

1 Changes in forest structure along an elevational gradient in the
2 Peruvian Andes cause species-specific stress responses in tree
3 seedlings

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6 **Abstract**

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- 8 We assessed the contribution of biotic competition factors to limiting elevational range
9 shifts of tree species along an Amazon to Andes elevational gradient, focussing on tree
10 seedlings as a key demographic bottleneck for future recruitment.
 - 11 Photosynthetic capacity measured using chlorophyll fluorescence estimated photosynthetic
12 stress experienced by naturally occurring seedlings of seven tree species spanning the el-
13 evational gradient. Physiognomic plant traits were also measured to assess the degree of
14 local acclimatory response to elevationally dependent environmental factors.
 - 15 We used linear mixed effects models to compare the effect sizes of individual biotic compe-
16 tition fixed effects against that of elevation. A matrix of multiple fixed effect mixed effects
17 models were compared statistically to ascertain the best combination of predictors affecting
18 seedling growth and stress metrics.
 - 19 •

20 **1 Introduction**

21 Rapid anthropogenic climate change is causing many species, across a wide range of taxa, to shift
22 their distributions in space (Chen et al., 2011; Hughes, 2000; Parmesan, 2006). The primary forces
23 driving this are an increase in temperature and changes in precipitation regime (Corlett and West-
24 cott, 2013; McCain and Colwell, 2011). Chen et al. (2011) estimates that globally, across a range
25 of taxonomic groups, species are experiencing mean latitudinal and altitudinal migration rates of
26 17.6 ± 2.9 km and 12.2 ± 1.8 m per decade, respectively. Previous studies have suggested that the
27 ability of species to respond to changes in mean annual temperature and precipitation regime will
28 be important in determining species success over the coming century (Colwell et al., 2008; Chen
29 et al., 2011; Feeley et al., 2012).

30 Species responses to climate change may occur either in the form of adaptation, *i.e.* changes
31 in phenology, physiology and morphology, or through range shifts over space (Bellard et al., 2012).
32 Range shifts have been observed in many studies across the world, particularly in temperate, sub-
33 arctic and mountainous regions where temperature change is the most extreme (Lenoir and Sven-
34 ning, 2015). The number of studies documenting adaptational responses are fewer, potentially
35 indicating that climate change is occurring so rapidly as to prevent effective adaptational responses

36 (Mantyka et al., 2012). Range shift rates vary between species depending on their sensitivity to cli-
37 mate and their fecundity, which affects rate of recruitment into newly suitable areas (MacLean and
38 Beissinger, 2017; Travis et al., 2013). This has the potential to create novel species assemblages as
39 species ranges begin to overlap more or less as they shift, with unknown consequences for ecosystem
40 functionality. Predicting range shifts across space has become an active field of research, (see Bell-
41 lard et al. 2012 and references therein), and is being used as a tool to inform conservation strategies
42 to mitigate the effects of climate change on biodiversity and ecosystem functionality (Dawson et al.,
43 2011).

44 The majority of species distribution models used to predict species range shifts as a conservation
45 tool have used bioclimatic envelopes to constrain species' ranges (Pearson and Dawson, 2003; Sinclair
46 et al., 2010). Bioclimatic envelopes are constructed by correlating current species range extent
47 with observed environmental conditions within those boundaries, then projecting spatially explicit
48 climate trends into the future under different climate change scenarios to predict how species range
49 boundaries will adjust in response (e.g. Araújo et al. 2006; Berry et al. 2002; Peterson et al. 2002;
50 Thuiller et al. 2005). These models have been criticised often for being overly simplistic, especially
51 when applied at the local scale (McMahon et al., 2011), where other factors that have not been
52 considered by the bioclimatic envelope model become important limiting factors for range shifts.
53 Such factors include unmeasured environmental variables, physical factors such as topography, and
54 biotic interactions with other species (Davis et al., 1998; Ettinger et al., 2011; Van der Putten et al.,
55 2010). In montane environments, range shifts do not consistently follow an expected upslope trend,
56 with ~25% of species showing a downslope movement and ~10% showing no movement (Lenoir
57 et al., 2010).

58 When range shifts in a rapidly changing climate are driven by a single environmental variable like
59 mean annual temperature, it is possible that a species will move into an area that is sub-optimal in
60 other ways than those predicted by the model if range shifts outstrip acclimatory/adaptive potential.
61 Range shifts into sub-optimal habitats may lead to reductions in local species abundance and/or
62 richness (Colwell et al., 2008), changes in community composition (Gibson-Reinemer et al., 2015),
63 ecosystem functioning (Bellard et al., 2012), and ecosystem service provision that are not predicted
64 by bioclimatic envelope models (Dobson et al., 2006; Isbell et al., 2011). In order to accurately
65 predict range shifts and their consequences for future ecosystem assembly, it is important that
66 predictive range models be expanded to include variables which describe habitat as well as climate
67 (Wisz et al., 2013).

68 For sessile taxa such as trees, range shifts occur as a result of differential recruitment and mor-
69 tality over space, at the leading and trailing edges of their range (Corlett and Westcott, 2013). In
70 communities of long-lived tree species however, the forest ecosystem may not shift in equilibrium
71 with the climate as individuals are resilient to gradual changes in climate, developing large root
72 systems and below-ground water and nutrient reserves to buffer against stressful conditions; adult
73 trees may persist where more sensitive seedlings perish (Bell et al., 2014; Lenoir et al., 2009). As
74 tree seedlings recruit upslope into areas that are newly suitable in terms of temperature, they will
75 encounter novel biotic environments consisting of canopy trees which first recruited into the area
76 when the climate was different. Forest trees, particularly those in moist tropical forests, often ex-
77 perience high levels of mortality during the seedling recruitment stage, creating a key demographic
78 bottleneck that can impact a species' success, potentially limiting upslope migration (Coomes and
79 Grubb, 2000). Seedling growth is affected by shade regimes created by adult tree canopy gaps.
80 There is abundant evidence that shading from adult tree canopies reduces seedling growth rate and
81 thus increases the probability of seedling mortality, with tropical forest tree seedlings frequently
82 growing into canopy gaps (Valladares et al., 2016). Additionally however, seedlings of many trop-
83 ical tree species are highly adapted to shade (Matsuraba et al., 2009), meaning that if a seedling
84 germinates under an open space in the canopy, especially in the tropics at a higher elevation, where
85 UV-B intensity is higher, mortality by UV-B and heat damage to photosynthetic machinery is quite

probable (Krause et al., 2001; Li et al., 2010). Many species found at high altitudes have specific adaptations to avoid UV-B damage to photosynthetic machinery, such as vertically stacked palisade mesophyll cells and thick cuticles to reduce UV-B absorption, and generally smaller thicker leaves (Prado et al., 2012). Species found at low altitudes however, are less adapted to high UV-B environments, instead possessing adaptations to make the most of the diminished light levels found under thick tree canopy, particularly during the seedling growth stage. If seedlings germinate in areas that have a different overstorey shade regime and forest structural type to that which they are adapted to grow in, damage may occur leading to loss of photosynthetic capacity, reducing growth rates and occasionally resulting in seedling mortality.

Montane forest physical structure also varies with elevation. Lowland forests often have lower tree density, with relatively few young trees in the light-deprived understorey, but a higher canopy cover due to adult trees being larger. Plant ground cover is generally greater at higher altitudes, with many epiphytic and ground-level herbaceous species (Martin et al., 2010). Tree seedlings moving upslope may also therefore compete with existing trees and herbaceous flora for nutrients and rootspace, although there is some separation between seedling and adult tree rooting depths for most species (Lewis and Tanner, 2000), especially for the largest trees. These factors acting as limitations to upslope migration of tree species in tropical montane forests may lead to species' ranges narrowing from the bottom up, with increased mortality due to temperature at the bottom of the elevational range, but without increased recruitment at the top end of the elevational range. This seedling mortality bottleneck provides a limiting factor to the success of tropical forest tree species experiencing range shifts and raises concern for their conservation as keystone species of these highly biodiverse ecosystems.

108 Talk more about the peculiarities and importance of studying the cloud forest elevational gradient

In this study, along a moist tropical forest elevational gradient in the Peruvian Andes, we investigated the role of biotic effects from existing forest structure on the physiology and physiognomy of tree seedlings across their elevational ranges, in order to increase our knowledge of the dynamics of montane cloud forest tree species elevational range shifts. We tested three hypotheses: 1) Within a species, seedlings growing at higher elevations would experience higher levels of photosynthetic stress than those at lower elevations, 2) Species would differ in their degree of acclimation to variation in adult tree forest structure, and 3) A combination of biotic and abiotic explanatory variables would best explain variation in seedling physiognomic and physiological traits across their elevational range.

118 2 Materials and Methods

119 2.1 Study Site

120 Data collection was conducted across 10 permanent 1 ha forest plots in the Kosñipata Valley of
121 Manú National Park, Peru (-13°N, -71°W, Figure 1, Table 1). The Kosñipata Valley has been
122 identified as a migration corridor for lowland species to migrate to higher elevations in response
123 to temperature increase (Feeley et al., 2011) and so is an appropriate location to study range shift
124 drivers. Plots are situated between 400 and 3200 m.a.s.l. along this migration corridor (Table 1,
125 Figure 2). The plots form part of a larger plot network established by the Andes Biodiversity and
126 Ecosystem Research Group (ABERG) in 2003 (Malhi et al., 2010; Girardin et al., 2014), and are
127 located within the Tropical Andes biodiversity hotspot identified in Myers et al. (2000). The plots
128 used in this study contain 719 tree species, and the valley as a whole contains an estimated 1167
129 tree species (ABERG unpublished data).

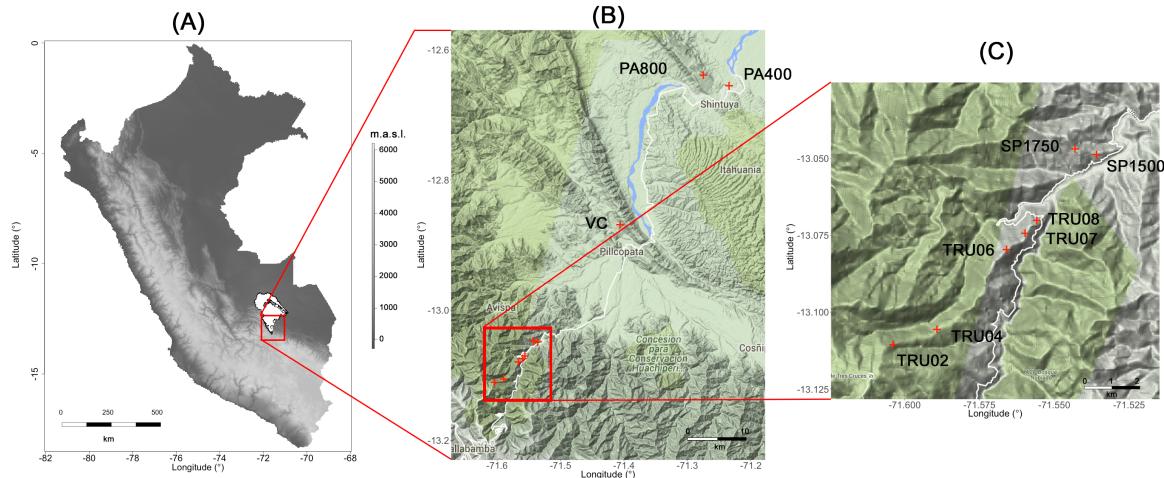


Figure 1: Maps showing the location of the study area and plot locations. (A) The site location within Peru with elevation shading, showing the proximity to Manú National Park (white area). (B) The location of the 1 ha plots within the Kosñipata Valley. (C) An enlargement of the Trocha Union and San Pedro plot groups. Red crosses indicate plot location, white lines in maps (B) and (C) indicate roads, text labels in (B) and (C) are plot codes, dark green areas in (B) and (C) denote the bounds of Manú National Park.

Table 1: Site environmental characteristics for each 1 ha plot sampled. NA indicates that no data was available. Adapted from Whitaker et al. (2014).

Site	Mean elev.	Precip. (mm y^{-1})	Mean temp. ($^{\circ}C$)	Soil C (%)	Soil N (%)	Soil pH	Trees ha^{-1}
PA400	406	NA	NA	NA	NA	NA	475
PA800	822	NA	NA	NA	NA	NA	690
VC	861	3087	20.7	16	1.4	3.8	645
SP1500	1497	2631	17.4	10.5	1	4	860
SP1750	1770	2631	15.8	26	1.8	4.2	887
TRU08	1839	2472	16	31	2	4.3	954
TRU07	2135	1827	NA	37	2.1	4	1060
TRU06	2281	NA	14.9	NA	NA	NA	1101
TRU04	2733	2318	11.1	28.5	1.8	3.9	1287
TRU02	3213	NA	8.9	44.5	2.6	3.8	1417

Add something about the environmental variation within the elevational gradient, presence of cloud zone etc.

2.2 Study species

We chose seven tree species for comparison from a total of 719 identified species within the 10 study plots. Species were selected according to their contrasting ranges (Figure 2), differences in genus migratory pattern (Feeley et al., 2011) (Figure 4), and because each species is dominant across its range in the Kosñipata Valley (ABERG, unpublished data) (Figure 3). Seedlings of *Myrcia* spp. are difficult to reliably identify to species in the field due to similar morphology and were instead sampled as a composite of three potential species: *Myrcia splendens*, *M. fallax*, and *M. rostrata*, the only *Myrcia* species known to be present in our plots from previous ABERG censuses. They

140 are referred to as *Myrcia* spp. from here onwards.

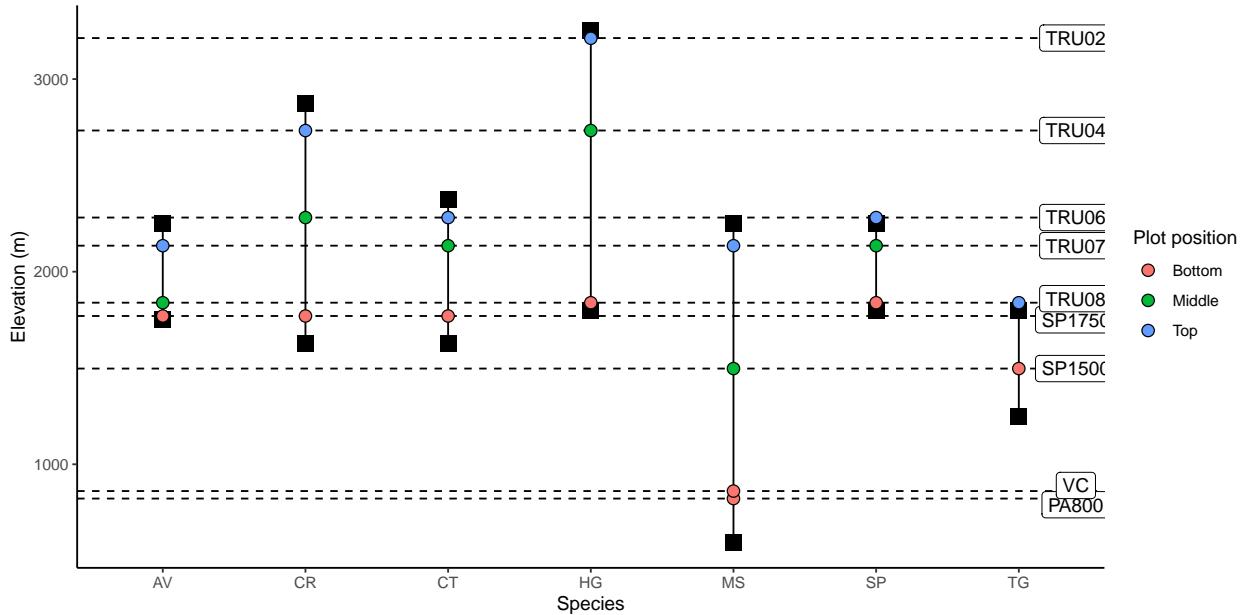


Figure 2: Elevations of study plots for each species (coloured points) with the upper and lower range extents for each species (black squares). Plot elevations are marked as dashed lines and labelled.

Table 2: The sites at which tree seedlings were sampled for each species, with the number of seedlings successfully sampled per site.

Species code	Species	Bottom	Middle	Top
AV	<i>Alzatea verticillata</i>	SP1750 =7	TRU08 =5	TRU07 =6
CR	<i>Clethra revoluta</i>	SP1750 =7	NA	TRU04 =8
CT	<i>Clusia thurifera</i>	SP1750 =9	TRU07 =9	NA
HG	<i>Hedyosmum goudotianum</i>	TRU08 =10	TRU04 =10	TRU02 =11
MS	<i>Myrcia</i> spp.	PA800 =10	SP1500 =8	TRU07 =10
SP	<i>Schefflera patula</i>	TRU08 =9	TRU07 =12	NA
TG	<i>Tapirira guianensis</i>	SP1500 =10	NA	TRU08 =10

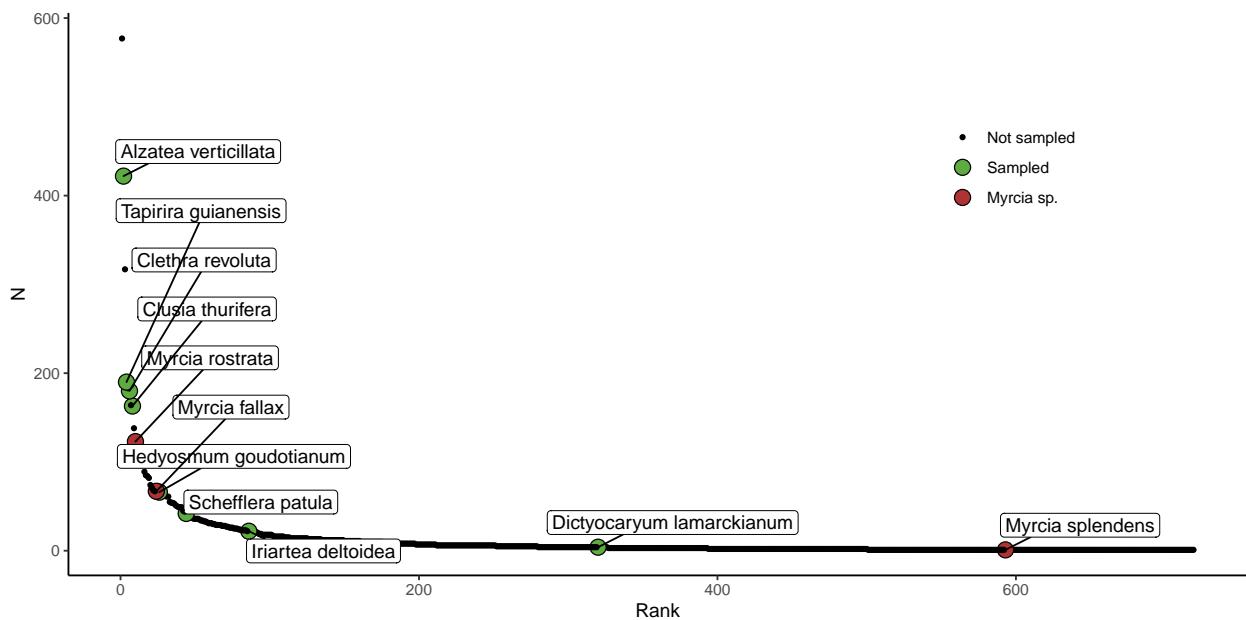


Figure 3: Rank abundance curve of all individuals >10 cm DBH of all species found in the plots measured in this study. Census data from 2014 (ABERG, unpublished data). Species sampled as part of this study are highlighted in red. *Myrcia* species which form the composite *Myrcia* spp. are highlighted in green.

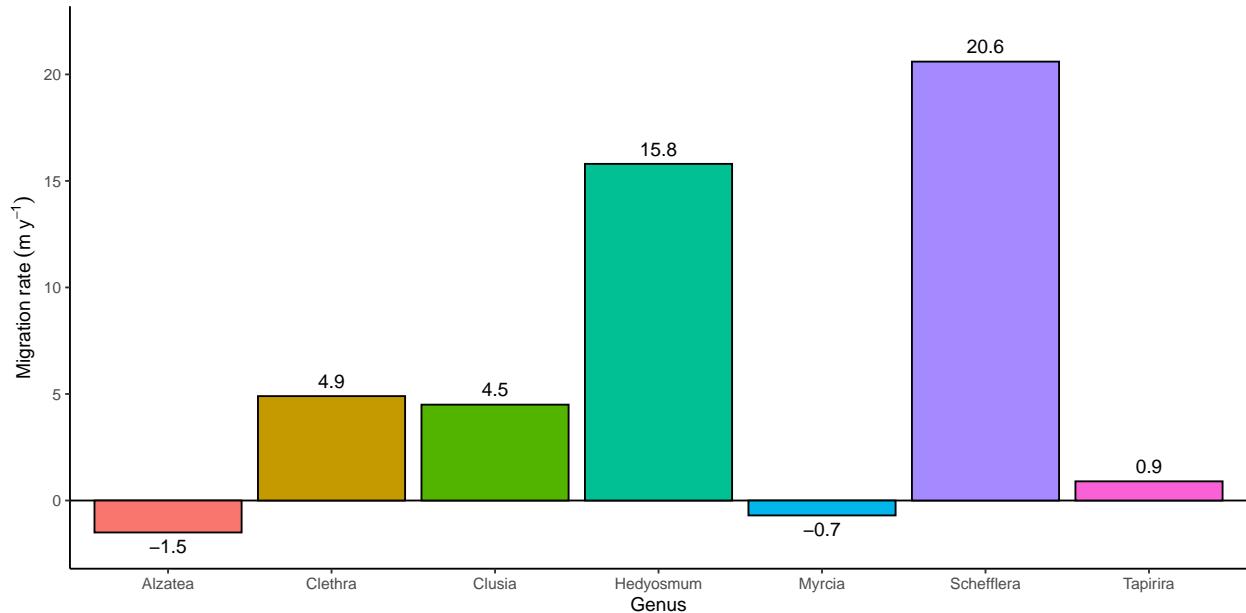


Figure 4: Estimated elevational migration rates within the Kosñipata valley for selected genera of which species are studied here. Migration rates are estimated using shifts in the centre of gravity of tree basal area as measured in the ABERG 1 Ha plot network.

141 2.3 Sampling and Measurement

142 Species were sampled in three plots representing the top, middle and bottom elevational extents of
 143 their ranges (Figure 2). Within each plot, a maximum of 10 seedlings were sampled. To minimise
 144 the chance of pseudo-replication of sampled seedlings, seedlings closer than 10 m to another sampled

seedling were excluded from the analysis, as it could not be guaranteed that the stems were not connected by a stolon or rhizome. It also ensured that competition measurements were truly independent. Within a cluster of seedlings within 5 m of each other, each seedling was assigned a number and a random number generator was used to choose a single seedling for measurement.

Proxies for photosynthetic capacity were measured on the highest fully-expanded leaf of each seedling to assess seedling stress. Seedlings under physiological stress may deactivate or lose chlorophyll photo-centres, lowering photosynthetic capacity. Chlorophyll- α fluorescence was measured to estimate photosynthetic capacity using a Walz Mini-PAM II (Walz Effeltrich, Germany), on a randomly selected area of adaxial leaf surface, avoiding prominent leaf veins. These measurements were used to calculate F_v/F_m according to Genty et al. (1989):

$$F_v/F_m = (F_m - F_o)/F_m \quad (1)$$

Where F_m is the maximal fluorescence in the dark and F_o is the minimal fluorescence in the dark (Maxwell and Johnson, 2000). Fluorescence measurements were taken after exposing the seedling to 30 minutes of total darkness by covering with an opaque black bag, to ensure complete dark adaptation (Campbell et al., 2007). Dark-adapted F_v/F_m measures the photosynthetic capacity of the leaf by relaxing the photo-centres prior to the fluorescence measurement. F_v/F_m is preferable to other chlorophyll fluorescence measures to estimate underlying physiological stress as it removes the noise created by environmental conditions at the time of measurement, instead providing a measure of the underlying photosynthetic capacity. A reduction in F_v/F_m is indicative of plant stress. Here, individuals with F_v/F_m values <0.7 are considered to be experiencing stress (Maxwell and Johnson, 2000).

In addition to F_v/F_m , leaf chlorophyll content was measured using a multi-spectral SPAD-meter (Minolta SPAD-502Plus, Spectrum Technologies, Plainfield, Illinois, USA). To account for variation in chlorophyll content across the leaf (Serrano, 2008), SPAD measurements were taken at three random points on the leaf. The leaf midvein, other prominent veins, and areas of obvious leaf necrosis were avoided in these measurements. The mean of the SPAD values was used to calculate an estimate leaf chlorophyll content using the conversion factor outlined in Coste et al. (2010) for neotropical broadleaf tree species:

$$Chl_{\alpha} = 117.1 \times \frac{\overline{SPAD}}{148.84 - \overline{SPAD}} \quad (2)$$

2.4 Leaf and whole-plant morphological measurements

After leaf physiological measurements, the same upper-most expanded leaf was removed from the seedling and hydrated for a minimum of 48 hours to reverse any leaf curling or contraction in thickness due to dessication. With the petiole removed, each leaf was photographed and the projected lead area was calculated using ImageJ Version 1.51 (Schneider et al., 2012). Mean leaf thickness was calculated using a digital micrometer (0-25 mm, Precision Technologies International, Tamworth, Staffordshire, UL) on three random points on the leaf, avoiding the midvein and prominent leaf veins. To quantify whole-seedling physiognomic characteristics we measured stem width below the lowest set of leaves using the digital micrometer and counted the number of fully expanded leaves (excluding cotyledons). We also measured seedling height from the base of the stem to the tip of the upper-most fully expanded leaf. Stem volume was calculated from stem width and seedling height assuming a cylinder of constant diameter. To account for differences in seedling growth stage and

184 to reduce the number of collinear variables in statistical analyses, stem height and number of leaves
185 was expressed as the ratio of number of leaves per unit stem height.

186 2.5 Competition measurements

187 To assess adult-seedling competition interactions we used two metrics, Leaf Area Index of canopy
188 foliage, and a metric approximating the degree of crowding from surrounding adult trees. Leaf Area
189 Index (LAI) was calculated from hemispherical photographs of the forest canopy above each seedling.
190 Photographs were captured under uniformly overcast cloud conditions to avoid lens flare and to aid
191 in delineation of foliage from sky during processing (Frazer et al., 2001). Images were taken with
192 a Coolpix 4500 compact camera, with a Nikon FC-E8 hemispherical fisheye converter lens. Images
193 were constrained to a 60° circular azimuthal field of view in order to restrict LAI calculations to
194 the part of the sky where the majority of photosynthetically active radiation penetrates the canopy
195 (Jupp et al., 2008; Jonckheere et al., 2004). Images were then converted to 8-bit grayscale and
196 binarized manually in ImageJ 1.51 to separate sky from plant material. Binarized images were then
197 analyzed using Hemiphot (ter Steege, 2018) in R to estimate LAI as the projected leaf area per unit
198 ground area ($\text{m}^2 \text{ m}^{-2}$).

199 To approximate crowding from adult trees, we used an adapted version of the Iterative Hegyi
200 Index (Hegyi, 1974; Lee et al., 2004; Seifert et al., 2014). Our adapted ‘Iterative Seedling Index’
201 (*ISI*) uses adult tree trunk diameter at ~1.3 m from ground level (Diameter at Breast Height,
202 DBH) and the distance of trees from the seedling to calculate an index for each seedling. Higher
203 *ISI* values may result from combinations of greater adult tree DBH and adult trees being closer to
204 the seedling, higher values indicate greater competition pressure from surrounding adult trees:

$$ISI_i = \log\left(\sum_{j=1}^n \left(\frac{1}{DIST_{ij}} D_j\right)\right) \quad (3)$$

205 where D_j is the DBH of a competitor tree and $DIST_{ij}$ is the euclidean distance between seedling
206 i and competitor tree j . *ISI* was log transformed for analysis, as results spanned multiple orders of
207 magnitude. The ‘iterative’ aspect refers to the selection of competitor trees. An iterative selection
208 method for competitive trees assumes that if the path between two trees is blocked by some obstacle,
209 e.g. another tree, the intensity of competition between them will be greatly reduced (Gadow and
210 Hui, 1999). The radius around the seedling is divided into 12 30° sectors, where only the nearest
211 tree >10 cm DBH within each sector is measured (Figure 5). The size of the competition radius
212 (C_R) is defined as:

$$C_R = 2 \times \sqrt{\frac{10000}{N}} \quad (4)$$

213 where N is the number of trees >10 cm DBH per ha (stand density). Stand density data
214 was taken from ABERG census data within each plot (ABERG unpublished data) and used to
215 interpolate the value of C_R for plot VC, for which no stand density data exists. We fitted a linear
216 regression between the elevation and trees ha^{-1} of each plot, and interpolated the trees ha^{-1} of plot
217 VC using the regression fit (Figure 6). C_R was rounded to the nearest metre for ease of measurement
218 (Table 3).

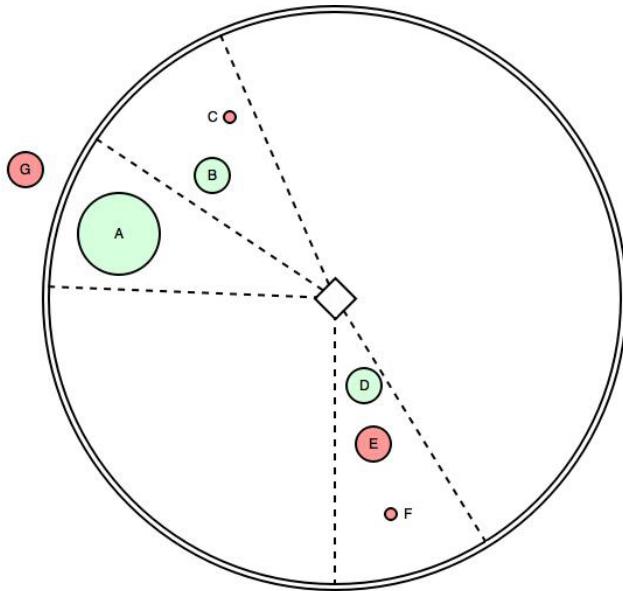


Figure 5: Schematic diagram showing the iterative selection of active competitor trees for the Iterative Seedling Index (ISI) (Equation 3). Trees marked in green (A, B, D) are active competitors for the tree of interest (black diamond). Trees marked in red (C, E, F, G) are non-active competitors, coloured circle radius represents tree DBH. The double circle defines the Competition Radius (C_R) (Table 3, Equation 4). Dashed lines represent 30° zones within which to choose one active competitor. D is the active competitor of its zone as it is the nearest competitor of a suitable DBH (>10 cm). F is not an active competitor as it is <10 cm DBH. G is not an active competitor as it is outside the competition radius. Adapted from Lee et al. (2004).

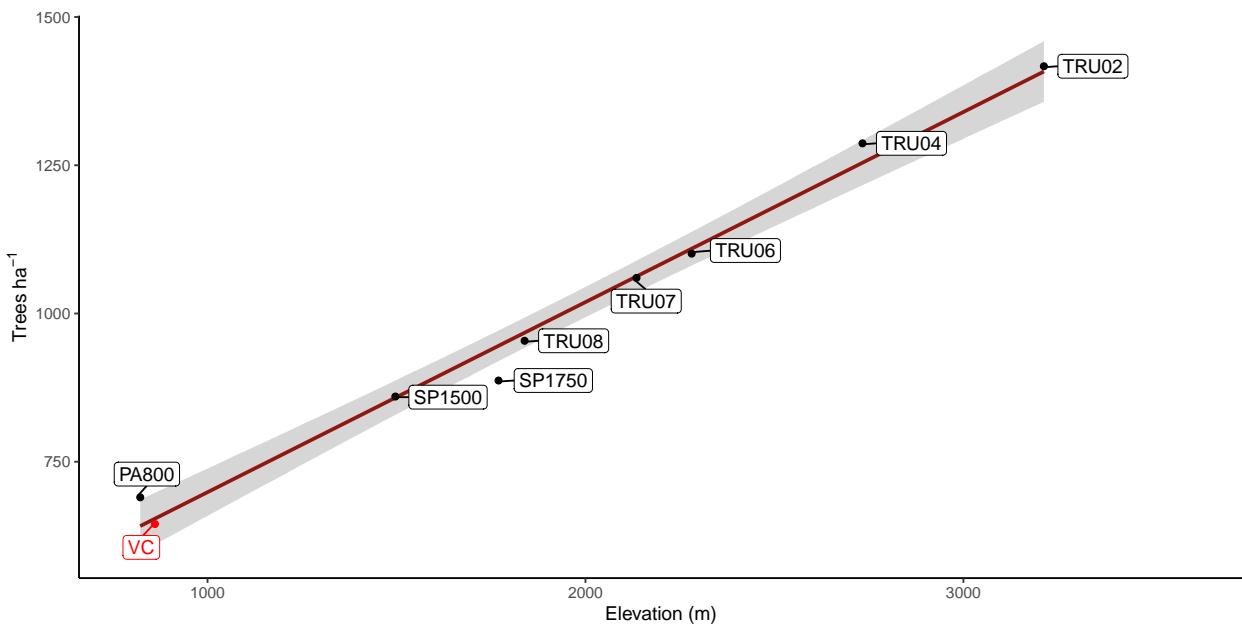


Figure 6: Linear regression with 95% confidence interval of number of trees per hectare for each site, used to estimate number of trees per hectare for site VC. $R^2 = 0.896$, $F_{(1,7)} = 579.5$, $p < 0.001$.

Table 3: Competition radius used for adult competition measurements for each site based on the number of trees per hectare.

Site	Trees ha ⁻¹	C_R (m)
PA800	690	8
VC	645	8
SP1500	860	7
SP1750	887	7
TRU08	954	6
TRU07	1060	6
TRU06	1101	6
TRU04	1287	6
TRU02	1417	5

219 2.6 Statistical Analysis

220 A matrix of single predictor linear mixed effects models were compared to test for the presence and
 221 strength of the causal relationship between each of the two competition variables and each of the six
 222 plant traits. The fixed effect of elevation was also included in this comparison in order to compare
 223 the effects of competition to that of other unmeasured elevationally dependent environmental effects.
 224 All fixed effects were standardised and rescaled with a mean of 0 and a standard deviation of 1 to
 225 allow easy comparison of effect sizes, according to (Gruber et al., 2011). Model comparison was
 226 performed on models fitted using Maximum Likelihood (ML) estimates (Bolker et al., 2008). Model
 227 quality was compared using Akaike Information Criteria (AIC) (Akaike, 1992), Akaike weights
 228 (W_i), and fixed effect marginal pseudo- R^2 values (R_M^2) using *r.squared.GLM()* from the *MuMIn*
 229 package (Bartoń, 2019). Random effects of site and species were added to the models. Site was
 230 added as a random intercept effect to account for pseudo-replication in site characteristics within
 231 which multiple seedlings per site were sampled. Tree species was added as a random slope effect to
 232 account for differences in morphology and physiology between seedlings and to allow for comparison
 233 of model slopes between species. As there were multiple species sampled within a single plot, but
 234 not all plots contained all species, these models have a partially crossed random effects structure.

235 The single fixed effect models were compared using ΔAIC_r against a random effects model to
 236 assess whether the fixed effects captured real variation in plant traits. Models were also analysed
 237 with an approximation of the variance explained by the model (R_C^2) using *r.squared.GLM()* from
 238 the *MuMIn* package (Bartoń, 2019), and slope coefficients (Figure ??, Figure 11) to compare their
 239 relative effect on plant traits. Single fixed effect model structures were as follows:

$$Y_{ij} = \beta_0 + \beta_1 X_{ij} + u_{0j} + u_{1j} X_{ij} + \epsilon_{ij} \quad (5)$$

240 where Y_{ij} is the response variable of species i at site j , X_{ij} is the fixed effect value of species
 241 i in site j . The random intercept grouping effect of site was used in all models to account for
 242 pseudo-replication in site characteristics for seedlings sampled along the elevation gradient.

243 To better understand the multiplicative effects of competition and other elevationally dependent
 244 environmental variables on plant traits we also compared linear mixed effects models with combi-
 245 nations of fixed effects, using AIC , W_i and R_C^2 , to find the model which best explained variation
 246 in each plant trait. These models used the same basic model specification as the single fixed effect
 247 random intercept models shown above, except with multiple additive fixed effects. To ensure all

248 models converged, these more complicated models only used random intercept effects for species
249 and sites. For initial model comparison, these models were fitted using Maximum Likelihood. All
250 statistical analyses were conducted using R, version 3.2.4 (R Core Team, 2019). Linear mixed effects
251 models were fitted using the *lme4* package (Bates et al., 2015).

252 3 Results

253 3.1 Variation in plant traits across elevation

254 All species except *Myrcia* spp. (MS) showed a general positive trend in photosynthetic efficiency
255 (F_v/F_m) across their respective elevational ranges, though the spread of F_v/F_m within species was
256 small. Random effect slopes of photosynthetic efficiency over elevation showed that none of the
257 species level regressions dipped below the critical stress threshold of 0.7. Of the 151 measured
258 seedlings, 12 had an F_v/F_m below 0.7. Of those 12 seedlings, 4 were *Alzatea verticillata* (AV), 2
259 were *Clethra revoluta* (CR) and 1 each from *Clusia thurifera* (CT) and *Myrcia* spp. (MS) (Figure
260 10).

261 Chlorophyll- α generally decreased with elevation, with negative trends in *A. verticillata* (AV),
262 *C. thurifera* (CT), *Myrcia* spp. (MS), *Schefflera patula* (SP) and *Tapirira guianensis* (TG). *C.*
263 *revoluta* (CR) and *Hedyosmum goudotianum* (HG) had positive trends (Figure 9).

264 The relationship between physiognomic plant traits and elevation varied between species. Leaf:height
265 ratio generally decreased with increasing elevation, with seedlings becoming less leafy with elevation,
266 except for *A. verticillata* (AV) which showed a negative U-shaped relationship, and *H. goudotianum*
267 (HG) which showed a positive hump-shaped relationship (Figure 8). Leaf area did not vary mean-
268 ingfully across elevation except in *S. patula* (SP) where it decreased and *T. guianensis* (TG) where
269 it increased. Leaf thickness generally increased or remained the same with elevation, except in *C.*
270 *thurifera* (CT), which had a weak negative relationship, with some individuals at the lower end
271 of the elevational range having particularly thick leaves. Stem volume was similarly unaffected by
272 elevation, except in *S. patula* (SP), where it decreased with elevation and in *H. goudotianum* (HG)
273 where it increased, driven by very large seedlings at the top end of the elevation gradient.

274 Species with restricted elevational ranges were more likely to have steeper relationships of both
275 physiological and physiognomic plant traits across elevation, e.g. *A. verticillata* (AV), *C. thurifera*
276 (CT), *S. patula* (SP) and *T. guianensis* (TG). These species with restricted elevational ranges also
277 inhabit the cloud zone of the forest elevational gradient (Figure 13).

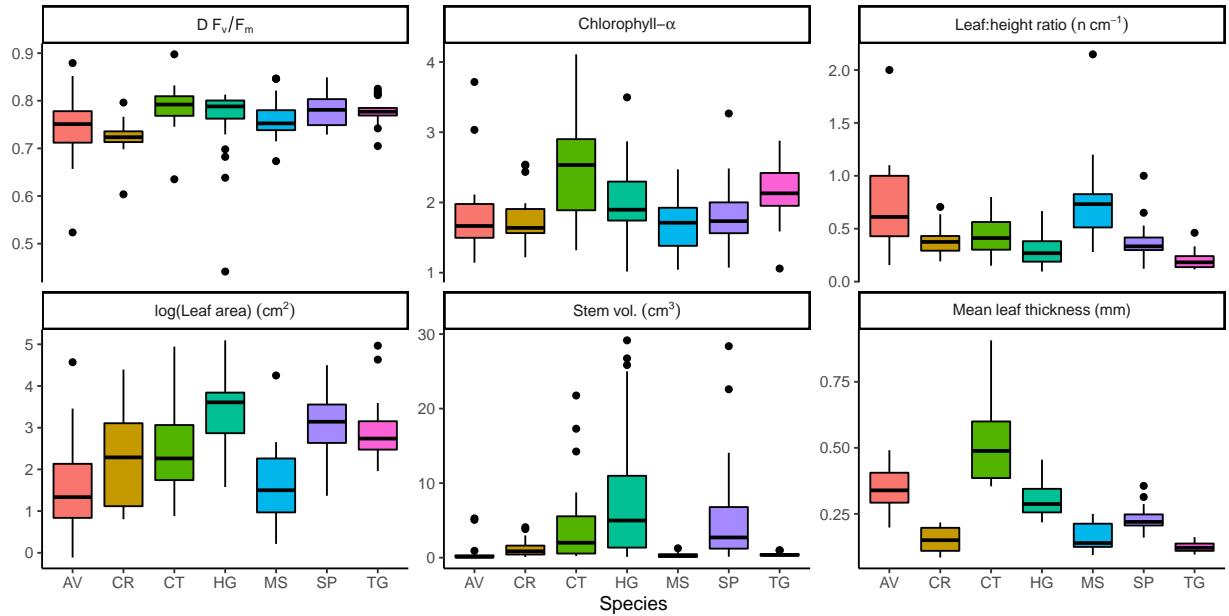


Figure 7: Box plots showing the variation in plant trait values within each species.

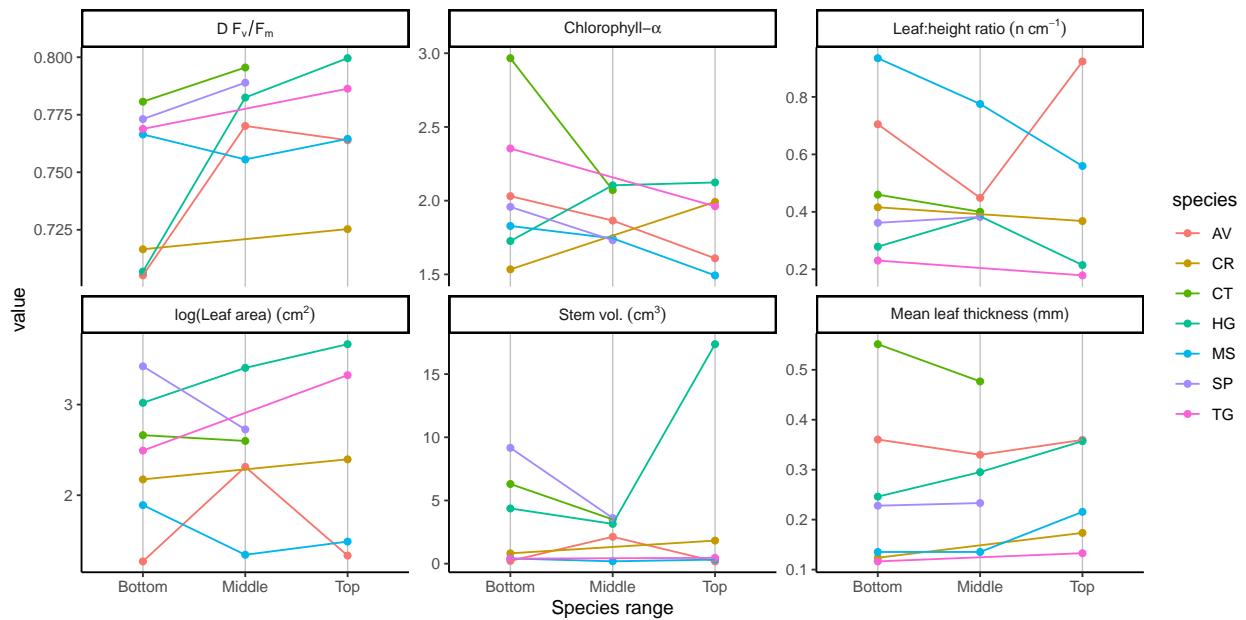


Figure 8: Interaction plots showing the variation in plant trait values within each species.

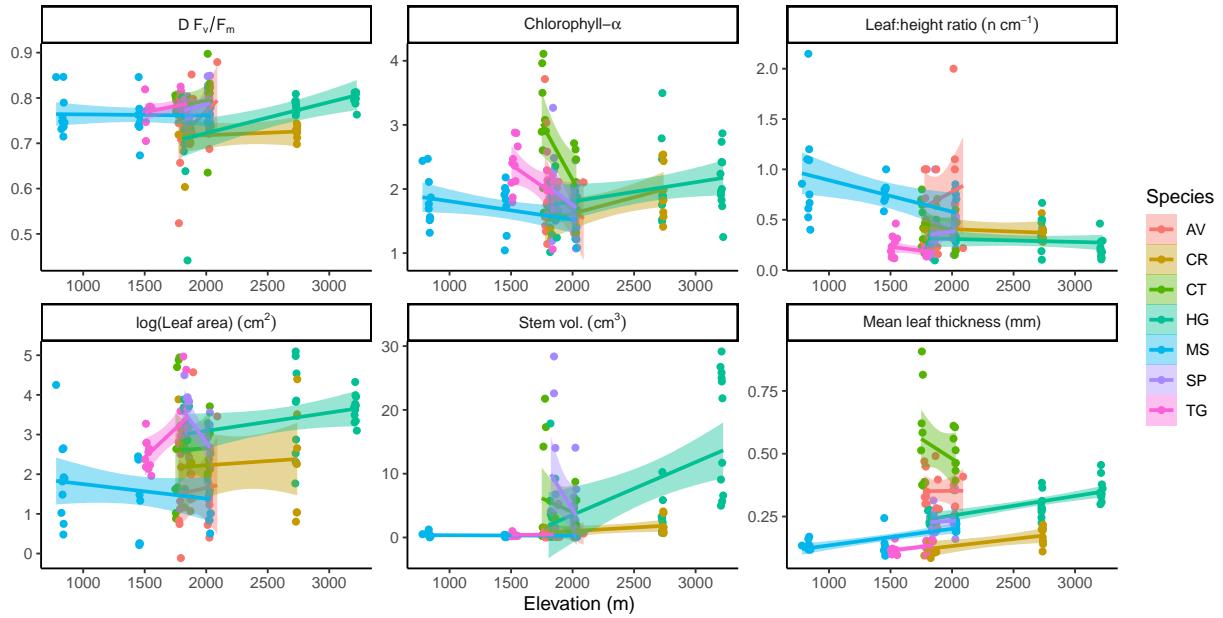


Figure 9: Scatter plots with linear model fits for each species, showing the variation in plant stress variables and plant traits across elevation.

278 3.2 Directional effects of adult competition on plant traits

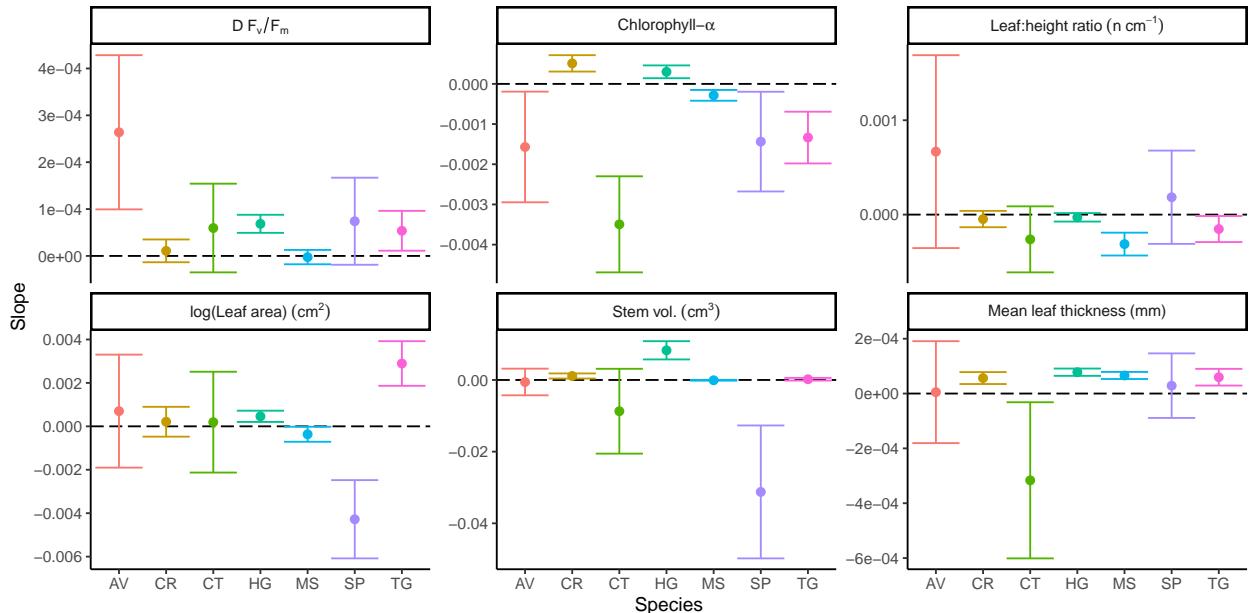


Figure 10: Interval plots showing the effect sizes (slopes) of each species in single fixed effect linear mixed effects models of plant traits against elevation variables.

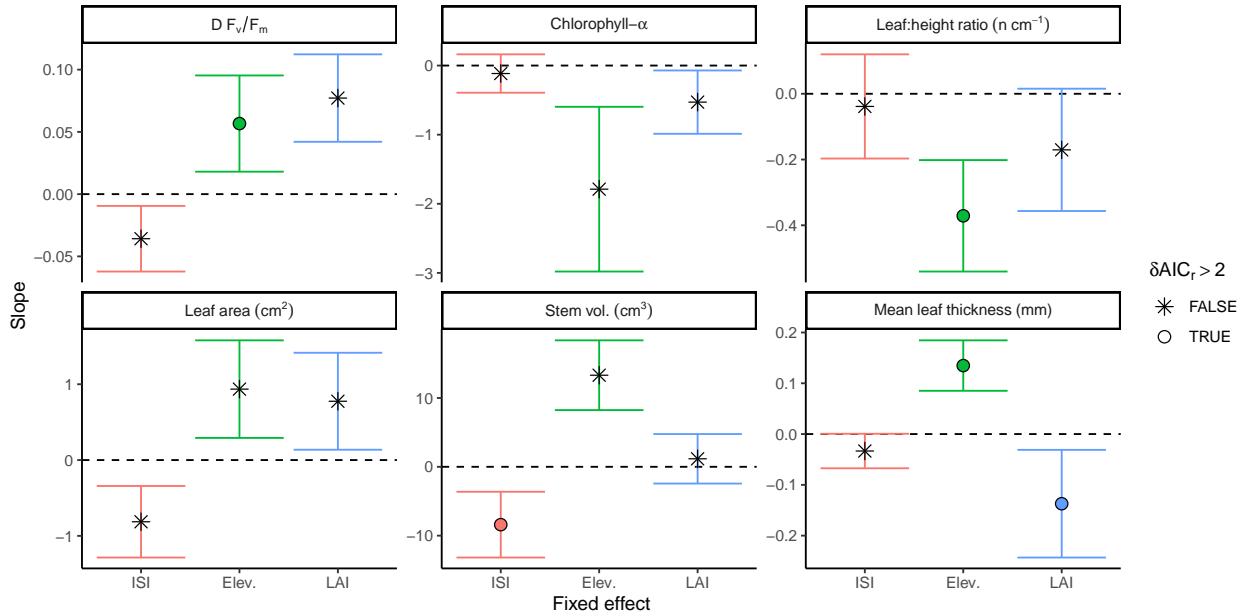


Figure 11: Model slopes for each single fixed effect model of plant traits predicted by elevation and competition variables. Error bars are ± 1 standard error. Models where δAIC_r was less than two and therefore not appreciably better than an equivalent random effects models are shown as asterisks.

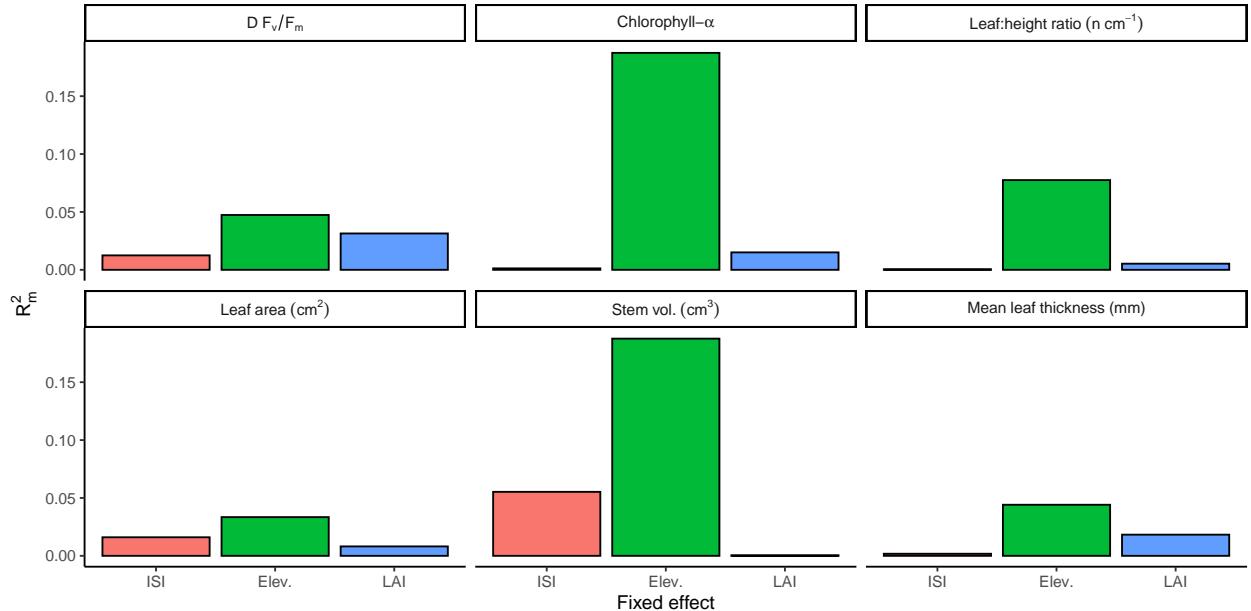


Figure 12: The approximation of marginal R^2 explained by the fixed effect of each single fixed effect model.

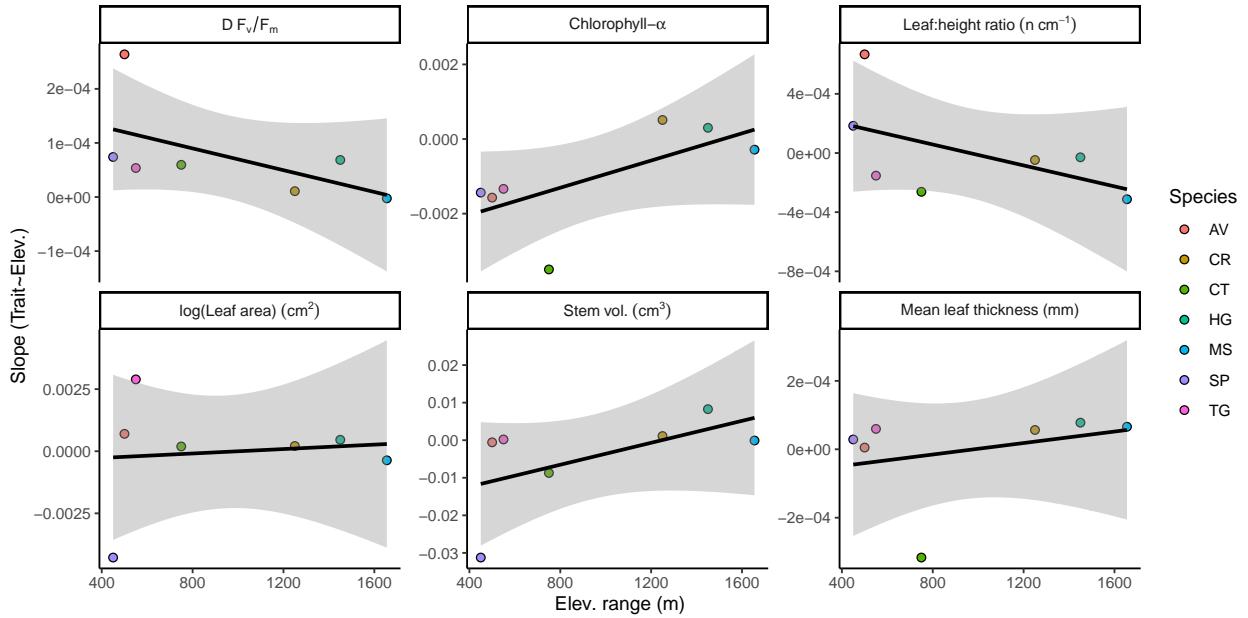


Figure 13: Slopes for each species in a linear model of plant trait vs. elevation, showing variation in model slope with elevational range.

279 Across species, models with single fixed effects poorly estimated variation in plant traits. Models
 280 with the fixed effect of elevation explained the greatest amount of variation in plant traits, notably
 281 stem volume and Chlorophyll- α , but neither of these models were appreciably better than a random
 282 effects model including only the effects of site and species (Figure 12). Models with ISI predicting
 283 stem volume and LAI predicting leaf thickness were the only two single fixed effect models using
 284 competition variables which provided adequate model fit (Figure 12). ISI caused a decrease in stem
 285 volume, LAI caused a decrease in leaf thickness.

286 Table 4 shows the fixed effects and model fit measures from the best fitting multiple fixed effect
 287 models used to predict plant traits. All of the best multiple predictor mixed effects models included
 288 elevation as a fixed effect. All of the best models, except the model predicting leaf Chlorophyll- α ,
 289 included both adult competition predictor variables, ISI and LAI, alongside that of elevation (Figure
 290 14). The variance explained by these best multiple predictor mixed effects models was higher than
 291 that for the single fixed effects models. F_v/F_m , Leaf:height ratio, Stem volume and Leaf thickness
 292 had models which were better than a random effects model with $\Delta AIC_r > 2$.

293 In the best fitting multiple fixed effect model for F_v/F_m , ISI and LAI had contrasting effects.
 294 An increase in ISI led to a decrease in F_v/F_m while an increase in LAI led to an increase in F_v/F_m .

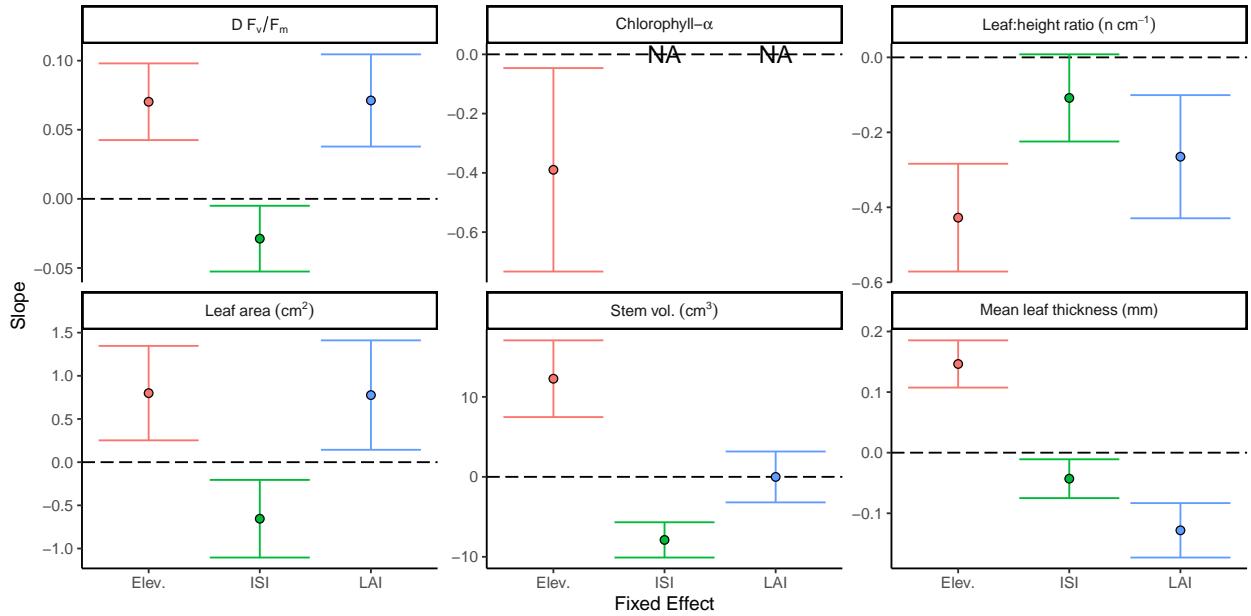


Figure 14

Table 4

Response	Fixed effects	ΔAIC_r	W_i	R_c^2	R_m^2
F_v/F_m	Elev. + ISI + LAI	-4.82	1	0.29	0.1
Chlorophyll- α	LAI	0.73	1	0.28	0.01
Leaf:height ratio	Elev. + ISI + LAI	-4.31	1	0.37	0.1
Leaf area _{log}	Elev. + ISI + LAI	-0.06	1	0.31	0.05
Stem vol.	Elev. + ISI + LAI	-11.6	1	0.57	0.23
Leaf thickness	Elev. + ISI + LAI	-12.59	1	0.78	0.08

295 Discussion

296 This study aimed to (a) determine whether tree seedling physiological and physiognomic plant
 297 traits were affected by competition from adult trees across an elevation gradient, (b) assess how
 298 the effects of competition compared to that of elevation, and (c) assess the degree to which plant
 299 trait-elevation relationships vary among species. It was found that adult competition variables
 300 never influence a given plant trait more than elevation, but that combinations of elevation and
 301 competition variables better predict variation in plant traits than elevation alone. Tree species with
 302 more restricted elevational ranges, which inhabit the cloud zone of the elevational gradient appear
 303 to be more sensitive to variation in biotic environmental factors. Interestingly however, according
 304 to F_v/F_m chlorophyll fluorescence measurements, seedlings did not experience greater physiological
 305 stress at higher elevations, instead all species exhibited model slopes >0 or approaching 0.

306 **3.3 Species specific responses to elevation dependent environmental variation**

307 **3.4 Photosynthetic efficiency (F_v/F_m)**

308 **3.5 Limits to future upslope migration**

309 **3.6 Species rarity**

310 Nine tree species were selected for this study. Although these species are common in the areas we
311 sampled (Appendix VI), there are many other species which may react more or less to the biotic
312 environment. There is evidence that rare species are more affected by environmental factors (??).
313 Rare species are more likely to occupy specialist niches, which are narrower on a local geographical
314 scale than those of generalist species (?). The evolutionary histories of specialists means they are
315 less likely to be able to acclimate to novel environments. Compared to the common species studied
316 here, rare species will not have such a large direct effect on globally significant ecosystem services
317 such as carbon sequestration, albedo, and drainage. This does not mean that rare species do not
318 have the potential to heavily influence ecosystem services indirectly. ?, and ? found that less
319 common species play vital supporting roles in maintaining ecosystem functions such as enhancing
320 invasion resistance and making limiting resources available to other species.

321 **3.7 Future studies**

322 On the basis of this study, which shows that adult-seedling competition intensity varies across
323 elevation and that this variation forms part of the observed plant trait response to elevation, it
324 is recommended that future studies aim to identify competition intensity thresholds beyond which
325 individuals cannot acclimate to the environmental conditions. The location of thresholds should be
326 confirmed using experimental transplantation of seedlings to different elevations to observe variation
327 in plant traits.

328 In order to determine whether changes in competition intensity also affect adult trees, and thus
329 recruitment, similar studies should be performed on adult trees. This would help to improve the
330 accuracy of species range-shift models by adding the potential variation found within populations
331 and allowing demographically explicit models.

332 **Conclusion**

333 **References**

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