal parameters and gas exchange parameters to species ecology, we used data from two trials in Harapan Rainforest (R.Kardiman, A. Ræbild, T. Swinfield, L. H. Schmidt, R. Afriandi, unpublished). In the first trial, eight of the late successional species were planted in a degraded forest area, with microhabitats ranging from open areas dominated by grasses to shaded habitats under secondary forest cover. The design was a randomized block design with species randomized within blocks. Light availability at each planting site was defined as shade for sites with less than 20% canopy openness and open with more than 20% canopy openness, determined from visual inspection of the density of vegetation/open sky in the 45° cone above each plant. The average height growth per year of surviving plants was calculated based on height recorded at planting and 2 years after planting. For this analysis, we use only the six species where the number of surviving plants was more than four in each category of light. In the second trial, seedlings of six of the late successional species (from the same batch) were grown in a nursery under open sky or a 70% shade net for 18 months. At the end of the trial, 10 randomly selected plants from each combination of species and light environment were harvested, and the final total biomass was recorded after drying at 75 °C for a minimum of 3 days.

Statistical analysis

Stomatal and gas exchange traits were analyzed using a General Linear Model (GLM) with two way interactions between species (11 levels) and light treatment (two levels), using the statistical software R version 3.3.3 (R Development Core Team 2017). The validity of the statistical models was evaluated through residuals plots, q-q plots, histograms of the residuals and the Shapiro–Wilk test. Logarithmic transformation was applied for stomatal density, speed of opening and $T\text{-}_{50\%}$. Tests were considered significant when the critical values (P) were less than 0.05, and marginally significant when P was less than 0.10. Where results indicated significant effects of light treatment or interactions between light and species, differences within species were analyzed with t-tests. Associations between anatomical traits, physiological measurements and growth in the two associated trials were analyzed using mean values across species and treatments. We applied analyses of co-variance with the dependent variables (e.g., growth) tested against the effect of light environment and its interaction with the independent variables (e.g., stomatal speed).

Since the plants were measured species by species, there is a possible confoundation between species and the time of measurement. The effect of this is likely to be small as anatomical traits tend to change little over short time periods. Furthermore, during the two and half months when the measurements took place, the weather conditions were relatively stable, making changes in the physiology of trees less likely. In any case, in the analyses of associations between stomatal characters, physiological traits and growth, such confoundation will add to the residual error and will not bias conclusions.

Results

All anatomical parameters of stomata were significantly different between species. While a significant effect of light was found for stomatal density, stomatal size showed significant interactions between species and light. For length and width of guard cells, these interactions were marginally significant (Table 1). Length of guard cells ranged from 17 to 24 μm, and width of guard cells ranged from 12 to 18 μm between species. The variation in guard cell form resulted in circular stomata in *Eusideroxylon zwageri* and *Irvingia malayana*, while other species tended to have elliptic stomata (Table 1). With respect to size, the range was from 236 to 364 μm^2 in the shade, and from 204 to 366 μm^2 in the sun. Within-species differences between shade and sun were significant only in *Aquilaria malaccensis*, where stomata in the shade were larger than in the sun. Stomatal density varied more than the other anatomical parameters and ranged between 62 and 371 mm^{-2} in the shade and between 52 and 486 mm^{-2} in the sun. The significant effect of light indicated that the stomatal density tended to increase in the sun. Within species, large differences of stomatal density between shade and sun were found in *I. malayana* and *Dyera costulata*. *Macaranga triloba* was the only species with stomata on both leaf surfaces. The average size on the upper surface was 260 μm^2 and on the lower surface it was 318 μm^2. The average number of stomata on the upper surface was 17 mm^{-2}, and on this side of the leaf there was no effect of light on the size and density of stomata. Compared with the densities on the lower sides (122 and 149 mm^{-2} for shade and sun plants, respectively), this corresponds to ratios between adaxial and abaxial densities of 0.11–0.14.

All gas exchange parameters were significantly different between species, and all parameters except $g_{s\text{-}op}$ had a significant interaction between species and light (Table 2). $g_{s\text{-}dark}$ ranged from 31 to 84 mmol m^{-2} s^{-1} in shade plants and in sun plants from 47 to 88 mmol m^{-2} s^{-1}. Within species, significant light effects were found in *A. malaccensis*. There was only a small differentiation in $g_{s\text{-}op}$ between species, which ranged from 127 to 152 mmol m^{-2} s^{-1}. However, speed of stomatal opening varied considerably and ranged from 0.06 to 0.19 mmol m^{-2} s^{-2} in the shade, while in the sun it ranged from 0.06 to 0.26 mmol m^{-2} s^{-2}. Three species were significantly affected by light, as *M. triloba* opened faster in shade than in the sun, whereas *Palaquium gutta* and *A. malaccensis* opened two times faster in the sun than in the shade. Also, $T\text{-}_{50\%}$ showed a large variation and ranged from 5 to 15 min for plants in the shade and from 5 to 17 min for plants in the sun (Table 2). The same three species that were significantly different with respect to speed of opening were also significantly different in $T\text{-}_{50\%}$, with fast opening indicated by short $T\text{-}_{50\%}$. The two included pioneer species were strongly different in stomata and gas exchange parameters, with small-sized stomata and fast stomatal opening in *M. paniculatus* and larger, slow-opening stomata in *M. triloba* (Tables 1 and 2). This also means that there were no significant differences between the group of late successional species and the two pioneers considered as a group (not shown).

Analyses of co-variance showed that smaller stomata opened faster than larger stomata (Figure 1A). There was a significant interaction between light and stomatal size ($F = 9.51$, P-value = 0.02), indicating that the light treatment affected the slope of this relation. In the same way $T\text{-}_{50\%}$ increased with the size of stomata (Figure 1B), but here the interaction between light and stomatal size was only marginally significant ($F = 3.68$, P-value = 0.09). Large stomata hence tended to open more slowly in the shade than in the sun (Figure 1).

Stomatal size and density were not correlated ($R^2 = 0.02$, P-value = 0.69), but the pioneer species *M. paniculatus* showed an outlier tendency by having small stomata and lower density compared with the rest of the species. When this species was excluded, the density and size were negatively correlated ($R^2 = 0.53$, P-value = 0.017, Figure 2A). As use of stomatal imprints may lead to underestimation of the true size of stomata if epidermal cells overlap with guard cells, we made cross-sections of leaves of *M. paniculatus.* These sections showed that the guard cells were fully exposed and the outlier tendency in this species is unexplained. Finally, $g_{s\text{-}op}$ showed a vague association with speed of opening ($R^2 = 0.28$, P-value = 0.092, Figure 2B), which was driven by the outlier tendency of *M. paniculatus.*

Differences between the annual height growth of the six species tested in Harapan Rainforest were highly significant and showed significant interactions with the light environment. Likewise, there were highly significant differences in dry weight of biomass between the six species in the shade/sun trial, and dry weight was significantly larger under shaded than under full sun conditions (Table 2). Height growth was not related to any of the stomatal or gas exchange parameters. However, biomass showed a significant association with $g_{s\text{-}dark}$, $g_{s\text{-}op}$ and speed of opening (Table 3, Figure 3), generally being larger for species with larger gas exchange and faster speed of opening. Furthermore the light environment had a significant influence, biomass being larger under shaded conditions.

Table 2. Gas exchange parameters of 11 rainforest species as affected by light treatments, coupled with height growth in a field trial, and biomass accumulation in a trial with light/shade (see Materials and methods). The lower part of the table shows results from analysis of variance of the parameters as a function of species and light treatments. Numbers indicate mean ± standard deviation. Significant effects of light within species (*t*-test) are represented by asterisks: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, (*) $P < 0.10$ and 'ns' non-significant.

Species	$g_{s\text{-}dark}$ (mmol m^{-2} s^{-1})		$g_{s\text{-}op}$ (mmol m^{-2} s^{-1})	Speed of opening (mmol m^{-2} s^{-2})		T-$_{50\%}$ (min)		Height growth rate (m $year^{-1}$)		Total biomass (g dry weight)	
	Shade	Sun		Shade	Sun	Shade	Sun	Shade	Sun	Shade	Sun
Early successional species											
Mallotus paniculatus	31 ± 5	49 ± 23	148 ± 19	0.19 ± 0.08	0.26 ± 0.11	7 ± 2.6(*)	5 ± 1.5	n.a	n.a	n.a	n.a
Macaranga triloba	65 ± 18	60 ± 7	138 ± 10	0.10 ± 0.04*	0.06 ± 0.02	8 ± 3.6*	14 ± 3.5	n.a	n.a	n.a	n.a
Late successional species											
Artocarpus elasticus	84 ± 11	84 ± 8	144 ± 24	0.15 ± 0.04(*)	0.11 ± 0.05	5 ± 1.8	6 ± 1.6	n.a	n.a	30.6 ± 16*	18.5 ± 12
Palaquium gutta	79 ± 19	73 ± 11	152 ± 19	0.06 ± 0.04*	0.10 ± 0.03	14 ± 5.9*	9 ± 1.4	n.a	n.a	n.a	n.a
Eusideroxylon zwageri	35 ± 8	47 ± 10	124 ± 22	0.06 ± 0.02	0.08 ± 0.06	12 ± 5.3	12 ± 5.1	0.09 ± 0.02	0.10 ± 0.03	12.3 ± 6*	7.9 ± 6
Hopea mengarawan	40 ± 7	48 ± 10	129 ± 16	0.06 ± 0.02	0.06 ± 0.01	15 ± 3.4	17 ± 3.4	0.13 ± 0.04*	0.23 ± 0.03	16.1 ± 6.8*	12.2 ± 12
Aquilaria malaccensis	57 ± 12*	76 ± 14	131 ± 23	0.06 ± 0.02*	0.12 ± 0.04	15 ± 5.9*	5 ± 2.3	0.20 ± 0.07	0.20 ± 0.06	26.5 ± 17(*)	19.1 ± 13
Dyera costulata	67 ± 10	58 ± 15	132 ± 19	0.10 ± 0.04	0.11 ± 0.05	9 ± 2.5	9 ± 4.9	0.15 ± 0.12*	0.21 ± 0.10	29.5 ± 16*	19.5 ± 15
Sterculia oblongata	78 ± 14	65 ± 16	140 ± 12	0.13 ± 0.07	0.12 ± 0.04	8 ± 1.8	8 ± 1.5	0.11 ± 0.02*	0.25 ± 0.10	33.6 ± 22*	21.7 ± 13
Shorea leprosula	48 ± 21	64 ± 14	138 ± 24	0.09 ± 0.04	0.07 ± 0.04	13 ± 4.4	11 ± 2.4	n.a	n.a	n.a	n.a
Irvingia malayana	74 ± 17	88 ± 13	137 ± 24	0.07 ± 0.03	0.07 ± 0.04	10 ± 3.9	9 ± 2	0.11 ± 0.03*	0.19 ± 0.08	n.a	n.a
F-values and significance levels from analysis of variance (df)											
Species (10)[1]	13.9***		1.94*	7.46***		8.69***		12.6***		13.7***	
Light (1)	3.58 (*)		0.03 ns	0.07 ns		2.54 ns		30.9***		43.9***	
Species × light (10)[1]	2.11*		1.09 ns	2.18*		4.92***		3.26**		0.39 ns	
Log transformation	No		No	Yes		Yes		Yes		Yes	

[1]Degrees of freedom for the variables height and growth were 5.

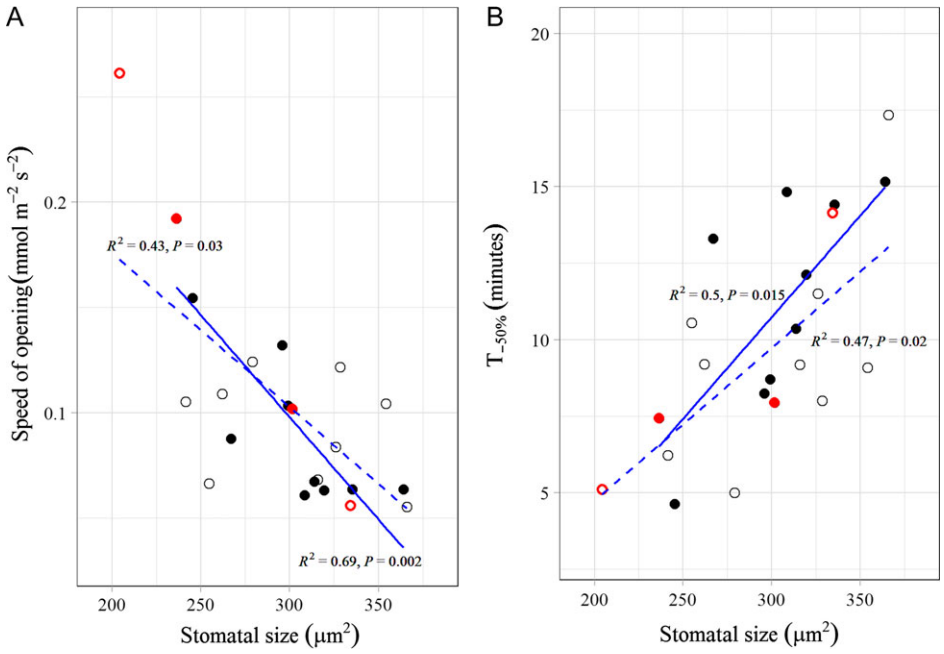

Figure 1. The relationship between stomatal size and speed of opening (A) and T-$_{50\%}$ (B) across species. Closed circles and the solid line indicate the shade plants, while open circles and the dashed line indicate sun plants. Each point represents the mean of a species. Note the logarithmic scales on the *Y*-axis. Symbols in red represent the two early successional species.

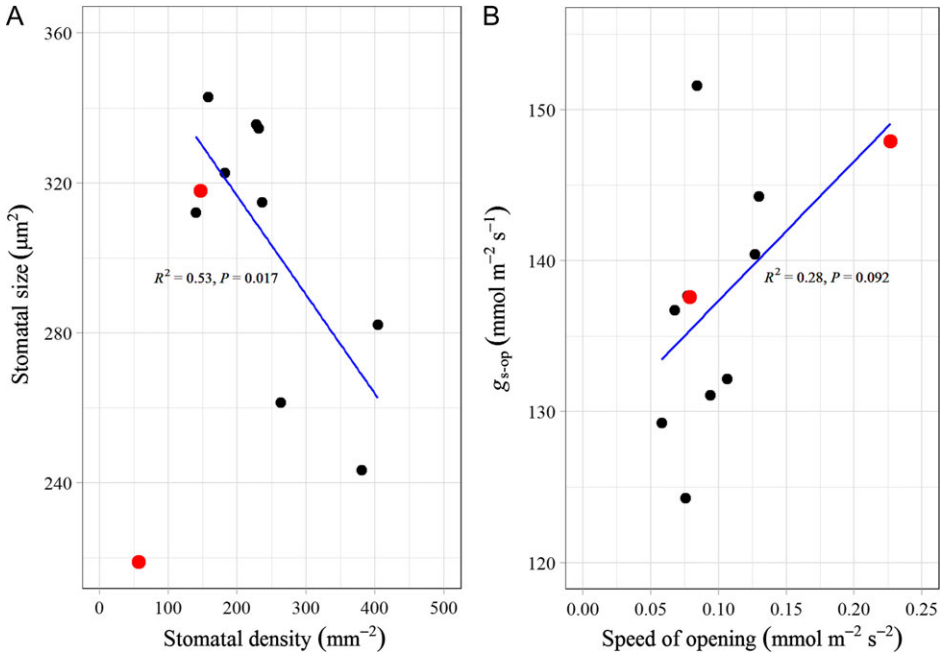

Figure 2. The relationships between stomatal density and stomatal size (A), and between speed of opening and $g_{s\text{-}op}$ across species (B). Each point represents the mean of a species. Symbols in red represent the two pioneer species. In (A), one species (*M. paniculatus*) was excluded from the correlation analysis (red symbol at the lower left part of the graph).

Discussion

Association between stomatal anatomy and dynamics

Our results confirmed a relation between size of stomata and the speed of opening as previously observed by Drake et al. (2013). Although the variation in stomatal size among the 11 species was relatively small and the smallest stomata were only 35–45% smaller than the largest, small stomata opened three to four times faster than large stomata (Figure 1). This was

also reflected in $T_{-50\%}$, which was reached three times faster for small than for large stomata. Whereas the study of Drake et al. (2013) was based on five species within the same genus (*Banksia*) occupying different ecological niches along a water availability gradient, our study was based on 11 species covering several families and genera, sharing the same habitat. Hence, there is reason to believe that the association between stomatal size and the speed of opening applies more generally. Similar relationships seem to apply within species, as studies of transpiration following leaf detachment in *Rosa hybrid* and *Solanum* introgression lines have shown that varieties and lines with small stomata tend to be more responsive than varieties and lines with large stomata (Giday et al. 2013, Fanourakis et al. 2014). Conversely, Elliott-Kingston et al. (2016) found no correlation between stomatal pore length and stomata responsiveness in a study of widely ranging taxa. However, while pore length may be an appropriate proxy for size in closely related plants where the dimensions of stomata scale with the pore lengths, it is likely less robust when species with different shapes of stomata are compared. If the limiting factor for stomatal speed of opening is the rate of change in osmotic potential in the guard cells (Raven 2014), volume or a related factor such as projected area (as in our case) are likely to be better proxies.

Table 3. Result from analysis of co-variance of biomass in response to physiological traits of six late successional species. Numbers in parenthesis indicate the numbers of degrees of freedom.

Effects	*F*-value	*P*-value
Stomatal conductance in the dark		
$g_{s\text{-}dark}$ (1)	24.41	0.001
Light (1)	19.0	0.002
$g_{s\text{-}dark}$ × light (1)	1.42	0.27
Operating stomatal conductance		
$g_{s\text{-}op}$ (1)	17.04	0.003
Light (1)	5.70	0.04
$g_{s\text{-}op}$ × light (1)	3.79	0.09
Speed of opening		
Speed (1)	11.76	0.009
Light (1)	11.64	0.009
Speed × light (1)	0.03	0.87

In angiosperms, stomatal opening appears to involve a displacement of the subsidiary cells. This has consequences for the speed of opening, as lycopod and fern species without subsidiary cells had less responsive stomata compared with a dicot and a grass, despite having close-to-similar sizes of stomata (Franks and Farquhar 2007). Likewise, Elliott-Kingston et al. (2016) found that stomatal closure was faster in taxa that diversified recently and under low atmospheric CO_2, and it is well known that grasses with their dumbbell-shaped stomata are more responsive than dicots (Vico et al. 2011). Generalizations beyond the group of angiosperm trees that we studied should therefore be treated with care.

Effect of light

The light treatment influenced both stomatal size and density, and our results show that the relationship between size and speed of stomatal opening also varied with the light climate, as the stomatal size had a larger influence on the speed in plants grown in shade compared with sun plants (Figure 1). In the absence of studies on this topic we speculate that this may be caused by changed stomatal micromorphology or biochemistry

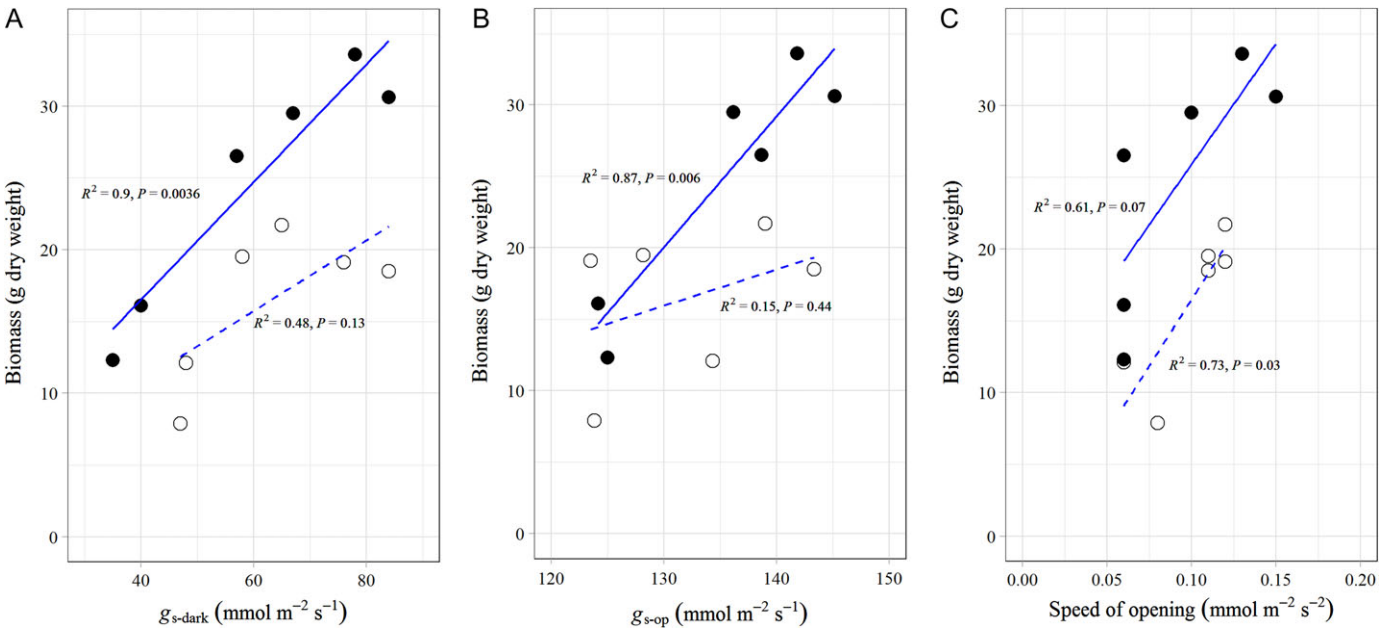

Figure 3. The relationships between dry biomass and $g_{s\text{-}dark}$ (A), $g_{s\text{-}op}$ (B) and speed of opening (C). Each point represents the mean of a species. Closed and open circles represent shade and open treatments, respectively.

in response to light conditions. Possible candidates could be changed activities of enzymes responsible for building up high levels of osmotic potential or changes in the concentrations of substrates needed for this (Raven 2014). While stomatal density generally was higher in plants in the sun compared with shade plants, some species had larger and some species smaller stomata in response to the light treatment (Table 1). Other studies have shown that light significantly increased stomatal density (Luken et al. 1995, Onwueme and Johnston 2000, Matos et al. 2009). In a range of temperate angiosperm trees, stomatal densities increased in open compared with shade conditions, but changes in guard cells length were both positive and negative (Abrams and Kubiske 1990). If the correlation between stomatal size and speed of opening applies generally, this suggests that in at least some species, increasing the speed of stomatal opening in response to light is not part of the acclimation strategy. In *A. malaccensis*, for example, speed of opening was highest in the light treatment, while in *M. triloba* it was lower, compared with the shaded plants. On the other hand, the values of $g_{s\text{-}dark}$ were high, varying between 21% and 63% of $g_{s\text{-}op}$, showing that even in darkness, stomata remain open. This would allow for immediate photosynthesis when light flecks appear on the leaves and lessen the need for (costly) stomatal adjustment. In humid rainforest environments, where risks of drought are small, this may help in achieving higher rates of photosynthesis.

Relations between growth, stomatal anatomy and gas exchange

A study on several rainforest tree species showed that growth positively correlated with stomatal conductance (Poorter and Bongers 2006). We found a similar association as species with higher $g_{s\text{-}op}$ also had larger accumulation of dry biomass (Figure 3B). This was observed despite a small variation in $g_{s\text{-}op}$ compared with other studies in rainforests, where much larger ranges of g_s were observed (e.g., Reich et al. 1999, Poorter and Bongers 2006). Interestingly, we also found associations between $g_{s\text{-}dark}$, speed of opening and the dry mass biomass (Figure 3A and C). In the small selection of species that we investigated, $g_{s\text{-}dark}$, $g_{s\text{-}op}$ and speed of opening were highly correlated, making it difficult to say whether large stomatal conductance, dynamic stomata or both were decisive for attaining high rates of primary production. Collectively, however, the findings support that under the well-watered condition, large stomatal conductance and fast opening leads to high rates of biomass accumulation.

On the other hand, height growth was not related to any of the anatomical or gas exchange parameters, giving no support for the hypothesis that species with fast height growth should possess smaller or faster stomata to facilitate life in open environments. Admittedly, our measurements of height did not include the two early-successional species with expected fast height growth, but these two species (*M. triloba* and *M. paniculatus*) differed widely in their stomatal morphology and physiology, suggesting different adaptive strategies in the species.

It is possible that since the investigated species potentially grow into big trees, their morphology may be adapted to conditions in the top of the canopy, rather than to the understory. On the other hand, Naumburg and Ellsworth (2000) found no evidence that shade-tolerant species had faster changes in stomatal conductance than shade-intolerant species, and their extensive review revealed no general tendencies in the literature. Hence, while within certain taxa there may be an association between induction time of stomata (as observed by Montgomery and Givnish 2008) and ecological niches of the species, it seems that across unrelated species, this is blurred by other factors that influence adaptation to shade.

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Conflict of interest

None declared.

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