

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     $17.6 \pm 2.9$  km and  $12.2 \pm 1.8$  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).

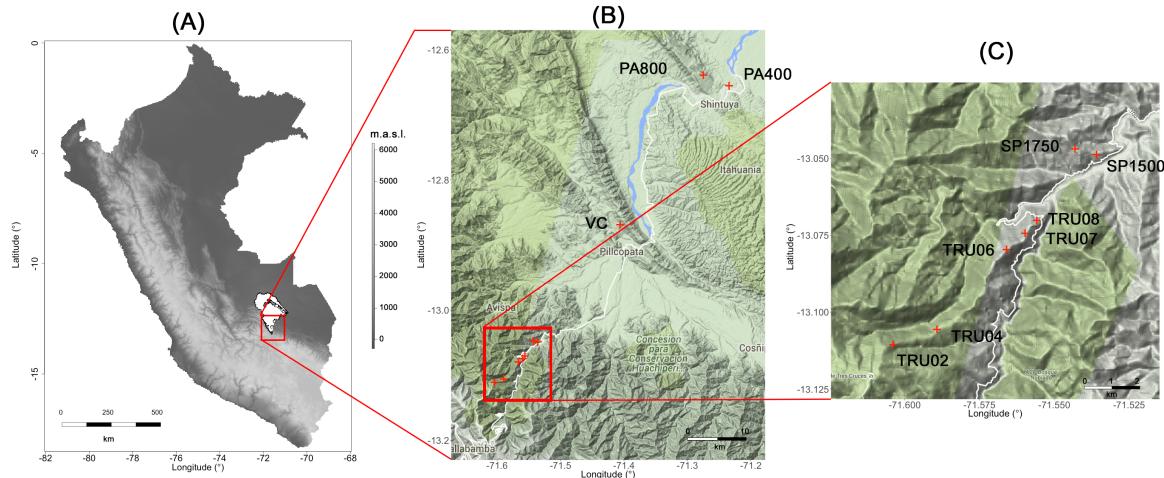


Figure 1: Maps showing the location of the study area and plot locations. (A) The site location within Peru with elevation shading, showing the proximity to Manú National Park (white area). (B) The location of the 1 ha plots within the Kosñipata Valley. (C) An enlargement of the Trocha Union and San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

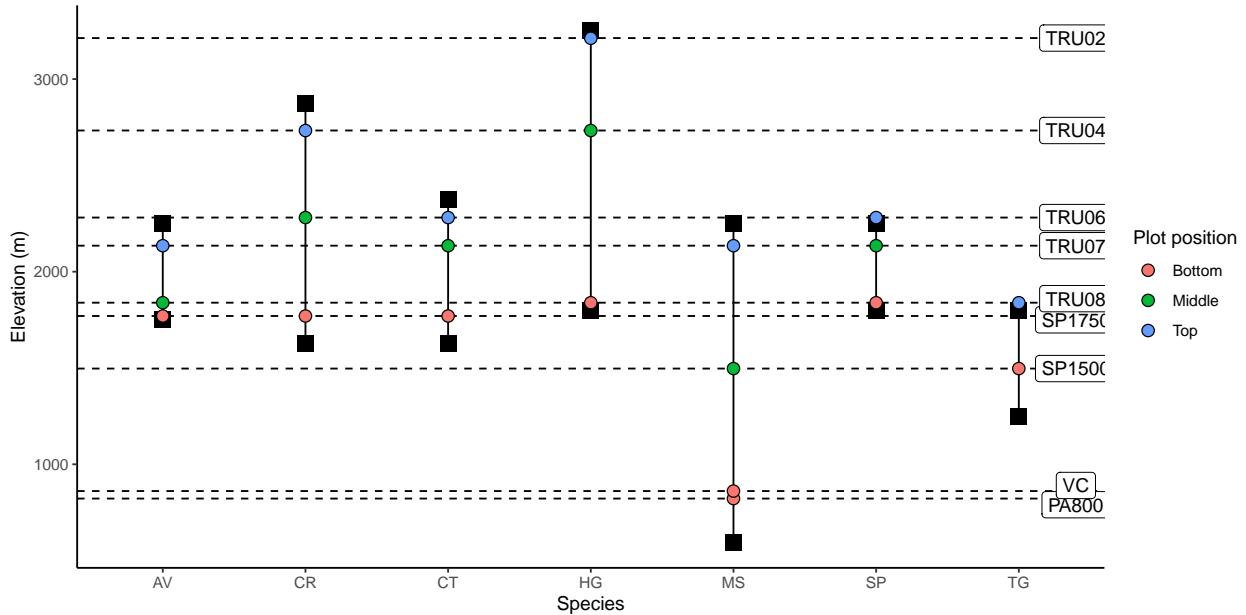


Figure 2: Elevations of study plots for each species (coloured points) with the upper and lower range extents for each species (black squares). Plot elevations are marked as dashed lines and labelled.

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

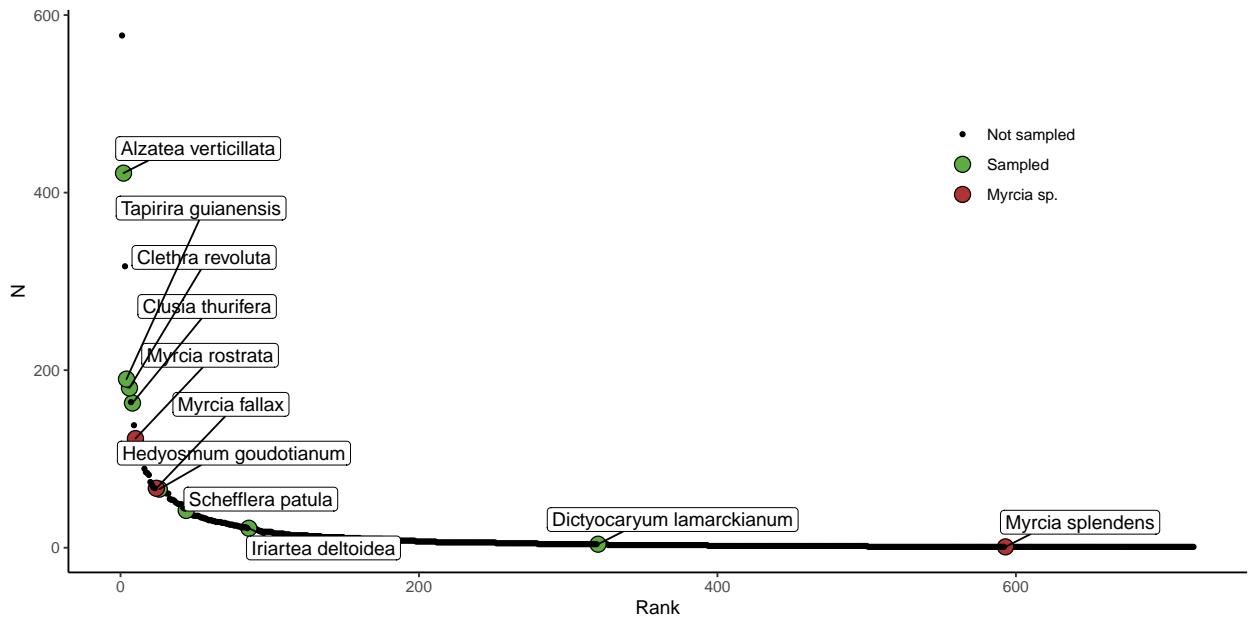


Figure 3: Rank abundance curve of all individuals >10 cm DBH of all species found in the plots measured in this study. Census data from 2014 (ABERG, unpublished data). Species sampled as part of this study are highlighted in red. *Myrcia* species which form the composite *Myrcia* spp. are highlighted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

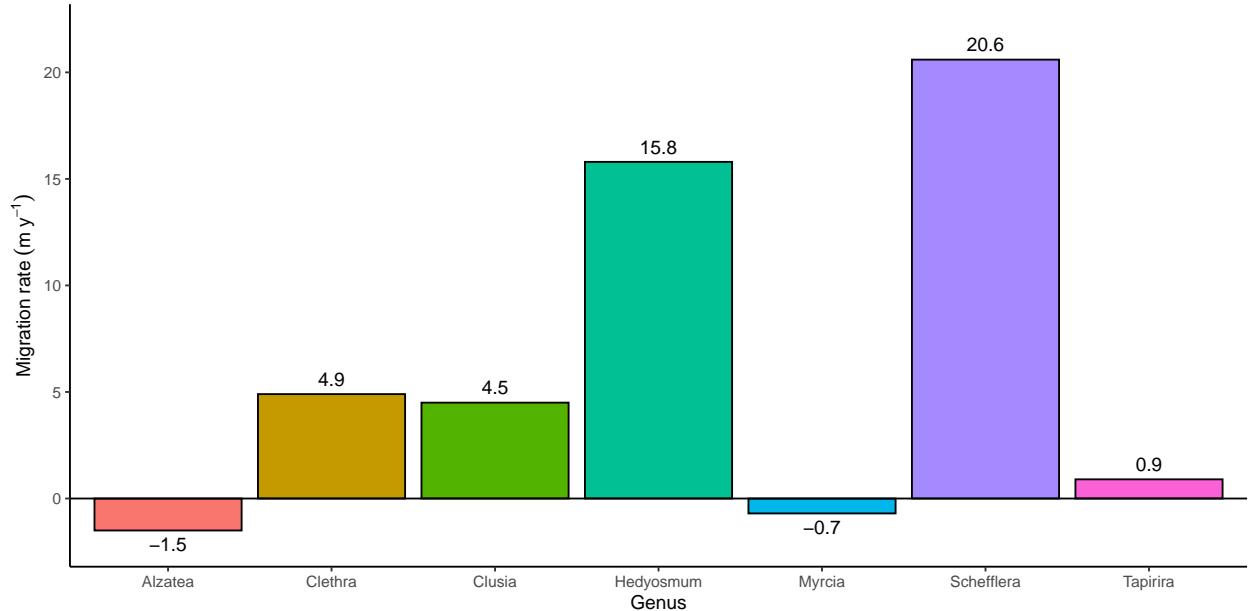


Figure 4: Estimated elevational migration rates within the Kosñipata valley for selected genera of which species are studied here. Migration rates are estimated using shifts in the centre of gravity of tree basal area as measured in the ABERG 1 Ha plot network.

### 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- $\alpha$  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  $\text{ha}^{-1}$  of each plot, and interpolated the trees  $\text{ha}^{-1}$  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).

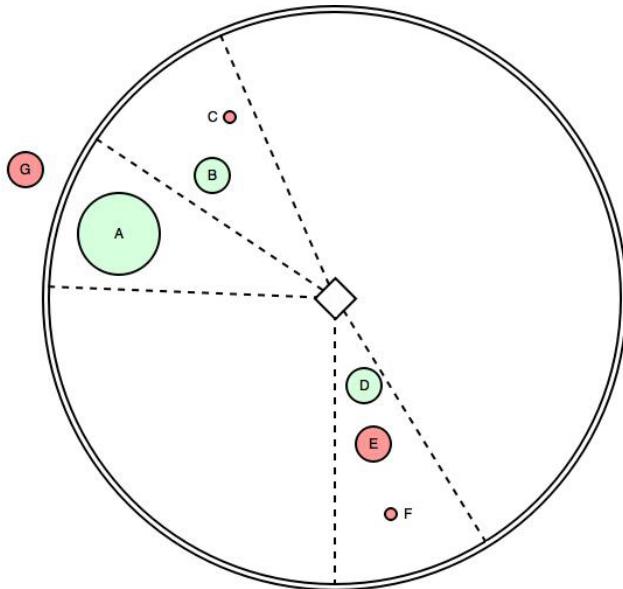


Figure 5: Schematic diagram showing the iterative selection of active competitor trees for the Iterative Seedling Index (ISI) (Equation 3). Trees marked in green (A, B, D) are active competitors for the tree of interest (black diamond). Trees marked in red (C, E, F, G) are non-active competitors, coloured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

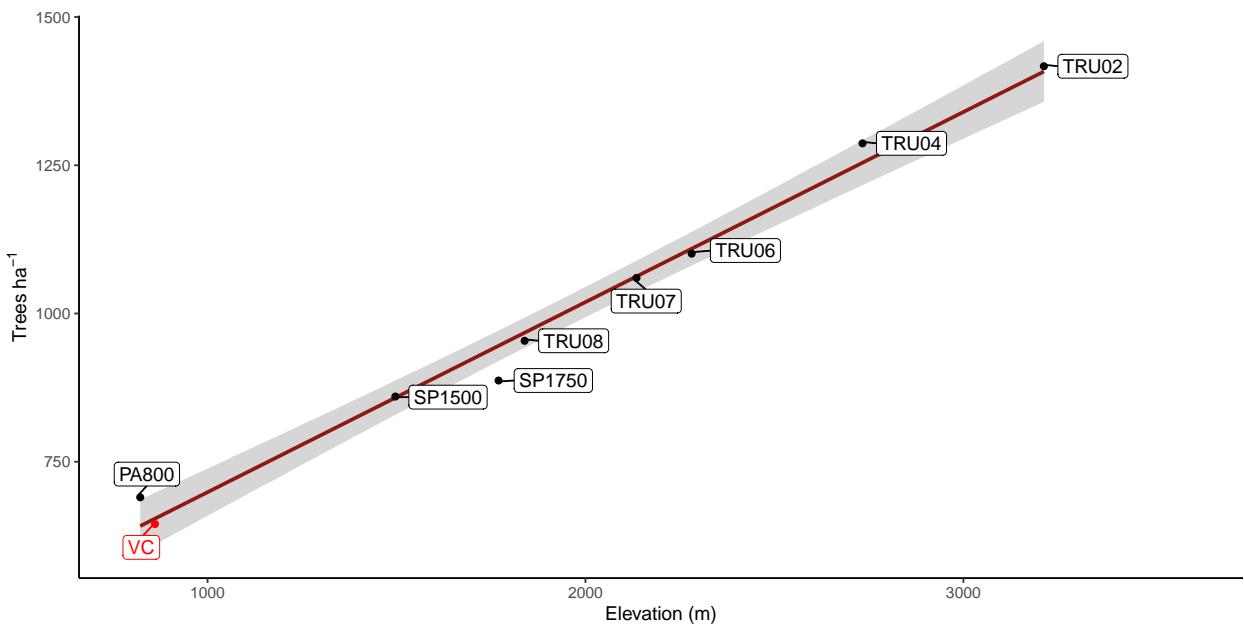


Figure 6: Linear regression with 95% confidence interval of number of trees per hectare for each site, used to estimate number of trees per hectare for site VC.  $R^2 = 0.896$ ,  $F_{(1,7)} = 579.5$ ,  $p < 0.001$ .

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

Site	Trees ha <sup>-1</sup>	$C_R$ (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  $\Delta AIC_r$  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 12, 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. Linear models of photosynthetic efficiency over elevation showed that none of the species  
257 level regressions dipped below the critical stress threshold of 0.7. Of the 151 measured seedlings,  
258 12 had an  $F_v/F_m$  below 0.7. Of those 12 seedlings, 4 were *Alzatea verticillata* (AV), 2 were *Clethra*  
259 *revoluta* (CR) and 1 each from *Clusia thurifera* (CT) and *Myrcia* spp. (MS). Chlorophyll- $\alpha$  generally  
260 decreased with elevation, with negative trends in *A. verticillata* (AV), *C. thurifera* (CT), *Myrcia*  
261 spp. (MS), *Schefflera patula* (SP) and *Tapirira guianensis* (TG). *C. revoluta* (CR) and *Hedyosmum*  
262 *goudotianum* (HG) had positive trends (Figure 9).

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

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

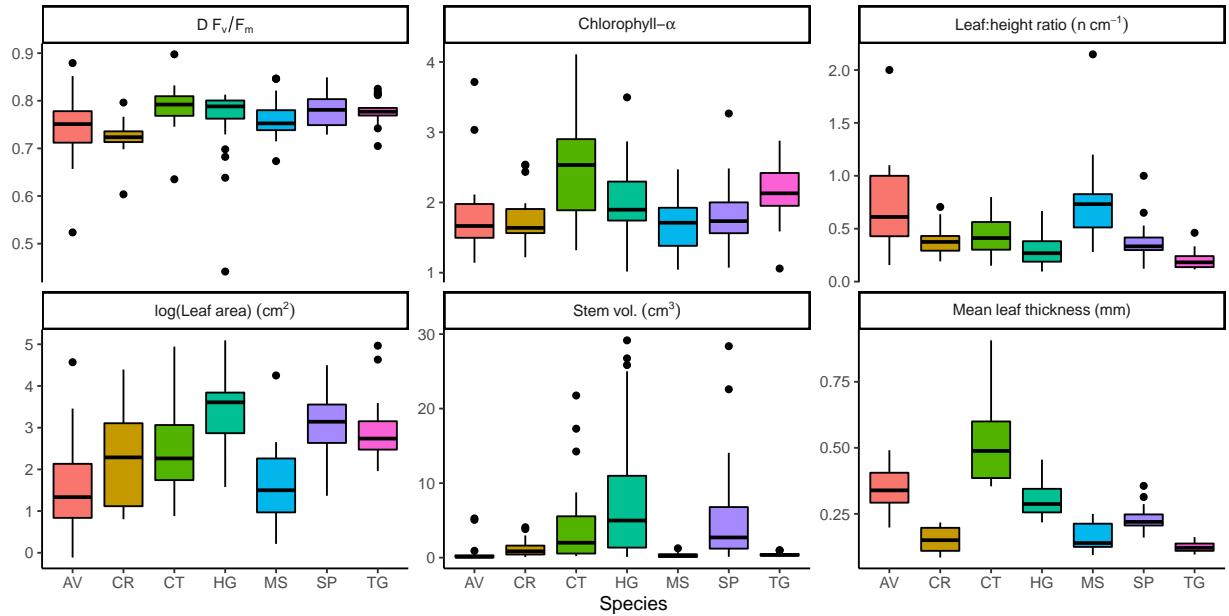


Figure 7: Box plots showing the variation in plant trait values within each species.

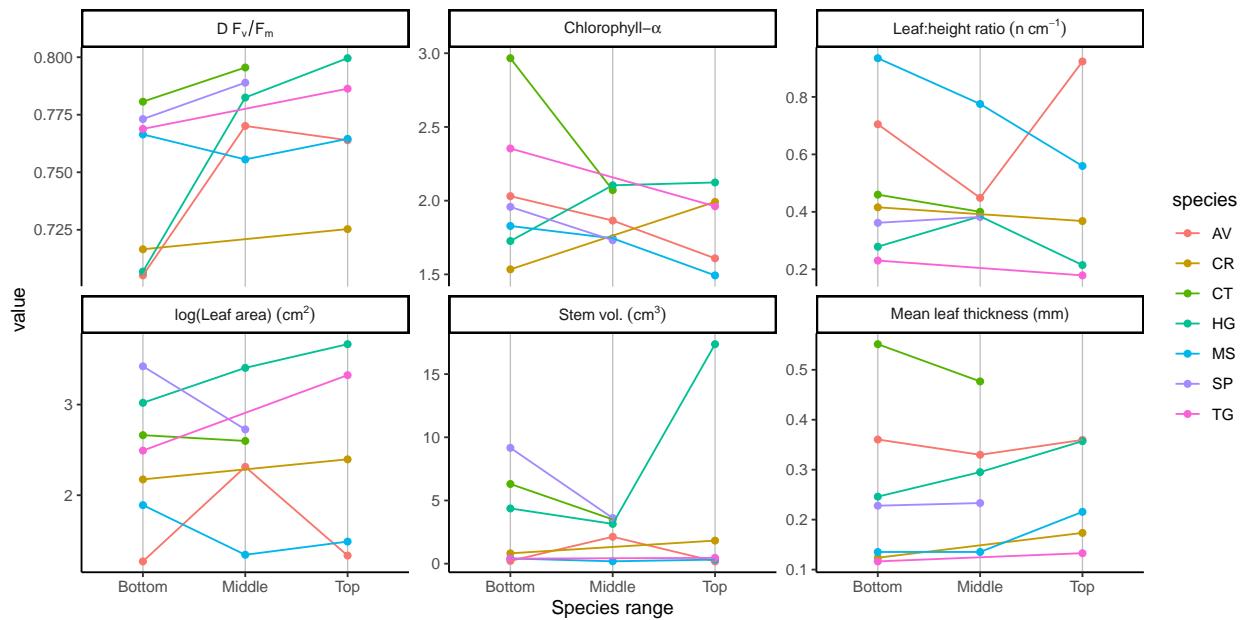


Figure 8: Interaction plots showing the variation in plant trait values within each species.

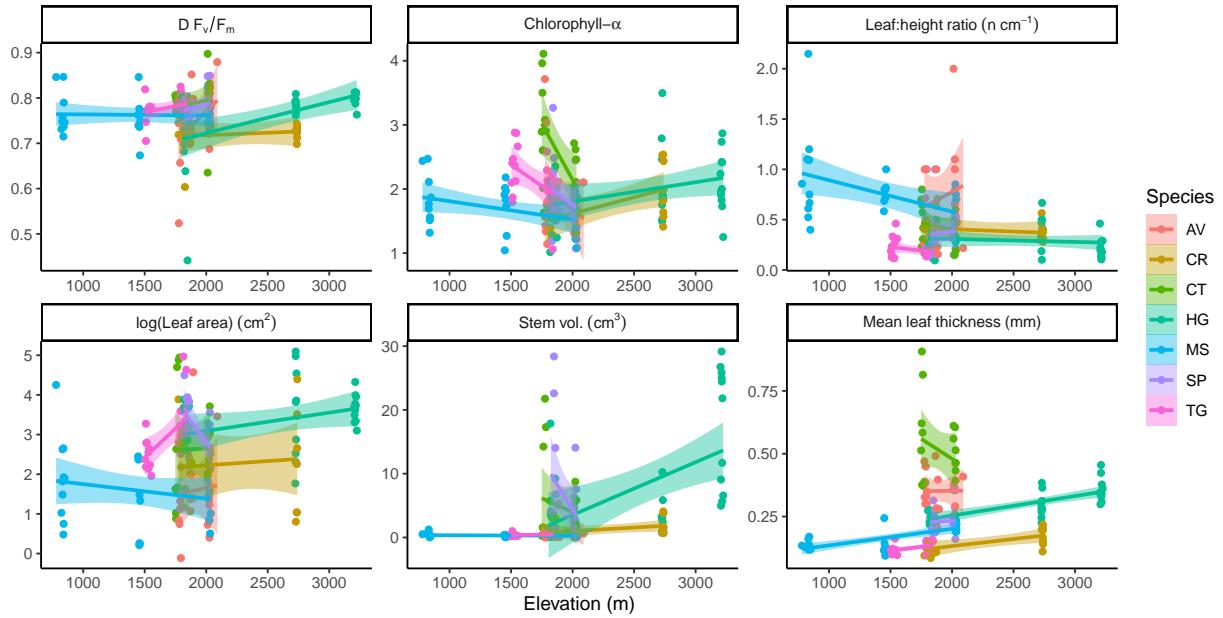


Figure 9: Scatter plots with linear model fits for each species, showing the variation in plant stress variables and plant traits across elevation.

### 276 3.2 Directional effects of adult competition on plant traits

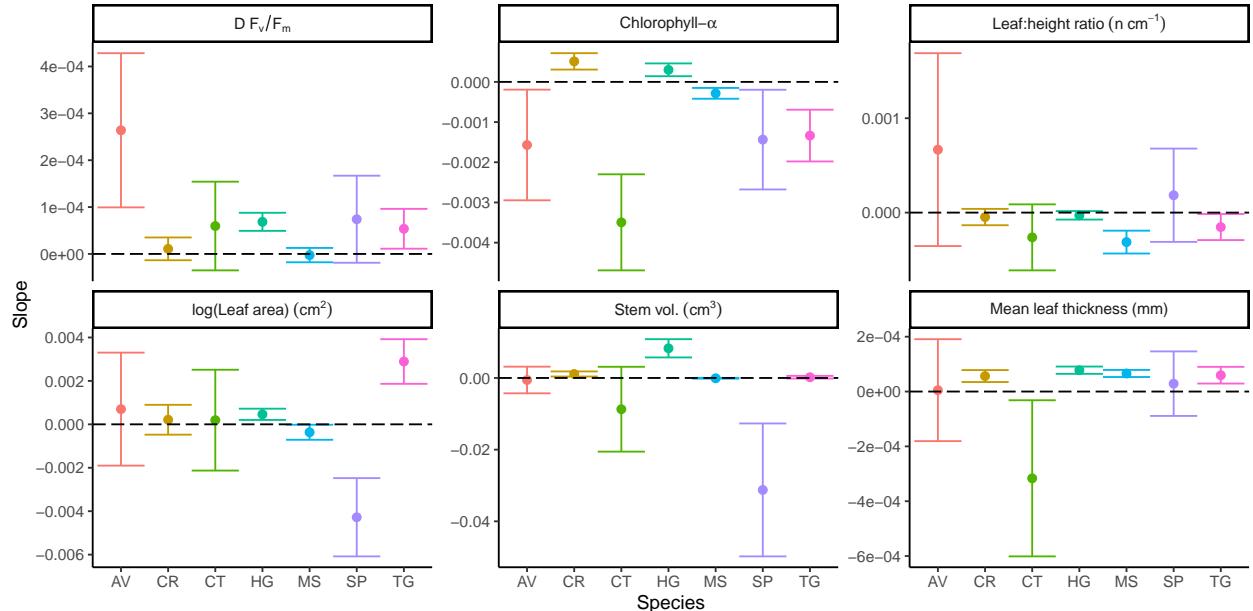


Figure 10: Interval plots showing the effect sizes (slopes) of each fixed effect in single fixed effect linear mixed effects models of plant traits against forest structure variables and elevation, for comparison.

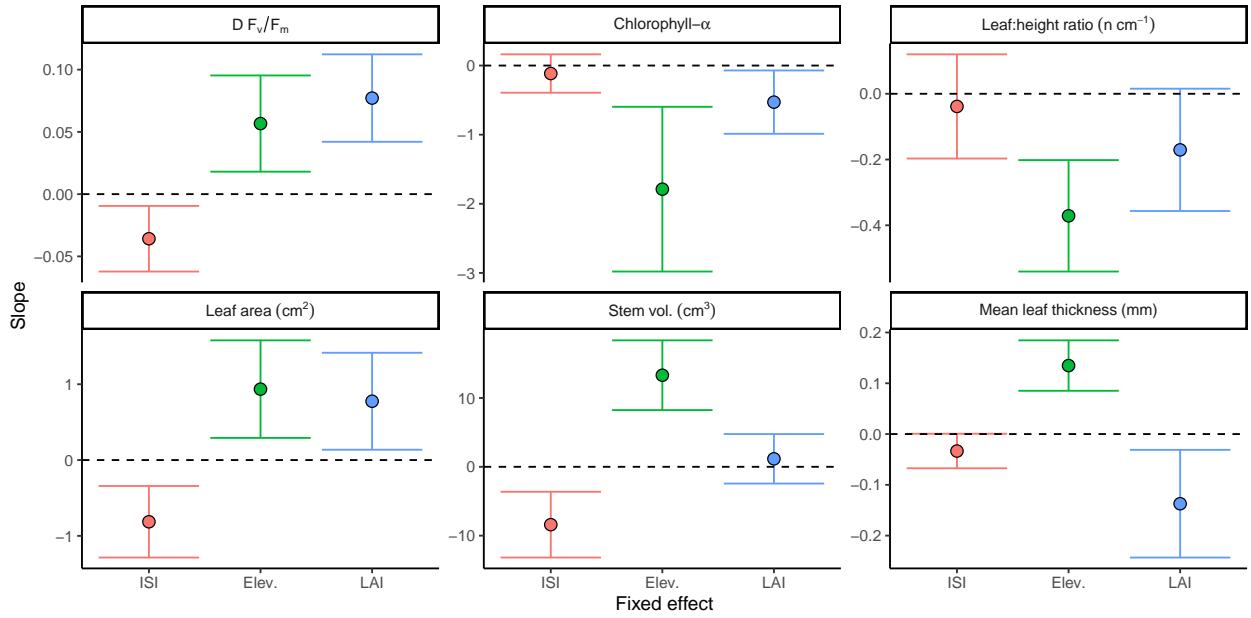


Figure 11

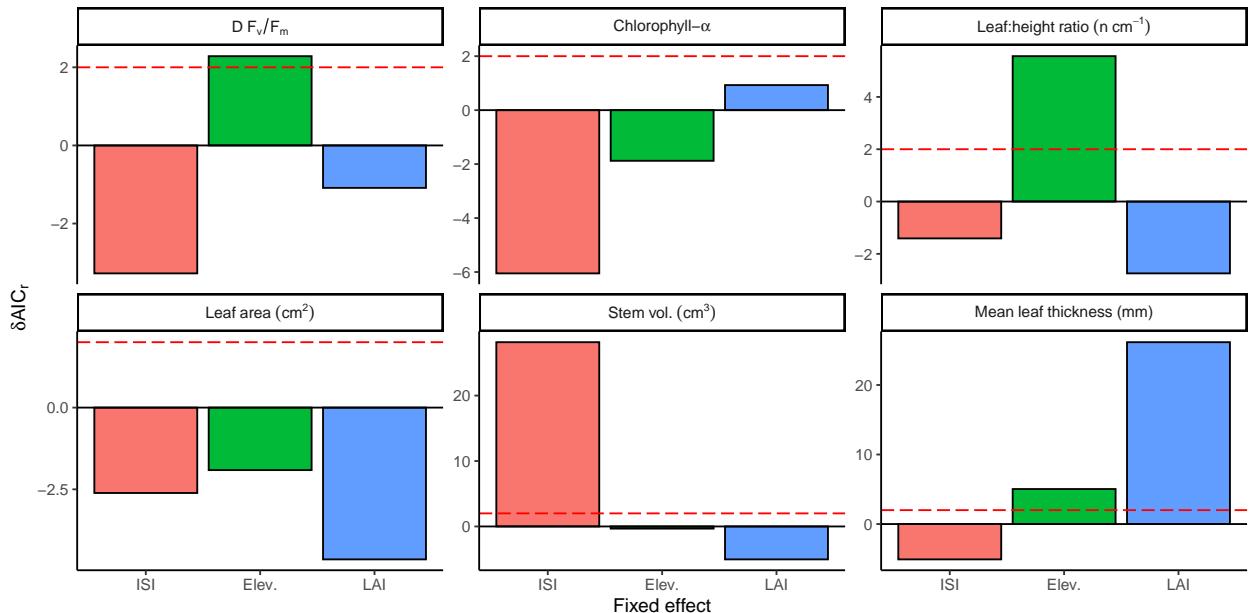


Figure 12: The difference in AIC values between each single fixed effect model and a corresponding random effects model using no fixed effects. A model with  $\Delta\text{AIC}_r > 2$  means the model is of appreciably higher quality than the random effects model. The horizontal dashed red line indicates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

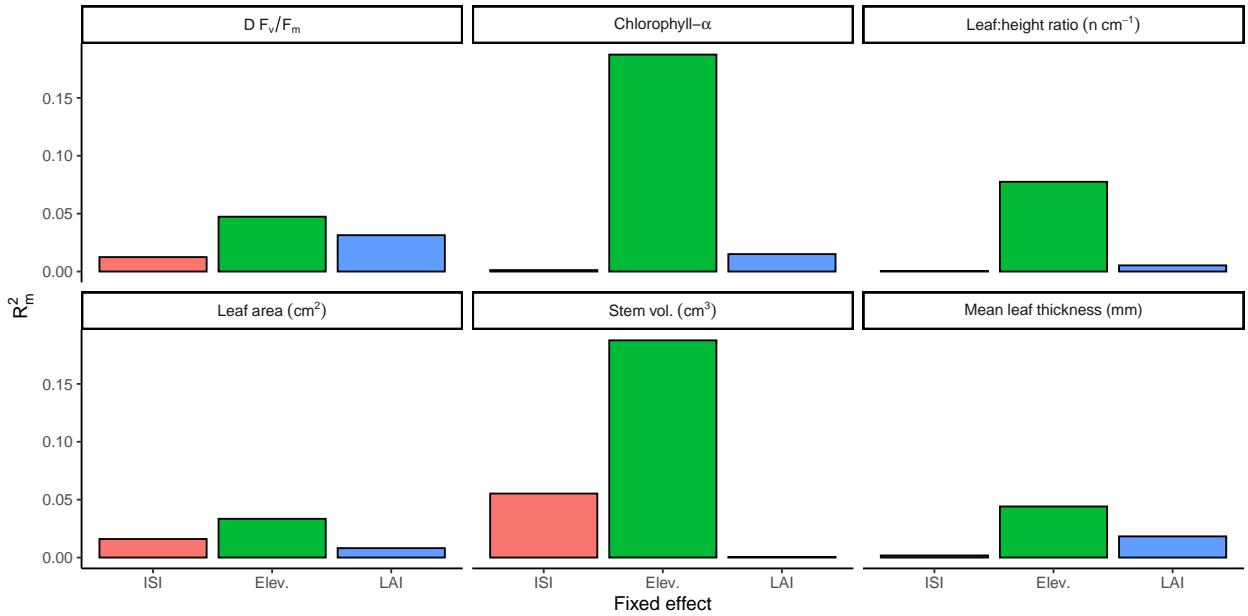


Figure 13: The variance explained by each single fixed effect model.

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- $\alpha$ , 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  $\Delta AIC_r > 2$

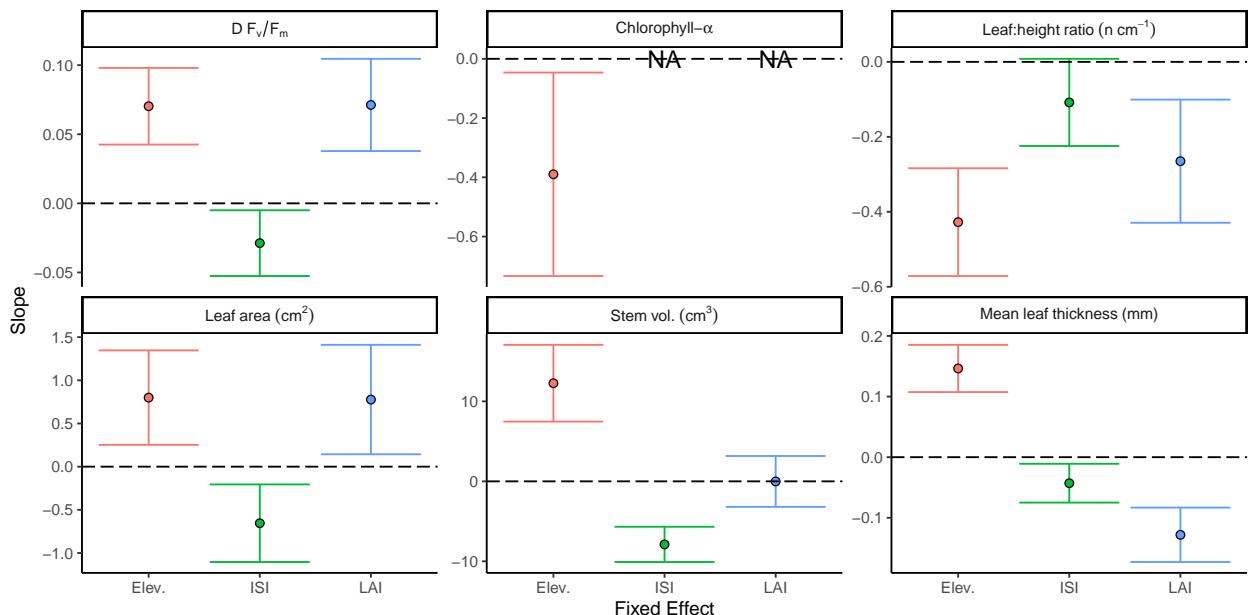


Figure 14

Table 4

Response	Fixed effects	$\Delta AIC_r$	$W_i$	$R_c^2$	$R_m^2$
$F_v/F_m$	Elev. + ISI + LAI	-4.82	1	0.29	0.1
Chlorophyll- $\alpha$	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

## 285 Discussion

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

## 296 4 Effect of biotic environment and elevation on plant traits

297 Single fixed effect models demonstrated that the three competition variables influence some plant  
 298 traits ( $\Delta AIC_r \geq 2$ , Figure 12a). The effect size of individual competition variables however, did  
 299 not exceed that of elevation for any plant traits (Figure 12b, Figure 11). The three competition  
 300 variables, which represent different types of competition, vary in their effects on seedling traits.  
 301 However, the estimated variance explained by the fixed effects of these models was low in all cases,  
 302 while the variance explained by the model as a whole varied according to the plant trait studied.  
 303 It would seem that unmeasured site level variation had a greater impact on plant traits than biotic  
 304 environmental variation *per se*.

305 **What were some of these unmeasured factors?**

## 306 Leaf physiology

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

315 Photosynthetic efficiency

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

330 Canopy density decreases with elevation (Figure ??), though this trend may be the result of  
331 wide within site variance ( $\Delta AIC_r \approx 2$ ). This trend concurs with more conclusive results from other  
332 studies which show a clear decrease in canopy density with elevation (??). The more variable  
333 relationship seen in this study may be the result of bias in the sampling strategy. LAI was not  
334 measured systematically across each site, instead being measured above each sampled seedling. It  
335 is expected that seedlings will grow successfully only under canopy where the average light intensity  
336 falls between a minimum needed for growth and a maximum that ensures temperature and UV-B  
337 stress does not cause the seedling to perish. In this study therefore, extreme canopy densities were  
338 probably not sampled. The presence of bias in our sampling strategy is supported by comparing the  
339 range of LAI measurements in other studies. For example, ?, in a review of 61 tropical evergreen  
340 forests, found that LAI ranged from 1.5 to 8. (after outlier exclusion), whereas our LAI estimate  
341 ranged from only 1.0 to 5.5, implying that a representative LAI sample was not achieved within  
342 each plot.

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

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

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

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

377 Maxwell and Johnson (2000) suggest that generally, optimum  $F_v/F_m$  is ~0.83, and that if  $F_v/F_m$   
378 falls below ~0.8, it is indicative of some kind of plant stress. It is important to note however, that  
379 this optimum is likely to vary markedly among species and has been criticised as yet another  
380 arbitrary threshold for a dynamic phenomenon (?). As a conservative estimate, here plants are  
381 defined as experiencing physiological stress when  $F_v/F_m < 0.7$ . Figure ?? shows that only a few  
382 individuals fall below this threshold, suggesting that few individuals along the elevational gradient  
383 are experiencing stress. Only *C. revoluta* features reduced photosynthetic capacity with elevation.  
384 *C. revoluta* also has the most individuals below the 0.7 threshold. This could be evidence that *C. revoluta*  
385 individuals experience greater stress at increasing elevations, but the relationship shown  
386 here is not strong enough to be conclusive, with large variation within each plot that *C. revoluta*  
387 seedlings were sampled. Alternatively other species which feature an increase in photosynthetic  
388 efficiency may be experiencing stress at lower elevations, giving support for the hypothesis given  
389 by Campbell et al. (2007), in which species ranges contract from the bottom up. Temperature  
390 increase is the most likely source of this increased stress at the lower limits of species ranges,  
391 though stress induced by antagonistic interactions from previously lower elevation species that have  
392 shifted upslope faster is also possible. Herbivores for example are expected to move upslope faster  
393 than tree species due to their mobility and shorter life-cycles (Chen et al., 2011).

#### 394 Chlorophyll- $\alpha$

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

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

405 The species with the smallest ranges show the steepest decrease in SPAD with elevation (Figure  
406 ??). From this one could suggest that specialists are more sensitive to increases in elevation in  
407 terms of their photosynthetic apparatus. Species with small ranges are interpreted as being more

408 specialist in their environmental requirements (Thuiller et al., 2005).

409 **Summary**

410 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  
411 on  $F_v/F_m$  while seedling-seedling competition had no effect. A decrease in ISI with elevation may  
412 have contributed to the observed increase in  $F_v/F_m$  with elevation though it is possible that this  
413 trend is actually a result of increased stress at lower elevations in response to temperature stress  
414 or herbivory stress.  $H_{n1}$  is therefore accepted for SPAD and rejected for  $F_v/F_m$ . The best multiple  
415 fixed effect model for  $F_v/F_m$  included all competition variables,  $H_{n2}$  is therefore rejected for  $F_v/F_m$ .  
416 SPAD is predicted equally poorly by elevation and competition variables.

418 **Leaf and plant morphology**

419 Leaf thickness increased with elevation. Other studies have also found positive correlations between  
420 leaf thickness and elevation, identifying climatic drivers such as mean daily insolation and diurnal  
421 temperature variation (?), which lead to reduced leaf pay-back times and a need to grow leaves that  
422 can survive the more variable environmental conditions found at higher elevations (?). Increased  
423 UV-B results in an increase in cuticle thickness, to reduce the concentration of UV-B absorbed by  
424 photosystem II (PSII) where it can cause damage and thus photoinhibition (??). In this study  
425 however, it is unclear whether the insolation UV-B fraction does increase with elevation as it was  
426 not measured. Additionally, it is expected that frequent cloud immersion in the high elevation  
427 sites would reduce UV-B absorption and thus the need for thick cuticles. Leaf thickness decreased  
428 under increased canopy density (Figure ??), adding support to the conclusion that increased direct  
429 sunlight is the cause of the decrease in leaf thickness with elevation.

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

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

445 Stem volume decreased with ISI (Figure ??). This may have contributed to the increase in  
446 stem volume with elevation, as ISI decreases with elevation (Figure ??). Other studies have found  
447 that stem volume increases with average wind speed in order to provide greater stem support (?),  
448 and that stems become more elongated as diurnal temperature range increases (?). Wind speed  
449 is expected to increase with elevation as is diurnal temperature range, providing further support  
450 for the trend seen here. An increase in stem volume with elevation suggests that tree seedlings are

allocating less biomass to other parts such as the leaves, meaning that plant growth may be slower at higher elevations. This is supported by the negative relationship between leaf area and elevation, and the negative relationship between leaf:height ratio and elevation, which suggests that seedlings produce fewer, smaller leaves as elevation increases.

## Summary

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

## 5 Variation in plant traits with elevation

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

## Variation among species

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

Leaf thickness had a similar positive relationship with elevation in 7/9 species, whereas *I. del-*

491 *toidea* and *S. patula* featuring reduced leaf thicknesses with elevation (Figure ??). *C. thurifera*  
492 had exceptionally high variance compared to other species, this is due to dense and prominent leaf  
493 vein structure in this species (Appendix V). For many *C. thurifera* individuals, the diameter of the  
494 micrometer used to measure leaf thickness was too wide to be placed between the prominent leaf  
495 veins, leading to an over-estimation of leaf thickness for these individuals. Regardless, *C. thurifera*  
496 showed a similar increase in leaf thickness with elevation. *I. deltoidea* had the steepest decrease  
497 in leaf thickness over elevation (Figure ??). This trend may be a peculiarity of the species or a  
498 result of environmental conditions at the upper sample plot for this species (VC). It is impossible  
499 to confirm whether site level variation at VC had a peculiar effect on *I. deltoidea* leaf thickness as *I.*  
500 *deltoidea* was the only species sampled at this site. Potentially, the greater leaf thickness at PA400  
501 compared to VC is due to an adaptation to increased herbivory pressure at PA400. There is no  
502 evidence for this increase in herbivory in lowland plots other than a general trend that herbivory  
503 pressure decreases with elevation in tropical forests (?).

## 504 Summary

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

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

## 519 6 Predictions for future species migration

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

532 Most species featured a decrease in photosynthetic efficiency at the bottom of their elevational  
533 ranges. This implies that these species may experience progressively greater plant stress at the

bottom of their ranges as temperature increases, and the bottom of their range will continue to shift upslope as a result. This study cannot infer whether the contraction of species' lower range limits will be faster or slower than the expansion of the upper range limit, though other studies have suggested that lower range limits will shift upslope faster than upper limits (Campbell et al., 2007), owing to climate change proceeding faster than micro-evolutionary processes to adapt to higher elevations. This will lead to an overall reduction in range size for many species.

## 7 Limitations of this study

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

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

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

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

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

## 581 8 Further research

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

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

## 592 Conclusion

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

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

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

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

615 Forest structure based competition affects physiological stress independently of elevation

## 616 References

- 617 Akaike, H. (1992), 'Information theory and an extension of the maximum likelihood principle',  
618 *Breakthroughs in Statistics* pp. 610–624.
- 619 Araújo, M. B., Thuiller, W. and Pearson, R. G. (2006), 'Climate warming and the decline of  
620 amphibians and reptiles in europe', *Journal of Biogeography* **33**, 1712–1728.
- 621 Bartoń, K. (2019), *MuMIn: Multi-Model Inference*. R package version 1.43.6.
- 622 Bates, D., Mächler, M., Bolker, B. M. and Walker, S. C. (2015), 'Fitting linear mixed-effects models  
623 using lme4', *Journal of Statistical Software* **67**(1), 1–48.
- 624 Bell, D. M., Bradford, J. B. and Lauenroth, W. K. (2014), 'Early indicators of change: diversgent  
625 climate envelopes between tree life stages imply range shifts in the western united states', *Global  
626 Ecology and Biogeography* **23**, 168–180.
- 627 Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. and Courchamp, F. (2012), 'Impacts of  
628 climate change on the future of biodiversity', *Ecology Letters* **15**, 365–377.
- 629 Berry, P. M., Dawson, T. P., Harrison, P. A. and Pearson, R. G. (2002), 'Modelling potential impacts  
630 of climate change on the bioclimatic envelope of species in britain and ireland', *Global Ecology &  
631 Biogeography* **11**, 453–462.
- 632 Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H.  
633 and White, J. S. (2008), 'Generalized linear mixed models: a practical guide for ecology and  
634 evolution', *Trends in Ecology and Evolution* **24**(3), 127–135.
- 635 Campbell, C., Atkinson, L., Zaragoza-Castells, J., Lundmark, M., Atkin, O. and Hurry, V. (2007),  
636 'Acclimation of photosynthesis and respiration is asynchronous in response to changes in temper-  
637 ature regardless of plant functional group', *New Phytologist* **176**, 375–389.
- 638 Chen, I., Hill, J. K., Ohlemüller, R., Roy, D. B. and Thomas, C. D. (2011), 'Rapid range of species  
639 associated with high levels of climate warming', *Science* **333**, 1024–1026.
- 640 Colwell, R. K., Brehm, G., Cardelús, C. L., Gilman, A. C. and Longino, J. T. (2008), 'Global  
641 warming, elevational range shifts and lowland biotic attrition in the wet tropics', *Science* **322**, 258–  
642 261.
- 643 Coomes, D. A. and Grubb, P. J. (2000), 'Impacts of root competition in forests and woodlands: a  
644 theoretical framework and review of experiments', *Ecological Monographs* **70**(2), 171–207.
- 645 Corlett, R. T. and Westcott, D. A. (2013), 'Will plant movemenets keep up with climate change?',  
646 *Trends in Ecology & Evolution* **28**(8), 482–488.
- 647 Coste, S., Baraloto, C., Leroy, C., Marcon, É., Renaud, A., Richardson, A. D., Roggy, J., Schimann,  
648 H., Uddling, J. and Hérault, B. (2010), 'Assessing foliar chlorophyll contents with the spad-502  
649 chlorophyll meter: a calibration test with thirteen tree species of tropical rainforest in french  
650 guiana', *Annals of Forest Science* **67**(6), 607–607.
- 651 Davis, M. A., Wrage, K. J. and Reich, P. B. (1998), 'Competition between tree seedlings and  
652 herbaceous vegetation: support for a theory of resource supply and demand', *Journal of Ecology*  
653 **86**, 652–661.
- 654 Dawson, T. P., Jackson, S. T., House, J. I., Prentice, I. C. and Mace, G. M. (2011), 'Beyond  
655 predictions: biodiversity conservation in a changing climate', *Science* **332**, 53–58.
- 656 Dobson, A., Lodge, D., Alder, J., Cumming, G. S., Keymer, J., McGlade, J., Mooney, H., Rusak,  
657 J. A., Sala, O., Wolters, V., Wall, D., Winfree, R. and Xenopoulos, M. A. (2006), 'Habitat loss,  
658 trophic collapse, and the decline of ecosystem services', *Ecology* **87**(8), 1915–1924.

- 659 Ettinger, A. K., Ford, K. R. and HilleRisLambers, J. (2011), 'Climate determines upper, but not  
660 lowe, altitudinal range limits of pacific northwest conifers', *Ecology* **92**(6), 1323–1331.
- 661 Feeley, K. J., Malhi, Y., Zelazowski, P. and Silman, M. R. (2012), 'The relative importance of defor-  
662 estation, precipitation change, and temperature sensitivity in determining the future distributions  
663 and diversity of amazonian plant species', *Global Change Biology* **18**, 2636–2647.
- 664 Feeley, K. J., Silman, M. R., Bush, M. B., Farfan, W., Cabrera, K. G., Malhi, Y., Meir, P., Revilla,  
665 N. S., Quisiyupanqui, M. N. R. and Saatchi, S. (2011), 'Upslope migration of andean trees',  
666 *Journal of Biogeography* **38**, 783–791.
- 667 Frazer, G. W., Fournier, R. A., Trofymow, J. A. and Hall, R. J. (2001), 'A comparison of digital  
668 and film fisheye photography for analysis of forest canopy structure and gap light transmission',  
669 *Agricultural and Forest Meteorology* **109**, 249–263.
- 670 Gadow, K. V. and Hui, G. (1999), *Modelling Forest Development*, Springer, The Netherlands.
- 671 Genty, B., Briantais, J. and Baker, N. R. (1989), 'The relationship between the quantum yield  
672 of photosynthetic electron transport and quenching of chlorophyll fluorescence', *Biochimica et  
673 Biophysica Acta* **990**, 87–92.
- 674 Gibson-Reinemeyer, D. K., Sheldon, K. S. and Rahel, F. J. (2015), 'Climate change creates rapid  
675 species turnover in montane communities', *Ecology and Evolution* **5**(12), 2340–2347.
- 676 Girardin, C. A. J., Espejob, J. E. S., Doughty, C. E., Huasco, W. H., Metcalfe, D. B., Durand-Baca,  
677 L., Marthews, T. R., Aragao, L. E. O. C., Farfan-Rios, W., García-Cabrera, K., Halladay, K.,  
678 Fisher, J. B., Galiano-Cabrera, D. F., Huaraca-Quispe, L. P., Alzamora-Tayne, I., Eguiluz-Mora,  
679 L., Salinas-Revilla, N., Silman, M. R., Meir, P. and Malhi, Y. (2014), 'Productivity and carbon  
680 allocation in a tropical montane cloud forest in the peruvian andes', *Plant Ecology & Diversity*  
681 **7**(1-2), 107–123.
- 682 Gruber, C. E., Nakagawa, S., Laws, R. J. and Jamieson, I. G. (2011), 'Multimodel inference in  
683 ecology and evolution: challenges and solutions', *Journal of Evolutionary Biology* **24**, 699–711.
- 684 Hegyi, F. (1974), A simulation model for managing jack-pine stands, in 'Royal College of Forestry,  
685 editor', Royal College of Forestry, Stockholm, Sweden, pp. 74–90.
- 686 Hughes, L. (2000), 'Biological consequences of global warming: is the signal already apparent?',  
687 *Trends in Ecology and Evolution* **15**(2), 56–61.
- 688 Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W. S., Reich, P. B., Scherer-Lorenzen, M.,  
689 Schmid, B., Tilman, D., van Ruijven, J., Weigelt, A., Wilsey, B. J., Zavaleta, E. S. and Loreau,  
690 M. (2011), 'Hugh plant diversity is needed to maintain ecosystem services', *Nature* **477**, 199–203.
- 691 Jonckheere, I., Fleck, S., Nackaerts, K., Muys, B., Coppin, P., Weiss, M. and Baret, F. (2004),  
692 'Review of methods for in situ leaf area index determination part i. theories, sensors and hemi-  
693 spherical photography', *Agricultural and Forest Meteorology* **121**, 19–35.
- 694 Jupp, D. L. B., Culvenor, D. S., Lovell, J. L., Newnham, G. J., Strahler, A. H. and Woodcock,  
695 C. E. (2008), 'Estimating forest lai profiles and structural parameters using a ground-based laser  
696 called "echidna"', *Tree Physiology* **29**, 171–181.
- 697 Krause, G. H., Koroleva, O. Y., Dalling, J. W. and Winter, K. (2001), 'Acclimation of tropical tree  
698 seedlings to excessive light in simulated tree-fall gaps', *Plant, Cell and Environment* **24**, 1245–  
699 1352.
- 700 Lee, W., von Gadow, K., Chung, D., Lee, J. and Shin, M. (2004), 'Dbh growth model for *Pinus*  
701 *densiflora* and *Quercus variabilis* mixed forests in central korea', *Ecological Modelling* **176**, 187–  
702 200.

- 703 Lenoir, J., Gégout, J., Guisan, A., Vittoz, P., Wohlgemuth, T., Zimmermann, N. E., Dullinger, S.,  
704 Pauli, H., Willner, W. and Svenning, J. (2010), 'Going against the flow: potential mechanisms for  
705 unexpected downslope range shifts in a warming climate', *Ecography* **33**, 295–303.
- 706 Lenoir, J., Gégout, J., Pierrat, J., Bontemps, J. and Dhôte, J. (2009), 'Differences between tree  
707 species seedling and adult altitudinal distribution in mountain forests during the recent warm  
708 period (1986–2006)', *Ecography* **32**, 765–777.
- 709 Lenoir, J. and Svenning, J. C. (2015), 'Climate-related range shifts - a global multidimensional  
710 synthesis and new research directions', *Ecography* **38**, 15–28.
- 711 Lewis, S. L. and Tanner, E. V. J. (2000), 'Effects of above- and belowground competition on growth  
712 and survival of rain forest tree seedlings', *Ecology* **81**(9), 2525–2538.
- 713 Li, F., Peng, S., Chen, B. and Hou, Y. (2010), 'A meta-analysis of the responses of woody and  
714 herbaceous plants to elevated ultraviolet-b radiation', *Acta Oecologia* **36**, 1–9.
- 715 MacLean, S. A. and Beissinger, S. R. (2017), 'Species' traits as predictors of range shifts under  
716 contemporary climate change: a review and meta-analysis', *Global Change Biology* **23**, 4094–  
717 4105.
- 718 Malhi, Y., Silman, M., Salinas, N., Bush, M., Meir, P. and Saatchi, S. (2010), 'Introduction: ele-  
719 vation gradients in the tropics: laboratories for ecosystem ecology and global change research',  
720 *Global Change Biology* **16**, 3171–3175.
- 721 Mantyka, C. S., Martin, T. G. and Rhodes, J. R. (2012), 'Interactions between climate and habi-  
722 tatt loss effects on biodiversity: a systematic review and meta-analysis', *Global Change Biology*  
723 **18**, 1239–1252.
- 724 Martin, P. H., Fahey, T. J. and Sherman, R. E. (2010), 'Vegetation zonation in a neotropical  
725 montane forest: environment, disturbance and ecotones', *Biotropica* **43**(5), 533–543.
- 726 Matsuraba, S., Krause, G. H., Aranda, J., Virgo, J., Beisel, K. G., Jahns, P. and Winter, K. (2009),  
727 'Sun-shade patterns of leaf carotenoid composition in 86 species of neotropical forest plants',  
728 *Functional Plant Biology* **36**, 20–36.
- 729 Maxwell, K. and Johnson, G. N. (2000), 'Chlorophyll fluorescence - a practical guide', *Journal of  
730 Experimental Botany* **51**(345), 659–668.
- 731 McCain, C. M. and Colwell, R. K. (2011), 'Assessing the threat to montane biodiversity from discon-  
732 tinuous shifts in temperature and precipitation in a changing climate', *Ecology Letters* **14**, 1236–1245.
- 733 McMahon, S. M., Harrison, S. P., Armbruster, W. C., Bartlein, P. J., Beale, C. M., Edwards,  
734 M. E., Kattge, J., Midgley, G., Morin, X. and Prentice, I. C. (2011), 'Improving assessment and  
735 modelling of climate change impacts on global terrestrial biodiversity', *Trends in Ecology and  
736 Evolution* **26**(5), 249–259.
- 737 Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. and Kent, J. (2000),  
738 'Biodiversity hotspots for conservation priorities', *Nature* **403**, 853–858.
- 739 Parmesan, C. (2006), 'Ecological and evolutionary responses to recent climate change', *Annual  
740 Review of Ecology, Evolution and Systematics* **37**, 637–669.
- 741 Pearson, R. G. and Dawson, T. P. (2003), 'Predicting the impacts of climate change on the dis-  
742 tribution of species: are bioclimate envelope models useful?', *Global Ecology & Biogeography*  
743 **12**, 361–371.
- 744 Peterson, A. T., Ball, L. G. and Cohoon, K. P. (2002), 'Predicting distributions of mexican birds  
745 using ecological niche modelling methods', *Ibis* **144**, E27–E32.

- 746 Prado, F. E., Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J. A. and Hilal, M.  
747 (2012), Uv-b radiation, its effects and defense mechanisms in terrestrial plants, *in* P. Ahmad and  
748 M. Prasad, eds, 'Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate  
749 Change', Springer, New York, USA, pp. 57–83.
- 750 R Core Team (2019), *R: A Language and Environment for Statistical Computing*, R Foundation for  
751 Statistical Computing, Vienna, Austria.
- 752 Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. (2012), 'Nih image to imagej: 25 years of  
753 image analysis', *Nat Methods* **9**(7), 671–675.
- 754 Seifert, T., Seifert, S., Seydack, A., Durrheim, G. and von Gadow, K. (2014), 'Competition effects  
755 in an afrotropical forest', *Forest Ecosystems* **1**(13), 1–15.
- 756 Serrano, L. (2008), 'Effects of leaf structure on reflectance estimates of chlorophyll content', *Inter-  
757 national Journal of Remote Sensing* **29**, 17–18.
- 758 Sinclair, S. J., White, M. D. and Newell, G. R. (2010), 'How useful are species distribution models  
759 for managing biodiversity under future climates?', *Ecology and Society* **15**(1), 1–13.
- 760 ter Steege, H. (2018), *Hemiphot.R: Free R scripts to analyse hemispherical photographs for canopy  
761 openness, leaf area index and photosynthetic active radiation under forest canopies*, Leiden, The  
762 Netherlands. Unpublished report.
- 763 Thuiller, W., Richardson, D. M., Pyšek, P., Midgley, G. F., Hughes, G. O. and Rouget, M. (2005),  
764 'Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale',  
765 *Global Change Biology* **11**, 2234–2250.
- 766 Travis, J. M. J., Delgado, M., Bocedi, G., Baguette, M., Bartoń, K., Bonte, D., Boulangeat, I.,  
767 Hodgson, J. A., Kubisch, A., Penteriani, V., Saastamoinen, M., Stevens, V. M. and Bullock,  
768 J. M. (2013), 'Dispersal and species' responses to climate change', *Oikos* **122**, 1532–1540.
- 769 Valladares, F., Laanisto, L., Niinemets, Ü. and Zavala, M. A. (2016), 'Shedding light on shade:  
770 ecological perspectives of understorey plant life', *Plant Ecology & Diversity* **9**(3), 237–251.
- 771 Van der Putten, W. H., Macel, M. and Visser, M. E. (2010), 'Predicting species distribution and  
772 abundance responses to climate change: why it is essential to include biotic interactions across  
773 trophic levels', *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 2025–  
774 2034.
- 775 Whitaker, J., Ostle, N., Nottingham, A. T., Ccahuana, A., Salinas, N., Bardgett, R. D., Meir,  
776 P. and McNamara, N. P. (2014), 'Microbial community composition explains soil respiration  
777 responses to changing carbon inputs along an andes-to-amazon elevation gradient', *Journal of  
778 Ecology* **102**, 1052–1071.
- 779 Wisz, M. S., Pottier, J., Kissling, W. D., Pellissier, L., Lenoir, J., Damgaard, C. F., Dormann,  
780 C. F., Forchhammer, M. C., Grytnes, J., Guisan, A., Heikkinen, R. K., Hoye, T. T., Kähn,  
781 I., Luoto, M., Maiorano, L., Nilsson, M., Normand, S., Öckinger, Schmidt, N. M., Ternansen,  
782 M., Timmermann, A., Wardle, D. A., Aastrup, P. and Svenning, J. (2013), 'The role of biotic  
783 interactions in shaping distributions and realised assemblages of species: implications for species  
784 distribution modelling', *Biological Reviews* **88**, 15–30.