

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

4 John L. Godlee

5 August 30, 2019

6 **Abstract**

- 7
- 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.

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.

2 Materials and Methods

2.1 Study Site

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used in this study contain 719 tree species, and the valley as a whole contains an estimated 1167 tree species (ABERG unpublished data).

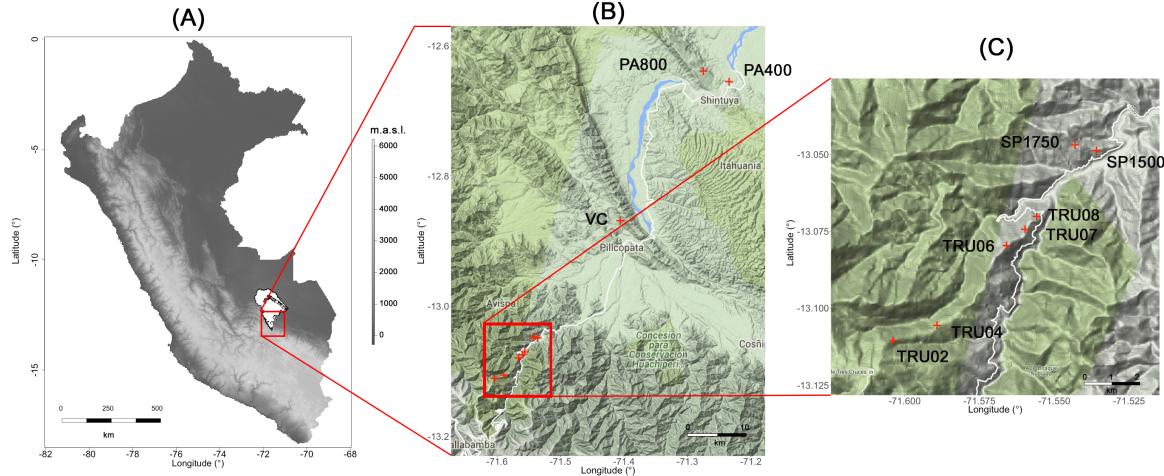


Figure 1: Maps showing the location of the study area and plot locations. (A) The site location within Peru with elevation shading, showing the proximity to Manú National Park (white area). (B) The location of the 1 ha plots within the Kosñipata Valley. (C) An enlargement of the Trocha Union and San 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

129 2.2 Study species

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 133 range in the Kosñipata Valley (ABERG, unpublished data) (Figure 3).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

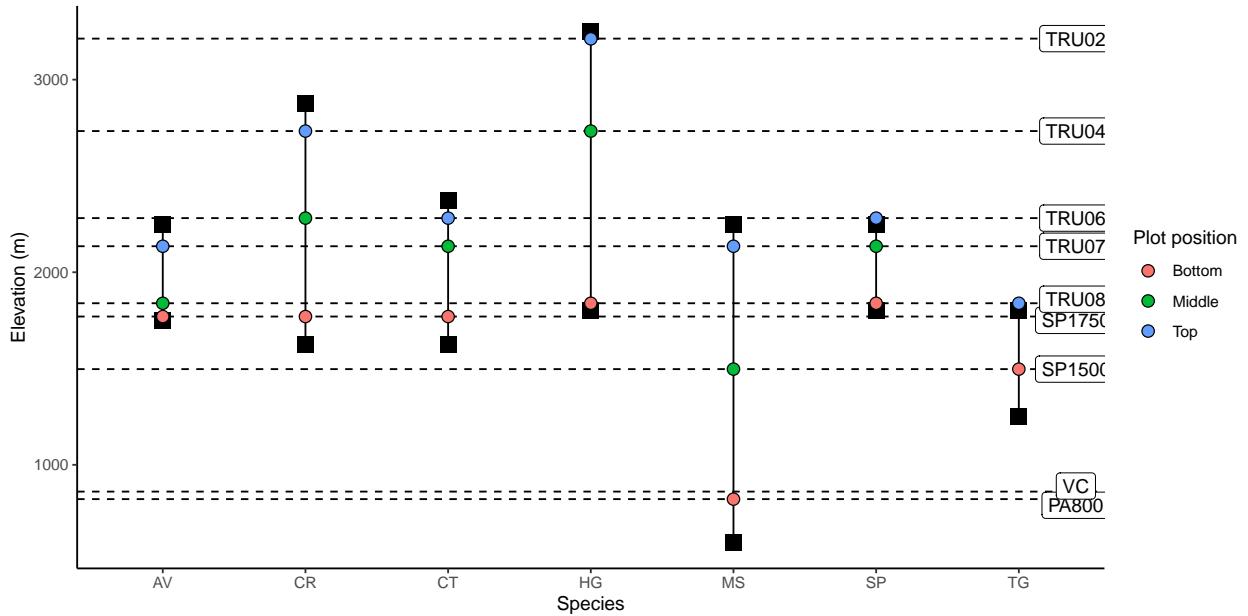


Figure 2: Elevations of study plots for each species (coloured points) with the upper and lower range extents for each species (black squares). Plot elevations are marked as dashed lines and labelled.

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

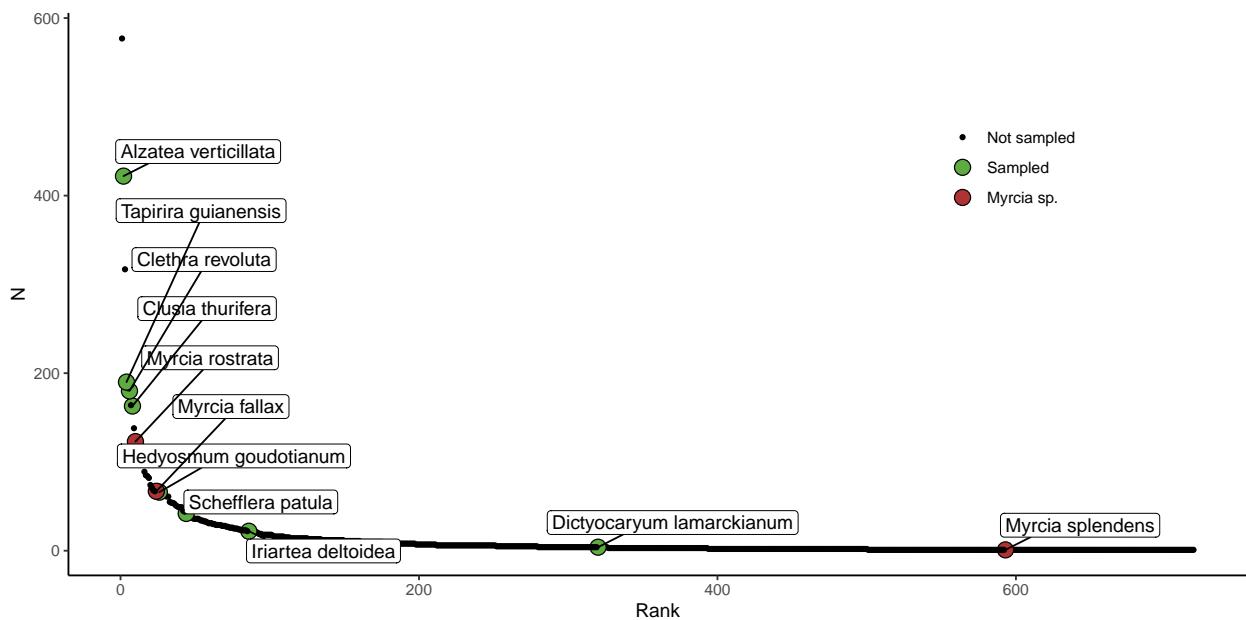


Figure 3: Rank abundance curve of all individuals >10 cm DBH of all species found in the plots measured in this study. Census data from 2014 (ABERG, unpublished data). Species sampled as part of this study are highlighted in red. *Myrcia* species which form the composite *Myrcia* spp. are highlighted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

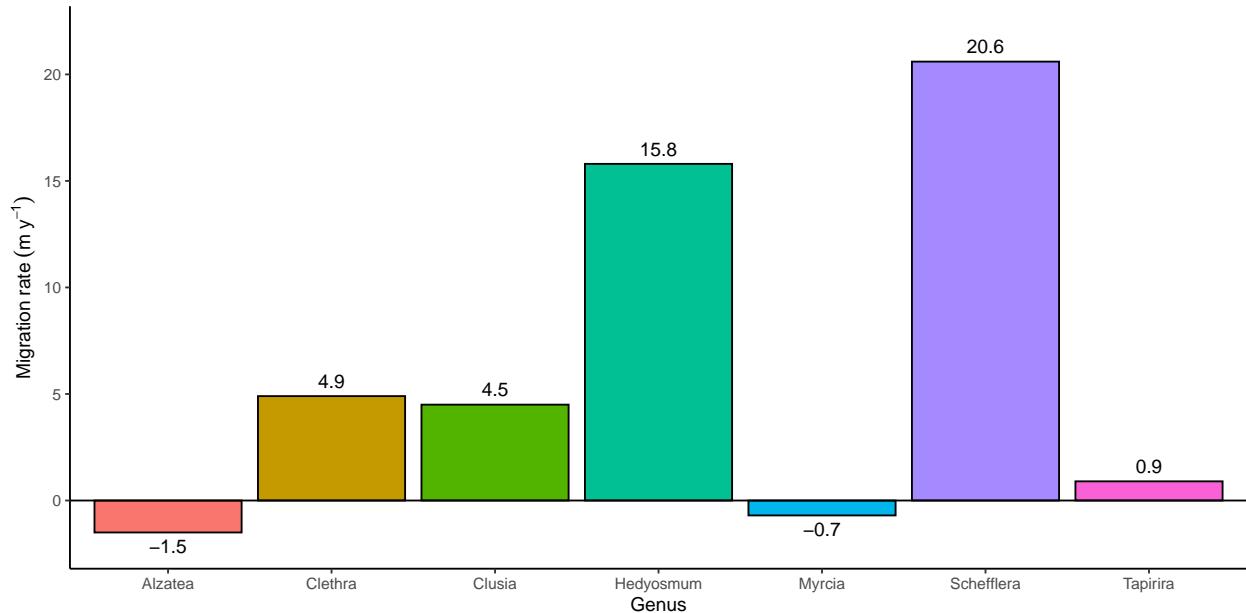


Figure 4: Estimated elevational migration rates within the Kosñipata valley for selected genera of which species are studied here. Migration rates are estimated using shifts in the centre of gravity of tree basal area as measured in the ABERG 1 Ha plot network.

138 2.3 Sampling and Measurement

139 Species were sampled in three plots representing the top, middle and bottom elevational extents of
 140 their ranges (Figure 2). Within each plot, a maximum of 10 seedlings were sampled. To minimise
 141 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

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

183 2.5 Competition measurements

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

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

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

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

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

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

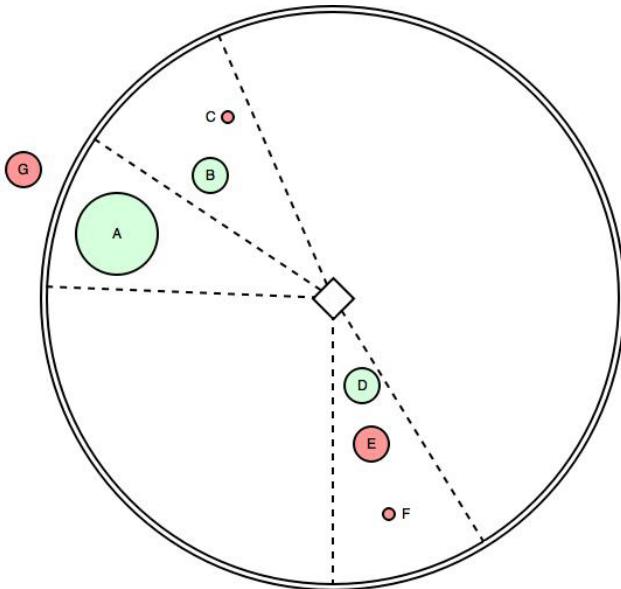


Figure 5: Schematic diagram showing the iterative selection of active competitor trees for the Iterative Seedling Index (ISI) (Equation 3). Trees marked in green (A, B, D) are active competitors for the tree of interest (black diamond). Trees marked in red (C, E, F, G) are non-active competitors, coloured 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)
PA400	475	9
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

216 2.6 Statistical Analysis

217 A matrix of single predictor linear mixed effects models were compared to test for the presence
 218 and strength of the causal relationship between each of the two competition variables and each of
 219 the six plant traits. The fixed effect of elevation was also included in order to compare the effects
 220 of competition to that of elevation. All fixed effects were standardised and rescaled with a mean
 221 of 0 and a standard deviation of 1 to allow easy comparison of effect sizes, according to (Gruber
 222 et al., 2011). Model comparison was performed on models fitted using Maximum Likelihood (ML)
 223 estimates (Bolker et al., 2008). Model quality was compared using Akaike Information Criteria
 224 (AIC) (Akaike, 1992), Akaike weights (W_i), and fixed effect marginal pseudo-R² values (R_M^2) using
 225 *r.squared.GLMM()* from the *MuMIn* package (Bartoń, 2019). Random effects of site and species
 226 were added to the models. The random effect of site accounted for pseudo-replication in site
 227 characteristics within which multiple seedlings per site were sampled. The random effect of tree
 228 species accounted for differences in morphology and physiology between seedlings and allowed for
 229 comparison between species in the effect of biotic environmental fixed effects in the model. As there
 230 were multiple species sampled within a single plot, but not all plots contained all species, these
 231 models have a partially crossed random effects structure.

232 In order to inform the error structure of single fixed effect mixed effects models, error structures
 233 were compared using AIC values on pairs of single fixed effect linear mixed effects models, where
 234 the model slopes of each species were allowed to vary by either intercept or by slope and intercept, to
 235 show whether species differ appreciably in their trait response to the various competition variables
 236 and elevation (fixed effects) (Figure ??). Where there was less than two ΔAIC_{rsri} points between
 237 pairs of models, a random intercept structure was maintained in the single fixed effect models, in
 238 order to maximise parsimoniousness. Single predictor models reported in the results use the optimal
 239 error structure. Pairs of models were fitted using Maximum Likelihood in order to allow comparison
 240 between models. The best models were re-fitted using Reduced Maximum Likelihood, according to
 241 Bolker et al. (2008).

242 The best quality single fixed effect models (using either independent intercepts or slopes for
 243 each species) were compared using ΔAIC_r against a random effects model, an approximation of the
 244 variance explained by the model (R_C^2) using *r.squared.GLMM()* from the *MuMIn* package (Bartoń,
 245 2019), and slope coefficients (Figure 12, Figure 11) to compare their relative effect on plant traits.
 246 Single predictor mixed effects model structures are as follows for random intercept models:

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

247 and for random slope models:

$$Y_{ij} = \quad (6)$$

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

251 To better understand the potential multiplicative effects of competition variables we also com-
 252 pared linear mixed effects models with combinations of fixed effects, using AIC , W_i and R_M^2 , to
 253 find the model which best explained variation in each plant trait. These models used the same
 254 basic model specification as the single predictor models shown above, except with multiple fixed
 255 effects. To ensure all models converged, these more complicated models only used random intercept
 256 effects for species and sites. For initial model comparison, these models were fitted using Maximum
 257 Likelihood.

258 To understand variation between species in their physiological and morphological response to
 259 competition effects, the best fitting models were re-estimated using Reduced Maximum Likelihood
 260 and the random effect slopes for each species were calculated and compared (Bolker et al., 2008).
 261 All statistical analyses were conducted using R, version 3.2.4 (R Core Team, 2019). Linear mixed
 262 effects models were conducted using the *lme4* package (Bates et al., 2015).

263 3 Results

264 3.1 Variation in plant traits across elevation

265 All species except *Myrcia* spp. (MS) showed a general positive trend in photosynthetic efficiency
 266 (F_v/F_m) across their respective elevational ranges. Linear models of photosynthetic efficiency over
 267 elevation showed that none of the species level regressions dipped below the critical threshold of 0.7,
 268 which indicates plant stress. Of the 151 measured seedlings, 12 had an F_v/F_m below 0.7. Of those
 269 12 seedlings, 4 were *Alzatea verticillata* (AV), 2 were *Clethra revoluta* (CR) and 1 each from *Clusia*
 270 *thurifera* (CT) and *Myrcia* spp.. Chlorophyll- α generally decreased with elevation, with negative
 271 trends in *A. verticillata* (AV), *C. thurifera* (CT), *Myrcia* spp. (MS), *Schefflera patula* (SP) and
 272 *Tapirira guianensis* (TG). *C. revoluta* (CR) and *Hedyosmum goudotianum* (HG) had positive trends
 273 (Figure 9).

274 The relationship between physiognomic plant traits and elevation varied between species. Leaf:height
 275 ratio generally decreased over elevation, except for *A. verticillata* (AV) which showed a negative
 276 U-shaped relationship, and *H. goudotianum* (HG) which showed a positive hump-shaped relation-
 277 ship (Figure 8). Leaf area generally increased with elevation, though *C. thurifera* (CT), *S. patula*
 278 (SP), and *Myrcia* spp. (MS) showed negative relationships. Leaf thickness was generally unaffected
 279 by elevation, except in *C. thurifera* (CT), which had a negative relationship. Stem volume was
 280 similarly unaffected by elevation, except in *S. patula*, where it decreased with elevation and in *H.*
 281 *goudotianum* (HG) where it increased, driven by very large seedlings at the top end of the elevation
 282 gradient.

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

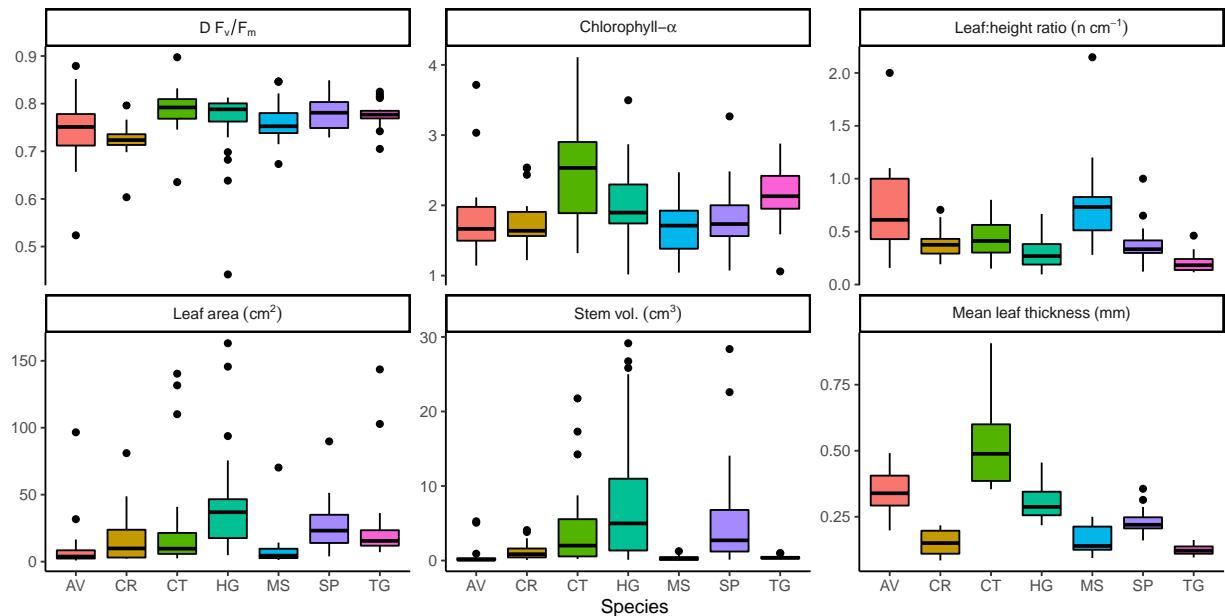


Figure 7: Box plots showing the variation in plant trait values within each species.

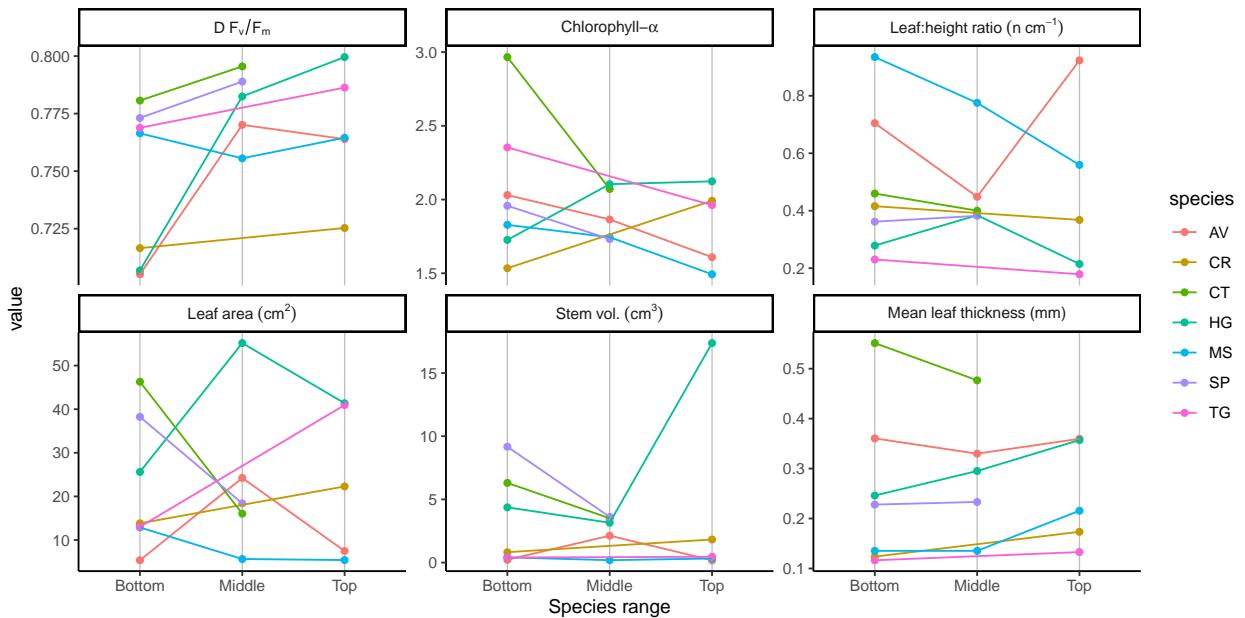


Figure 8: Interaction plots showing the variation in plant trait values within each species.

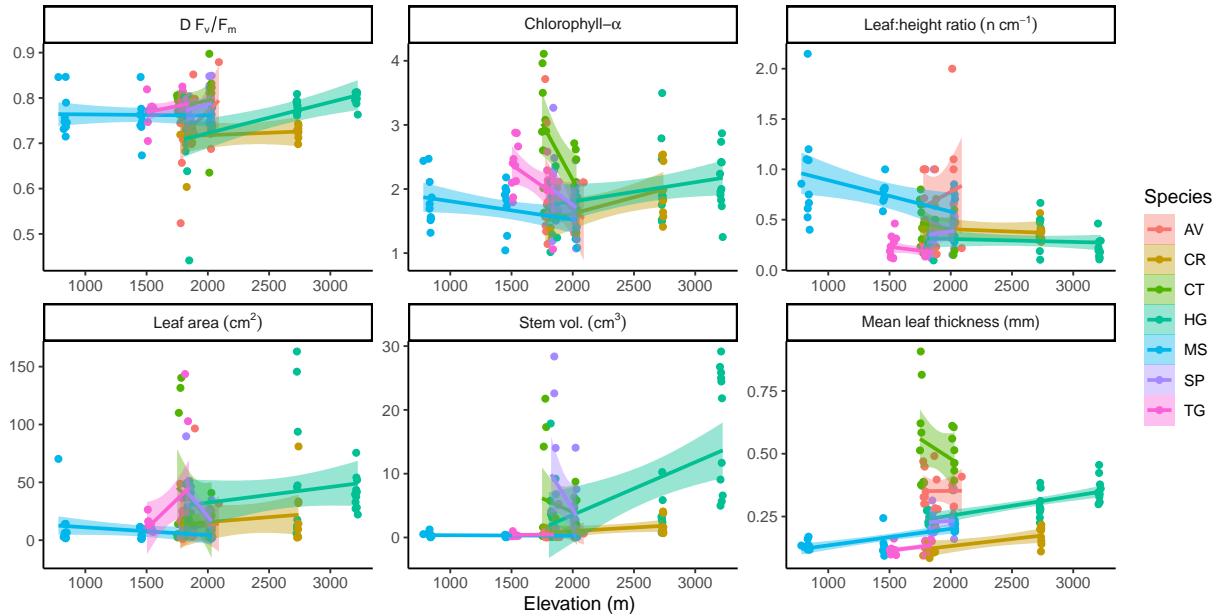


Figure 9: Scatter plots with linear model fits for each species, showing the variation in plant stress variables and plant traits across elevation.

287 3.2 Directional effects of adult competition on plant traits

288 Random slope terms per species for single fixed effects models between plant traits and environmental
 289 variables produced models of better quality than random intercept terms per species in 8/18 cases. All models with F_v/F_m as the response variable were of better quality when the random slope
 290 term was included (Figure 11). Only $F_v/F_m \sim \text{Elev.}$, Leaf:Height ratio $\sim \text{Elev.}$, Stem vol. $\sim \text{ISI}$, Leaf
 291 thickness $\sim \text{Elev.}$ and Leaf thickness $\sim \text{LAI}$ produced models better than equivalent random effects
 292 models. Even so, these models explained very little of the variation in plant traits, with the highest
 293 R_m^2 being for the model of Stem vol. $\sim \text{Elev.}$ ($R_m^2 = 0.19$). In the single predictor mixed effects
 294 models, models with elevation as the fixed effect consistently outperformed other single predictor
 295 mixed effects models

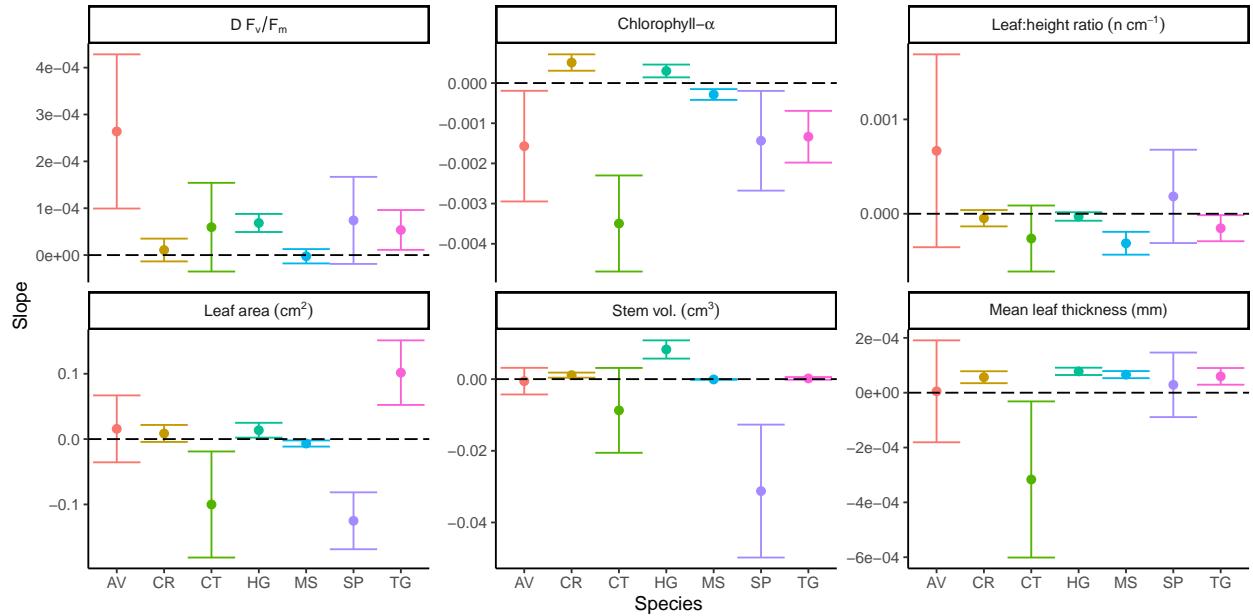


Figure 10: Interval plots showing the effect sizes (slopes) of each fixed effect in single fixed effect linear mixed effects models of plant traits against forest structure variables and elevation, for comparison.

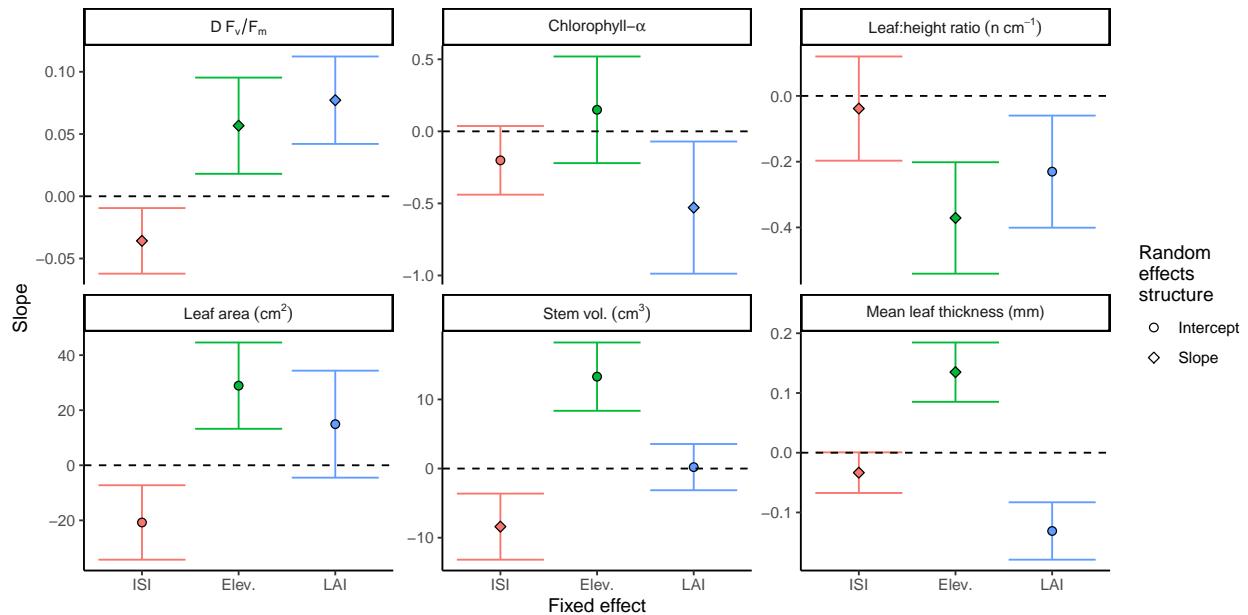


Figure 11

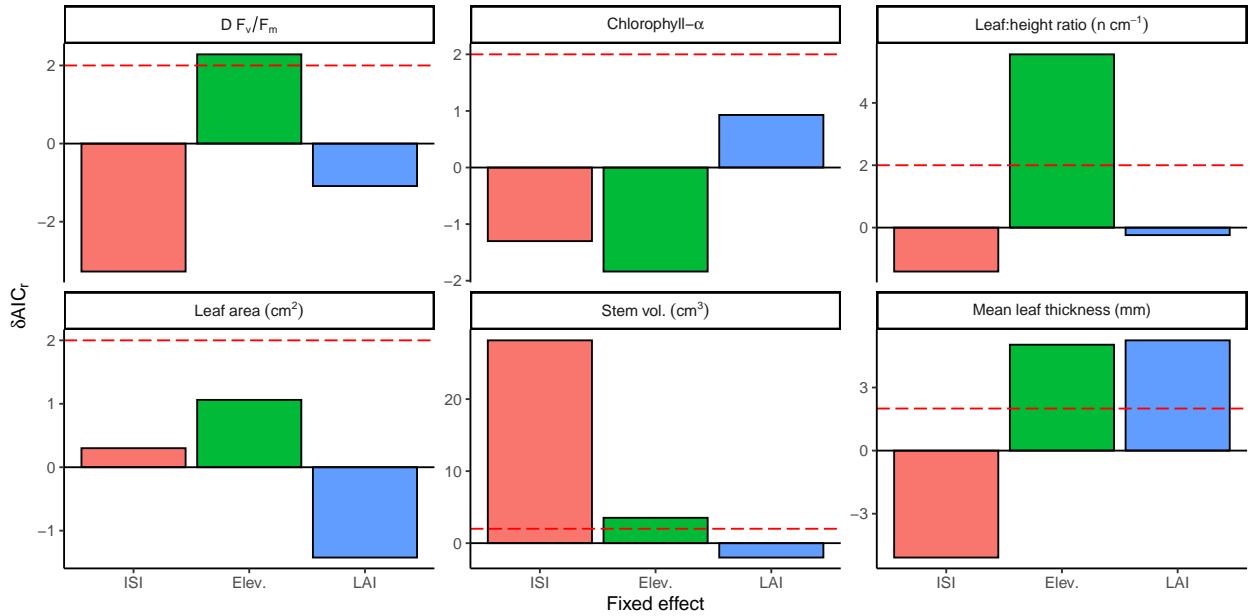


Figure 12: The difference in AIC values between each single fixed effect model and a corresponding random effects model using no fixed effects. A model with $\Delta AIC_r > 2$ means the model is of appreciably higher quality than the random effects model. The horizontal dashed red line indicates this threshold.

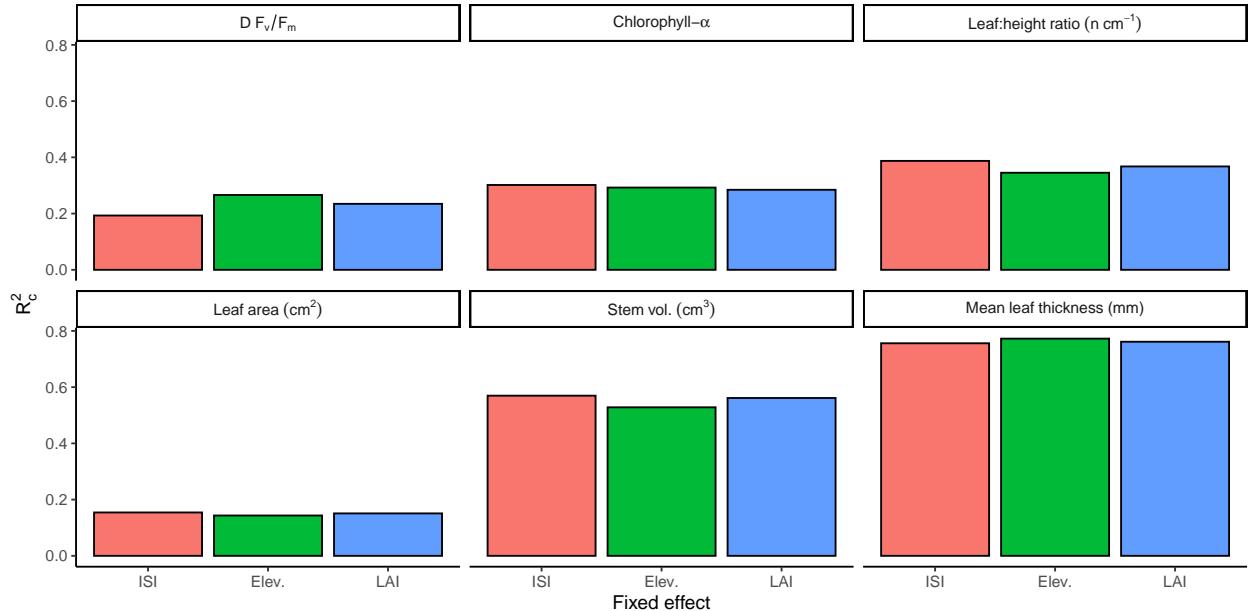


Figure 13: The variance explained by each single fixed effect model.

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

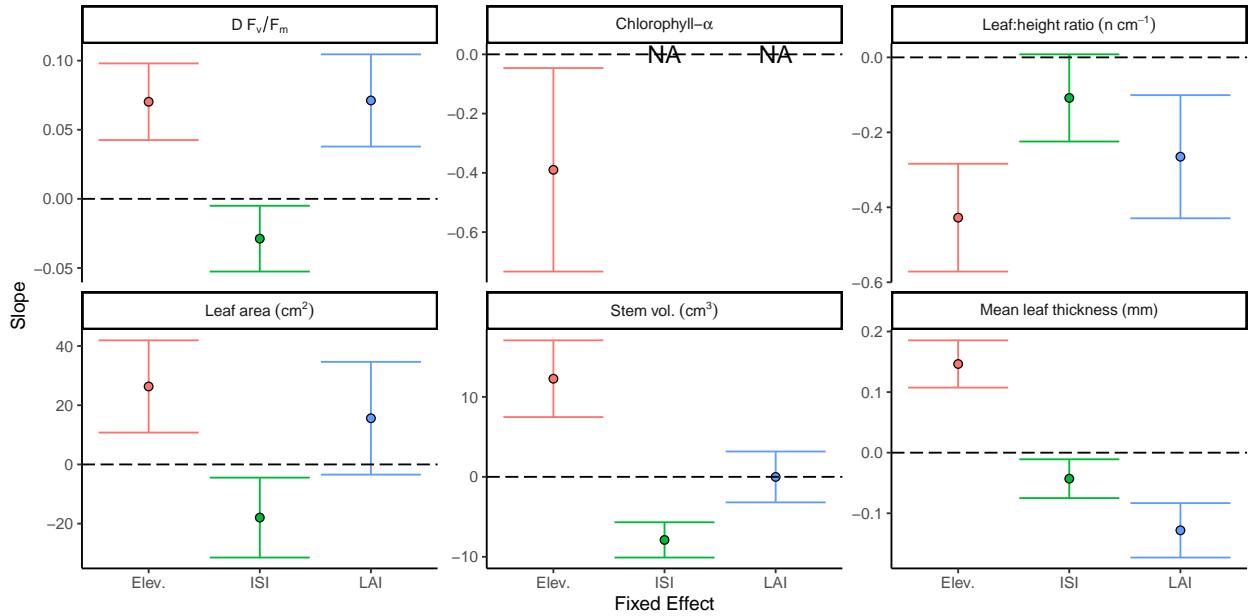


Figure 14

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	Elev. + ISI + LAI	0.52	1	0.16	0.06
Stem vol.	Elev. + ISI + LAI	-11.6	1	0.57	0.23
Leaf thickness	Elev. + ISI + LAI	-12.59	1	0.78	0.08

305 Discussion

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

316 4 Effect of biotic environment and elevation on plant traits

317 Single fixed effect models demonstrated that the three competition variables influence some plant
318 traits ($\Delta\text{AIC}_r \geq 2$, Figure 12a). The effect size of individual competition variables however, did
319 not exceed that of elevation for any plant traits (Figure 12b, Figure 11). The three competition
320 variables, which represent different types of competition, vary in their effects on seedling traits.
321 However, the estimated variance explained by the fixed effects of these models was low in all cases,
322 while the variance explained by the model as a whole varied according to the plant trait studied.
323 It would seem that unmeasured site level variation had a greater impact on plant traits than biotic
324 environmental variation *per se*.

325 What were some of these unmeasured factors?

326 Leaf physiology

327 Together, leaf chlorophyll- α content and F_v/F_m are useful measures of a plant's health and the in-
328 tegrity of its photosynthetic apparatus, which are necessary for further growth (?). This study found
329 contrasting effects of elevation on chlorophyll- α and F_v/F_m . As elevation increased, photosynthetic
330 efficiency increased or remained the same in all species, but chlorophyll- α content decreased or re-
331 mained the same (Figure ??). There is however, large variation in chlorophyll- α values within sites
332 and elevation explains little of the variance in chlorophyll- α (Figure ??), meaning this relationship
333 may be erroneous. Biotic environmental variables explained comparatively little variation in F_v/F_m
334 or chlorophyll- α compared to morphological leaf traits (Figure ??b).

335 Photosynthetic efficiency

336 Single fixed effect models showed that an increase in canopy density (LAI) caused an increase in
337 photosynthetic efficiency (F_v/F_m) (Figure ??). Specifically, an increase in photosynthetic efficiency
338 under denser canopy may be the result of a more temporally constant microclimate (?). A denser
339 canopy regulates diurnal temperature oscillations by more effectively trapping warm air between
340 the canopy and the forest floor, reducing temperature stress on the plant (?). Increased shading
341 under denser canopy also reduces the potential for seedling desiccation and cavitation, which can
342 cause damage to seedling leaves. As Sun-flecks move across the forest floor they result in rapid leaf
343 temperature increase (??). Additionally, a reduction in direct sunlight reduces the potential for
344 UV-B damage to photosynthetic apparatus (?). Diurnal temperature oscillations are generally of
345 greater range at higher elevations (?) as is the UV-B insolation fraction (?), suggesting that the
346 beneficial effects of increased canopy density on photosynthetic efficiency may become greater at
347 higher elevations. In this region however, persistent cloud cover at higher elevations throughout the
348 day may result in no increase in incident UV-B, the majority being absorbed by cloud condensation
349 nuclei before it reaches the leaf (?).

350 Canopy density decreases with elevation (Figure ??), though this trend may be the result of
351 wide within site variance ($\Delta\text{AIC}_r \geq 2$). This trend concurs with more conclusive results from other
352 studies which show a clear decrease in canopy density with elevation (??). The more variable
353 relationship seen in this study may be the result of bias in the sampling strategy. LAI was not
354 measured systematically across each site, instead being measured above each sampled seedling. It
355 is expected that seedlings will grow successfully only under canopy where the average light intensity
356 falls between a minimum needed for growth and a maximum that ensures temperature and UV-B
357 stress does not cause the seedling to perish. In this study therefore, extreme canopy densities were

358 probably not sampled. The presence of bias in our sampling strategy is supported by comparing the
359 range of LAI measurements in other studies. For example, ?, in a review of 61 tropical evergreen
360 forests, found that LAI ranged from 1.5 to 8. (after outlier exclusion), whereas our LAI estimate
361 ranged from only 1.0 to 5.5, implying that a representative LAI sample was not achieved within
362 each plot.

363 It is expected that a decrease in canopy density with elevation will lead to more individuals
364 showing signs of stress at higher elevations, due to the factors discussed above. An increase in plant
365 stress limits overall fitness as energy is allocated more to acclimation processes than to fecundity
366 (?). This may hinder further upward migration, especially in species with limited dispersal distance
367 such as *I. deltoidea* which relies on seed dispersal by large mammals (predominantly primates) (??)
368 over short distances. In this instance however, there is no clear decrease in F_v/F_m with elevation
369 within any species ($\Delta AIC_r = 1.61$), with 8/9 species show an increase in F_v/F_m with elevation
370 (Figure ??). This suggests that the effect of canopy density in decreasing photosynthetic efficiency
371 across elevation is masked by other environmental variables.

372 In contrast to the effects of LAI, ISI caused a decrease in photosynthetic efficiency. This suggests
373 that the mechanisms by which LAI may affect photosynthetic efficiency (shading, temperature
374 regulation) differ from those of ISI (nutrient competition, water competition, predation mutualisms)
375 (Lewis and Tanner, 2000). Other studies have shown a nutrient competition effect between adult
376 trees and nearby seedlings. ? demonstrated that adult trees of greater basal area (equivalent to
377 DBH) cause a larger reduction in soil available nitrogen which subsequently decreased the growth
378 of pine seedlings. Similarly, ? showed that trenching around neotropical tree seedlings in order to
379 decrease root competition increased the growth and leaf nutrient content of the seedlings. In this set
380 of plots, soil moisture is rarely a limiting factor, and insect predators are much rarer in cloud forests
381 than lowland forests (?). This suggests that any negative effect of increased ISI on photosynthetic
382 efficiency would be the result of nutrient competition by adult trees.

383 ISI decreases with elevation (Figure ??) and a decrease in ISI causes an increase in photosynthetic
384 efficiency. The increase in F_v/F_m with elevation may therefore be partly the result of decreased
385 adult-seedling nutrient competition at higher elevations. The large effect of elevation however,
386 implies that other unmeasured environmental variables influence this trend more than simply a
387 decrease in ISI.

388 Herbaceous plant density had little effect on F_v/F_m . In the single predictor models, the slope
389 was the smallest of all the environmental variables and explained the least variance (Figure 12,
390 Figure ??). In the multi-predictor models the best fitting model did not include herbaceous plant
391 density (Table ??). Other studies have shown that size-asymmetric competition with adults has
392 a much greater role in structuring forest ecosystems than seedling-seedling competition, especially
393 in tropical forests where seedlings are relatively scarce compared to adult trees (??). ? estimated
394 the area around tree seedlings in neotropical forests within which seedlings affect the availability of
395 resources both above- and below-ground to other seedlings, finding that most zones did not overlap
396 at all. This implies that seedling-seedling competition in neotropical forests is insignificant.

397 Maxwell and Johnson (2000) suggest that generally, optimum F_v/F_m is ~0.83, and that if F_v/F_m
398 falls below ~0.8, it is indicative of some kind of plant stress. It is important to note however, that
399 this optimum is likely to vary markedly among species and has been criticised as yet another
400 arbitrary threshold for a dynamic phenomenon (?). As a conservative estimate, here plants are
401 defined as experiencing physiological stress when $F_v/F_m < 0.7$. Figure ?? shows that only a few
402 individuals fall below this threshold, suggesting that few individuals along the elevational gradient
403 are experiencing stress. Only *C. revoluta* features reduced photosynthetic capacity with elevation.
404 *C. revoluta* also has the most individuals below the 0.7 threshold. This could be evidence that *C.*
405 *revoluta* individuals experience greater stress at increasing elevations, but the relationship shown

here is not strong enough to be conclusive, with large variation within each plot that *C. revoluta* seedlings were sampled. Alternatively other species which feature an increase in photosynthetic efficiency may be experiencing stress at lower elevations, giving support for the hypothesis given by Campbell et al. (2007), in which species ranges contract from the bottom up. Temperature increase is the most likely source of this increased stress at the lower limits of species ranges, though stress induced by antagonistic interactions from previously lower elevation species that have shifted upslope faster is also possible. Herbivores for example are expected to move upslope faster than tree species due to their mobility and shorter life-cycles (Chen et al., 2011).

414 Chlorophyll- α

SPAD value was not clearly influenced by any of the measured competition variables, or elevation (Figure ??). SPAD varied largely both within and among species, with large standard errors surrounding the estimates of each species (Figure ??, Table ??). The best fitting multiple fixed effect LMM for SPAD did not include elevation (Figure ??), though this model was only 14.2% more likely to be the best model than the next best model and the fixed effect of LAI accounted for only 0.4% of the variance in SPAD (Figure ??).

The lack of meaningful variation in SPAD contrasts other studies that have shown increases in chlorophyll content in response to shading (?????) and soil nitrogen content (?). In this study however, SPAD did not vary with LAI (shading), ISI (soil nutrient availability) or herbaceous plant abundance.

The species with the smallest ranges show the steepest decrease in SPAD with elevation (Figure ??). From this one could suggest that specialists are more sensitive to increases in elevation in terms of their photosynthetic apparatus. Species with small ranges are interpreted as being more specialist in their environmental requirements (Thuiller et al., 2005).

429 Summary

Most species demonstrated an increase in F_v/F_m with elevation, while SPAD showed little meaningful variation in response to elevation. Adult-seedling competition variables had contrasting effects on F_v/F_m while seedling-seedling competition had no effect. A decrease in ISI with elevation may have contributed to the observed increase in F_v/F_m with elevation though it is possible that this trend is actually a result of increased stress at lower elevations in response to temperature stress or herbivory stress. H_{n1} is therefore accepted for SPAD and rejected for F_v/F_m . The best multiple fixed effect model for F_v/F_m included all competition variables, H_{n2} is therefore rejected for F_v/F_m . SPAD is predicted equally poorly by elevation and competition variables.

438 Leaf and plant morphology

Leaf thickness increased with elevation. Other studies have also found positive correlations between leaf thickness and elevation, identifying climatic drivers such as mean daily insolation and diurnal temperature variation (?), which lead to reduced leaf pay-back times and a need to grow leaves that can survive the more variable environmental conditions found at higher elevations (?). Increased UV-B results in an increase in cuticle thickness, to reduce the concentration of UV-B absorbed by photosystem II (PSII) where it can cause damage and thus photoinhibition (??). In this study however, it is unclear whether the insolation UV-B fraction does increase with elevation as it was

446 not measured. Additionally, it is expected that frequent cloud immersion in the high elevation
447 sites would reduce UV-B absorption and thus the need for thick cuticles. Leaf thickness decreased
448 under increased canopy density (Figure ??), adding support to the conclusion that increased direct
449 sunlight is the cause of the decrease in leaf thickness with elevation.

450 Leaf area variation was explained poorly by both competition variables. Previous studies have
451 shown a clear decrease in leaf area with elevation, citing decreases in canopy density and an increase
452 in nutrient competition with elevation as drivers of this variation (?). Plants with access to higher
453 resource levels generally invest in leaves which can achieve a higher photosynthetic rate per energy
454 input in leaf construction, at the expense of leaf longevity (?). In the plots studied here however,
455 available nitrogen does not decrease with elevation, though elevational variation in other nutrients
456 is not known.

457 Leaf:height ratio decreased with elevation (Figure ??) meaning that plants became less leafy
458 per unit stem height as elevation increased. However this relationship explained very little of the
459 variance in leaf:height ratio (Table ??). Competition variables had little effect on leaf:height ratio
460 (Figure ??). Few studies have focussed specifically on measures of leaf:height ratio or number of
461 leaves as an adaptive/acclimatory trait though we may interpret that a reduction in “leafiness” is
462 an extension of the trend seen in reduced leaf area with elevation. Seedlings may be more likely to
463 produce fewer leaves in order to allocate more biomass to structural support in those leaves that
464 are grown (?).

465 Stem volume decreased with ISI (Figure ??). This may have contributed to the increase in
466 stem volume with elevation, as ISI decreases with elevation (Figure ??). Other studies have found
467 that stem volume increases with average wind speed in order to provide greater stem support (?),
468 and that stems become more elongated as diurnal temperature range increases (?). Wind speed
469 is expected to increase with elevation as is diurnal temperature range, providing further support
470 for the trend seen here. An increase in stem volume with elevation suggests that tree seedlings are
471 allocating less biomass to other parts such as the leaves, meaning that plant growth may be slower
472 at higher elevations. This is supported by the negative relationship between leaf area and elevation,
473 and the negative relationship between leaf:height ratio and elevation, which suggests that seedlings
474 produce fewer, smaller leaves as elevation increases.

475 Summary

476 Stem volume was the only morphological plant trait that showed clear variation with a competition
477 variable (ISI), therefore H_{n1} is accepted for all other morphological plant traits. All morpholog-
478 ical plant traits were best explained by a multiple fixed effect model including elevation and a
479 combination of competition variables, therefore H_{n2} is accepted for all morphological plant traits.
480 Morphological plant traits varied across elevation in a manner similar to that identified by previous
481 studies, responding to elevation dependent abiotic environmental variables such as temperature and
482 nutrient availability. The strength of the relationships seen here is not as great as that demonstrated
483 by other studies, possibly because of the comparatively low sample size per species in this study
484 compared to larger reviews and the presence of confounding environmental variables that were not
485 accounted for in statistical analysis.

486 **5 Variation in plant traits with elevation**

487 Within each species, plant traits vary across elevation, with slope standard errors overlapping zero
488 in only a few instances (Figure ??). H_{n4} can therefore be rejected, and it can be concluded that
489 the individuals sampled in this study are acclimating their morphology in response to elevationally
490 dependent environmental variables. The difference in magnitude and direction of the relationships
491 shows that species are responding differently to changes in elevation. Supporting the observations
492 and predictions of other studies that species are likely to migrate at different rates to climate
493 change. Those species showing increased morphological change with elevation are expected to be
494 more sensitive to changes in climate and are thus more likely to show greater migration rates.

495 **Variation among species**

496 Species varied largely in the direction, magnitude and variance of their plant trait response to
497 elevation (Figure ??), therefore H_{n5} is rejected. Variation among species in slope implies that species
498 differ in their sensitivity to changing environmental conditions across elevation. *D. lamarckianum*
499 and *I. deltoidea*, the two monocot species, show no similarity in their plant trait response to elevation,
500 often having different slope directions for a given plant trait. Together, *D. lamarckianum* and *I.*
501 *deltoidea* show no difference to dicot species in terms of their plant trait-elevation relationship.
502 *A. verticillata* has a comparatively large variance for all trait-elevation relationships except stem
503 volume. This implies that *A. verticillata* is either more sensitive to changes in climate, or that it
504 has a larger acclimatory range than other species; both may be true. *A. verticillata* has a very small
505 elevational range (Figure ??) but is also one of the most common tree species found along this set of
506 plots (Appendix VI). This supports the theory that common species have a wider acclimatory range
507 and that species with small ranges are sensitive to environmental variation. In contrast, *Myrcia*
508 spp. has little variation in plant traits compared to other species but has the largest elevational
509 range, the *Myrcia* spp. species sampled are among the rarer species sampled.

510 Leaf thickness had a similar positive relationship with elevation in 7/9 species, whereas *I. del-*
511 *toidea* and *S. patula* featuring reduced leaf thicknesses with elevation (Figure ??). *C. thurifera*
512 had exceptionally high variance compared to other species, this is due to dense and prominent leaf
513 vein structure in this species (Appendix V). For many *C. thurifera* individuals, the diameter of the
514 micrometer used to measure leaf thickness was too wide to be placed between the prominent leaf
515 veins, leading to an over-estimation of leaf thickness for these individuals. Regardless, *C. thurifera*
516 showed a similar increase in leaf thickness with elevation. *I. deltoidea* had the steepest decrease
517 in leaf thickness over elevation (Figure ??). This trend may be a peculiarity of the species or a
518 result of environmental conditions at the upper sample plot for this species (VC). It is impossible
519 to confirm whether site level variation at VC had a peculiar effect on *I. deltoidea* leaf thickness as *I.*
520 *deltoidea* was the only species sampled at this site. Potentially, the greater leaf thickness at PA400
521 compared to VC is due to an adaptation to increased herbivory pressure at PA400. There is no
522 evidence for this increase in herbivory in lowland plots other than a general trend that herbivory
523 pressure decreases with elevation in tropical forests (?).

524 **Summary**

525 Tree seedlings are responding to changes in elevationally dependent environmental variables by
526 altering their morphology. Additionally, the strength of the plant trait response varies between
527 species, suggesting that some species are more sensitive to environmental change than others.

528 The lack of a clear relationship between plant traits and competition intensity, suggests that
529 tree seedlings are not affected by the biotic environment at the extremes of their ranges more than
530 they are by other environmental variation. Species will therefore continue to migrate upslope,
531 largely unimpeded by changes in biotic environment. It is possible that species will encounter biotic
532 environmental thresholds beyond which adaptation and acclimation are no longer able to prevent
533 stress and increased mortality. In order to answer these questions experimental transplantation is
534 recommended, in order to place individuals outside of their current range. Even then, experimental
535 transplantations do not account for potentially rapid micro-evolution that may occur as species
536 migrate into novel environments. Sufficiently rapid micro-evolution could result in species being
537 able to migrate upslope almost indefinitely, as they adapt and become more able to acclimate to
538 changing climates.

539 **6 Predictions for future species migration**

540 This study confirms that adult-seedling competition intensity decreases with elevation (H_{n3}), and
541 that this decrease causes some proportion of the effect of elevation on plant traits, though this
542 proportion is likely to be small as LMMs show that elevation still has the greatest influence over
543 plant traits, despite including competition variables alongside elevation in multiple fixed effect
544 models. As such, species may continue to move upslope as temperature increases, without being
545 negatively affected physiologically at the upper limits of their ranges by adapting their morphology
546 to the changing environment. The results from this study however, cannot be used to determine
547 what will happen if a species reaches its adaptational limits as its range shifts. Given that few species
548 experienced physiological stress, it is suggested that none of the species sampled have reached this
549 limit yet. The exception being *C. revoluta*, which shows some evidence of increased physiological
550 stress with elevation and relatively flat relationships between elevation and plant traits, though this
551 trend cannot be confirmed without more study.

552 Most species featured a decrease in photosynthetic efficiency at the bottom of their elevational
553 ranges. This implies that these species may experience progressively greater plant stress at the
554 bottom of their ranges as temperature increases, and the bottom of their range will continue to
555 shift upslope as a result. This study cannot infer whether the contraction of species' lower range
556 limits will be faster or slower than the expansion of the upper range limit, though other studies
557 have suggested that lower range limits will shift upslope faster than upper limits (Campbell et al.,
558 2007), owing to climate change proceeding faster than micro-evolutionary processes to adapt to
559 higher elevations. This will lead to an overall reduction in range size for many species.

560 **7 Limitations of this study**

561 This study sampled seedling physiology over a narrow time period. While F_v/F_m and SPAD are
562 unlikely to vary on a daily basis, they may do over the course of a season (?). Seedlings are likely to
563 alter their leaf physiology and morphology in response to a temporally heterogeneous environment
564 throughout the course of their life. As canopy gaps open and close the light and precipitation
565 regime will change. The measured physiological responses of individuals therefore may not be
566 representative of its physiology over a lifetime. Furthermore, this study only measured seedlings,
567 ignoring other life stages. This means the results of this study cannot be used to directly infer
568 the effects of biotic interactions on plant traits across entire populations. It is likely however, that
569 established adult trees will be less sensitive to competition from other adult trees and completely
570 insensitive to competition from seedlings (?).

571 Nine tree species were selected for this study. Although these species are common in the areas
572 we sampled (Appendix VI), there are many other species which may react more or less to the biotic
573 environment. There is evidence that rare species are more affected by environmental factors (??).
574 Rare species are more likely to occupy specialist niches, which are narrower on a local geographical
575 scale than those of generalist species (?). This means however that measurements of rare species
576 would need to be particularly sensitive to small changes from potentially multiple environmental
577 factors to effectively model effects on seedling health. The evolutionary histories of specialists means
578 they are less likely to be able to acclimate to novel environments. Compared to the common species
579 studied here, rare species will not have such a large direct effect on globally significant ecosystem
580 services such as carbon sequestration, albedo, and drainage. This does not mean that rare species
581 do not have the potential to heavily influence ecosystem services indirectly. ?, and ? found that less
582 common species play vital supporting roles in maintaining ecosystem functions such as enhancing
583 invasion resistance and making limiting resources available to other species .

584 There is large potential for falsely inferring causation from the results of this study. Along
585 elevational gradients many environmental factors both abiotic and biotic co vary. For example,
586 this study concluded that an increase in ISI caused a decrease in photosynthetic efficiency. How-
587 ever, it was found that ISI covaries with elevation, along with many other potential unmeasured
588 environmental variables, therefore photosynthetic efficiency may have merely inversely correlated
589 with ISI rather than ISI causing the variation in photosynthetic efficiency, despite well-documented
590 supporting evidence.

591 This study is deliberately wide in its scope, using competition intensity proxies in order to infer
592 the influences of many ecosystem processes such as nutrient competition, shading, etc.. By not
593 explicitly testing the effects of these mechanistic processes, which are complex in their effects, we
594 cannot determine the relative contribution of each process implicit in each competition proxy. It
595 is recommended therefore that experiments under constant environmental conditions explicitly test
596 the effect of variation in ecosystem processes which are implied to change as a result of variation in
597 the competition proxies measured here, such as nutrient availability and shading.

598 The study did not use experimental treatments. It could be argued therefore that measured
599 seedlings would have been unlikely to show stress at all, as seedlings would not have grown to the
600 minimum size needed for measurement otherwise.

601 8 Further research

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

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

612 Conclusion

613 This study has provided an estimation of the relative effects of seedling-seedling and adult-seedling
614 competition on neotropical tree seedling plant traits, thereby evaluating the potential for competition
615 effects to limit vertical range shifts in response to anthropogenically induced temperature
616 increase. This study found that the intensity of adult-seedling competition affected photosynthetic
617 efficiency, stem volume and leaf thickness. Investigation of the variation in these competition proxies
618 over elevation showed that competition effects form part of a complement of environmental variables
619 that covary across elevation, resulting in an overall variation in plant traits with elevation.

620 Multiple fixed effect models were of better quality when including competition variables alongside
621 elevation as predictors of plant traits. In light of this, it is suggested that adult-seedling competition
622 proxies or more direct measures of adult-seedling competition are included in future species
623 distribution models alongside climatic variables in order to more accurately and precisely predict
624 species migrations.

625 This study cannot make direct predictions of how species will react to environmental conditions
626 outside of those measured here. Instead it is suggested that future studies focus on experimental
627 transplantation of seedlings to elevations outside of their current ranges in order to build more
628 realistic predictions of future range shift potential.

629 There was marked variation between species in their plant trait response to elevation. This
630 provides supporting evidence for conclusions of other studies which either predict or demonstrate
631 that species differ in their sensitivity to variation in environment and will therefore be likely to vary
632 in their rate of upslope migration. The presence of species specific range shift trends supports the
633 conclusion that biotic environmental effects should be included in range-shift models, as they are
634 only likely to become stronger over time as species ranges overlap.

635 Forest structure based competition affects physiological stress independently of elevation

636 References

- 637 Akaike, H. (1992), ‘Information theory and an extension of the maximum likelihood principle’,
638 *Breakthroughs in Statistics* pp. 610–624.
- 639 Araújo, M. B., Thuiller, W. and Pearson, R. G. (2006), ‘Climate warming and the decline of
640 amphibians and reptiles in europe’, *Journal of Biogeography* **33**, 1712–1728.
- 641 Barton, K. (2019), *MuMIn: Multi-Model Inference*. R package version 1.43.6.
- 642 Bates, D., Mächler, M., Bolker, B. M. and Walker, S. C. (2015), ‘Fitting linear mixed-effects models
643 using lme4’, *Journal of Statistical Software* **67**(1), 1–48.
- 644 Bell, D. M., Bradford, J. B. and Lauenroth, W. K. (2014), ‘Early indicators of change: divergent
645 climate envelopes between tree life stages imply range shifts in the western united states’, *Global
646 Ecology and Biogeography* **23**, 168–180.
- 647 Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. and Courchamp, F. (2012), ‘Impacts of
648 climate change on the future of biodiversity’, *Ecology Letters* **15**, 365–377.
- 649 Berry, P. M., Dawson, T. P., Harrison, P. A. and Pearson, R. G. (2002), ‘Modelling potential impacts
650 of climate change on the bioclimatic envelope of species in britain and ireland’, *Global Ecology &
651 Biogeography* **11**, 453–462.

- 652 Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H.
653 and White, J. S. (2008), 'Generalized linear mixed models: a practical guide for ecology and
654 evolution', *Trends in Ecology and Evolution* **24**(3), 127–135.
- 655 Campbell, C., Atkinson, L., Zaragoza-Castells, J., Lundmark, M., Atkin, O. and Hurry, V. (2007),
656 'Acclimation of photosynthesis and respiration is asynchronous in response to changes in temper-
657 ature regardless of plant functional group', *New Phytologist* **176**, 375–389.
- 658 Chen, I., Hill, J. K., Ohlemüller, R., Roy, D. B. and Thomas, C. D. (2011), 'Rapid range of species
659 associated with high levels of climate warming', *Science* **333**, 1024–1026.
- 660 Colwell, R. K., Brehm, G., Cardelús, C. L., Gilman, A. C. and Longino, J. T. (2008), 'Global
661 warming, elevational range shifts and lowland biotic attrition in the wet tropics', *Science* **322**, 258–
662 261.
- 663 Coomes, D. A. and Grubb, P. J. (2000), 'Impacts of root competition in forests and woodlands: a
664 theoretical framework and review of experiments', *Ecological Monographs* **70**(2), 171–207.
- 665 Corlett, R. T. and Westcott, D. A. (2013), 'Will plant movements keep up with climate change?',
666 *Trends in Ecology & Evolution* **28**(8), 482–488.
- 667 Coste, S., Baraloto, C., Leroy, C., Marcon, É., Renaud, A., Richardson, A. D., Roggy, J., Schimann,
668 H., Uddling, J. and Héault, B. (2010), 'Assessing foliar chlorophyll contents with the spad-502
669 chlorophyll meter: a calibration test with thirteen tree species of tropical rainforest in french
670 guiana', *Annals of Forest Science* **67**(6), 607–607.
- 671 Davis, M. A., Wrage, K. J. and Reich, P. B. (1998), 'Competition between tree seedlings and
672 herbaceous vegetation: support for a theory of resource supply and demand', *Journal of Ecology*
673 **86**, 652–661.
- 674 Dawson, T. P., Jackson, S. T., House, J. I., Prentice, I. C. and Mace, G. M. (2011), 'Beyond
675 predictions: biodiversity conservation in a changing climate', *Science* **332**, 53–58.
- 676 Dobson, A., Lodge, D., Alder, J., Cumming, G. S., Keymer, J., McGlade, J., Mooney, H., Rusak,
677 J. A., Sala, O., Wolters, V., Wall, D., Winfree, R. and Xenopoulos, M. A. (2006), 'Habitat loss,
678 trophic collapse, and the decline of ecosystem services', *Ecology* **87**(8), 1915–1924.
- 679 Ettinger, A. K., Ford, K. R. and HilleRisLambers, J. (2011), 'Climate determines upper, but not
680 lowe, altitudinal range limits of pacific northwest conifers', *Ecology* **92**(6), 1323–1331.
- 681 Feeley, K. J., Malhi, Y., Zelazowski, P. and Silman, M. R. (2012), 'The relative importance of defor-
682 estation, precipitation change, and temperature sensitivity in determining the future distributions
683 and diversity of amazonian plant species', *Global Change Biology* **18**, 2636–2647.
- 684 Feeley, K. J., Silman, M. R., Bush, M. B., Farfan, W., Cabrera, K. G., Malhi, Y., Meir, P., Revilla,
685 N. S., Quisiyupanqui, M. N. R. and Saatchi, S. (2011), 'Upslope migration of andean trees',
686 *Journal of Biogeography* **38**, 783–791.
- 687 Frazer, G. W., Fournier, R. A., Trofymow, J. A. and Hall, R. J. (2001), 'A comparison of digital
688 and film fisheye photography for analysis of forest canopy structure and gap light transmission',
689 *Agricultural and Forest Meteorology* **109**, 249–263.
- 690 Gadow, K. V. and Hui, G. (1999), *Modelling Forest Development*, Springer, The Netherlands.
- 691 Genty, B., Briantais, J. and Baker, N. R. (1989), 'The relationship between the quantum yield
692 of photosynthetic electron transport and quenching of chlorophyll fluorescence', *Biochimica et
693 Biophysica Acta* **990**, 87–92.

- 694 Gibson-Reinemer, D. K., Sheldon, K. S. and Rahel, F. J. (2015), 'Climate change creates rapid
695 species turnover in montane communities', *Ecology and Evolution* **5**(12), 2340–2347.
- 696 Girardin, C. A. J., Espejob, J. E. S., Doughty, C. E., Huasco, W. H., Metcalfe, D. B., Durand-Baca,
697 L., Marthews, T. R., Aragao, L. E. O. C., Farfàn-Rios, W., García-Cabrera, K., Halladay, K.,
698 Fisher, J. B., Galiano-Cabrera, D. F., Huaraca-Quispe, L. P., Alzamora-Taype, I., Eguiluz-Mora,
699 L., Salinas-Revilla, N., Silman, M. R., Meir, P. and Malhi, Y. (2014), 'Productivity and carbon
700 allocation in a tropical montane cloud forest in the peruvian andes', *Plant Ecology & Diversity*
701 **7**(1-2), 107–123.
- 702 Gruber, C. E., Nakagawa, S., Laws, R. J. and Jamieson, I. G. (2011), 'Multimodel inference in
703 ecology and evolution: challenges and solutions', *Journal of Evolutionary Biology* **24**, 699–711.
- 704 Hegyi, F. (1974), A simulation model for managing jack-pine stands, in 'Royal College of Forestry,
705 editor', Royal College of Forestry, Stockholm, Sweden, pp. 74–90.
- 706 Hughes, L. (2000), 'Biological consequences of global warming: is the signal already apparent?',
707 *Trends in Ecology and Evolution* **15**(2), 56–61.
- 708 Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W. S., Reich, P. B., Scherer-Lorenzen, M.,
709 Schmid, B., Tilman, D., van Ruijven, J., Weigelt, A., Wilsey, B. J., Zavaleta, E. S. and Loreau,
710 M. (2011), 'Hugh plant diversity is needed to maintain ecosystem services', *Nature* **477**, 199–203.
- 711 Jonckheere, I., Fleck, S., Nackaerts, K., Muys, B., Coppin, P., Weiss, M. and Baret, F. (2004),
712 'Review of methods for in situ leaf area index determination part i. theories, sensors and hemi-
713 spherical photography', *Agricultural and Forest Meteorology* **121**, 19–35.
- 714 Jupp, D. L. B., Culvenor, D. S., Lovell, J. L., Newnham, G. J., Strahler, A. H. and Woodcock,
715 C. E. (2008), 'Estimating forest lai profiles and structural parameters using a ground-based laser
716 called 'echidna", *Tree Physiology* **29**, 171–181.
- 717 Krause, G. H., Koroleva, O. Y., Dalling, J. W. and Winter, K. (2001), 'Acclimation of tropical tree
718 seedlings to excessive light in simulated tree-fall gaps', *Plant, Cell and Environment* **24**, 1245–
719 1352.
- 720 Lee, W., von Gadow, K., Chung, D., Lee, J. and Shin, M. (2004), 'Dbh growth model for *Pinus*
721 *densiflora* and *Quercus variabilis* mixed forests in central korea', *Ecological Modelling* **176**, 187–
722 200.
- 723 Lenoir, J., Gégout, J., Guisan, A., Vittoz, P., Wohlgemuth, T., Zimmermann, N. E., Dullinger, S.,
724 Pauli, H., Willner, W. and Svenning, J. (2010), 'Going against the flow: potential mechanis,s for
725 unexpected downslope range shifts in a warming climate', *Ecography* **33**, 295–303.
- 726 Lenoir, J., Gégout, J., Pierrat, J., Bontemps, J. and Dhôte, J. (2009), 'Differences between tree
727 species seedling and adult altitudinal distribution in mountain forests during the recent warm
728 period (1986-2006)', *Ecography* **32**, 765–777.
- 729 Lenoir, J. and Svenning, J. C. (2015), 'Climate-related range shifts - a global multidimensional
730 synthesis and new research directions', *Ecography* **38**, 15–28.
- 731 Lewis, S. L. and Tanner, E. V. J. (2000), 'Effects of above- and belowground competition on growth
732 and survival of rain forest tree seedlings', *Ecology* **81**(9), 2525–2538.
- 733 Li, F., Peng, S., Chen, B. and Hou, Y. (2010), 'A meta-analysis of the responses of woody and
734 herbaceous plants to elevated ultraviolet-b radiation', *Acta Oecologia* **36**, 1–9.
- 735 MacLean, S. A. and Beissinger, S. R. (2017), 'Species' traits as predictors of range shifts under
736 contemporary climate change: a review and meta-analysis', *Global Change Biology* **23**, 4094–
737 4105.

- 738 Malhi, Y., Silman, M., Salinas, N., Bush, M., Meir, P. and Saatchi, S. (2010), 'Introduction: ele-
739 vation gradients in the tropics: laboratories for ecosystem ecology and global change research',
740 *Global Change Biology* **16**, 3171–3175.
- 741 Mantyka, C. S., Martin, T. G. and Rhodes, J. R. (2012), 'Interactions between climate and habi-
742 tatt loss effects on biodiversity: a systematic review and meta-analysis', *Global Change Biology*
743 **18**, 1239–1252.
- 744 Martin, P. H., Fahey, T. J. and Sherman, R. E. (2010), 'Vegetation zonation in a neotropical
745 montane forest: environment, disturbance and ecotones', *Biotropica* **43**(5), 533–543.
- 746 Matsuraba, S., Krause, G. H., Aranda, J., Virgo, J., Beisel, K. G., Jahns, P. and Winter, K. (2009),
747 'Sun-shade patterns of leaf carotenoid composition in 86 species of neotropical forest plants',
748 *Functional Plant Biology* **36**, 20–36.
- 749 Maxwell, K. and Johnson, G. N. (2000), 'Chlorophyll fluorescence - a practical guide', *Journal of*
750 *Experimental Botany* **51**(345), 659–668.
- 751 McCain, C. M. and Colwell, R. K. (2011), 'Assessing the threat to montane biodiversity from discon-
752 cordant shifts in temperature and precipitation in a changing climate', *Ecology Letters* **14**, 1236–1245.
- 753 McMahon, S. M., Harrison, S. P., Armbruster, W. C., Bartlein, P. J., Beale, C. M., Edwards,
754 M. E., Kattge, J., Midgley, G., Morin, X. and Prentice, I. C. (2011), 'Improving assessment and
755 modelling of climate change impacts on global terrestrial biodiversity', *Trends in Ecology and*
756 *Evolution* **26**(5), 249–259.
- 757 Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. and Kent, J. (2000),
758 'Biodiversity hotspots for conservation priorities', *Nature* **403**, 853–858.
- 759 Parmesan, C. (2006), 'Ecological and evolutionary responses to recent climate change', *Annual*
760 *Review of Ecology, Evolution and Systematics* **37**, 637–669.
- 761 Pearson, R. G. and Dawson, T. P. (2003), 'Predicting the impacts of climate change on the dis-
762 tribution of species: are bioclimate envelope models useful?', *Global Ecology & Biogeography*
763 **12**, 361–371.
- 764 Peterson, A. T., Ball, L. G. and Cohoon, K. P. (2002), 'Predicting distributions of mexican birds
765 using ecological niche modelling methods', *Ibis* **144**, E27–E32.
- 766 Prado, F. E., Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J. A. and Hilal, M.
767 (2012), Uv-b radiation, its effects and defense mechanisms in terrestrial plants, in P. Ahmad and
768 M. Prasad, eds, 'Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate
769 Change', Springer, New York, USA, pp. 57–83.
- 770 R Core Team (2019), *R: A Language and Environment for Statistical Computing*, R Foundation for
771 Statistical Computing, Vienna, Austria.
- 772 Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. (2012), 'Nih image to imagej: 25 years of
773 image analysis', *Nat Methods* **9**(7), 671–675.
- 774 Seifert, T., Seifert, S., Seydack, A., Durrheim, G. and von Gadow, K. (2014), 'Competition effects
775 in an afrotropical forest', *Forest Ecosystems* **1**(13), 1–15.
- 776 Serrano, L. (2008), 'Effects of leaf structure on reflectance estimates of chlorophyll content', *Inter-
777 national Journal of Remote Sensing* **29**, 17–18.
- 778 Sinclair, S. J., White, M. D. and Newell, G. R. (2010), 'How useful are species distribution models
779 for managing biodiversity under future climates?', *Ecology and Society* **15**(1), 1–13.

- 780 ter Steege, H. (2018), *Hemiphot.R: Free R scripts to analyse hemispherical photographs for canopy*
781 *openness, leaf area index and photosynthetic active radiation under forest canopies*, Leiden, The
782 Netherlands. Unpublished report.
- 783 Thuiller, W., Richardson, D. M., Pyšek, P., Midgley, G. F., Hughes, G. O. and Rouget, M. (2005),
784 'Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale',
785 *Global Change Biology* **11**, 2234–2250.
- 786 Travis, J. M. J., Delgado, M., Bocedi, G., Baguette, M., Bartoń, K., Bonte, D., Boulangeat, I.,
787 Hodgson, J. A., Kubisch, A., Penteriani, V., Saastamoinen, M., Stevens, V. M. and Bullock,
788 J. M. (2013), 'Dispersal and species' responses to climate change', *Oikos* **122**, 1532–1540.
- 789 Valladares, F., Laanisto, L., Niinemets, Ü. and Zavala, M. A. (2016), 'Shedding light on shade:
790 ecological perspectives of understorey plant life', *Plant Ecology & Diversity* **9**(3), 237–251.
- 791 Van der Putten, W. H., Macel, M. and Visser, M. E. (2010), 'Predicting species distribution and
792 abundance responses to climate change: why it is essential to include biotic interactions across
793 trophic levels', *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 2025–
794 2034.
- 795 Whitaker, J., Ostle, N., Nottingham, A. T., Ccahuana, A., Salinas, N., Bardgett, R. D., Meir,
796 P. and McNamara, N. P. (2014), 'Microbial community composition explains soil respiration
797 responses to changing carbon inputs along an andes-to-amazon elevation gradient', *Journal of
798 Ecology* **102**, 1052–1071.
- 799 Wisz, M. S., Pottier, J., Kissling, W. D., Pellissier, L., Lenoir, J., Damgaard, C. F., Dormann,
800 C. F., Forchhammer, M. C., Grytnes, J., Guisan, A., Heikkinen, R. K., Hoye, T. T., Kähn,
801 I., Luoto, M., Maiorano, L., Nilsson, M., Normand, S., Öckinger, Schmidt, N. M., Termansen,
802 M., Timmermann, A., Wardle, D. A., Aastrup, P. and Svenning, J. (2013), 'The role of biotic
803 interactions in shaping distributions and realised assemblages of species: implications for species
804 distribution modelling', *Biological Reviews* **88**, 15–30.