

1 **Title:**

2 Stomatal anatomy, leaf structure and nutrients of tropical rainforest tree species respond to altitude
3 in a coordinated manner in accordance with the leaf economics spectrum

4 **Running title:**

5 Response of stomatal traits to altitude

6

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18

19 **Abstract**

20 Understanding taxon level responses of key plant traits to environmental variation across tropical
21 rainforests (TRFs) is important to determine their response to climate change. We used an
22 altitudinal gradient (from 150 to 2100 m above sea level, asl) across TRFs in Sri Lanka to answer
23 the following questions: (a) Does the response to altitude by stomatal traits differ among plant taxa
24 in TRFs?; (b) Are the altitudinal responses of key leaf structural traits (e.g. leaf mass per area,
25 LMA) and major leaf nutrient (nitrogen, N, and phosphorus, P) concentrations linked to the
26 corresponding variation in stomatal traits in a coordinated response across taxa?; (c) How strong
27 is the influence of climatic variation on responses of leaf traits to altitude?. Leaf samples were
28 collected in permanent sampling plots within rainforest reserves at Kanneliya (150 m asl),
29 Sinharaja- Enasalwatta (1050 m), Hakgala (1800 m) and Pidurutalagala (2100 m) from 19 species
30 in three plant genera (*Calophyllum*, *Semecarpus* and *Syzygium*). Stomatal density, guard cell
31 length and epidermal density showed variation among taxa, but did not respond to altitude.
32 Potential conductance index (PCI), a proxy for photosynthetic capacity, decreased with increasing
33 altitude, in a common response across taxa. We found evidence that altitudinal responses of LMA,
34 leaf N and P were linked to stomatal responses in a coordinated manner, where key features were
35 the negative correlations between PCI and LMA and between proxy photosynthetic N- and P-use
36 efficiencies ('PNUE' and 'PPUE') and LMA. We found strong responses to climatic variation
37 across taxa and altitudes, where PCI, 'PNUE' and 'PPUE' increased and LMA decreased with
38 increasing temperature, precipitation and solar irradiance. We conclude that stomatal traits of tree
39 species in TRFs form part of a coordinated leaf trait response to environmental change which is in
40 accordance with the leaf economics spectrum.

41 **Key words:** *leaf traits, tropical rainforests, Sri Lanka, climate variation, stomatal anatomy*

42 **Introduction**

43 Tropical rainforests (TRFs) are one of the most sensitive ecosystems to environmental change
44 (Malhi et al. 2009, 2014, Zelazowski et al. 2011, Hubau et al. 2020). The high diversity of plant
45 species in TRFs are likely to elicit different responses by different species to changes in climatic
46 and soil variables (Hofhansl et al. 2020). Therefore, it is important to determine the species/genus
47 level variation of responses to understand and predict ecosystem responses to environmental
48 perturbations such as long-term climate change and climate extremes.

49 Altitudinal gradients are used as a means of detecting the influence of environmental change on
50 tropical ecosystems, their processes and productivity (Malhi et al. 2010, 2017). Ecosystems such
51 as TRFs which span a wide range of altitudes experience changes in several key environmental
52 variables that vary with altitude. For example, air and soil temperatures, atmospheric pressure,
53 vapour pressure deficit and partial pressure of CO₂ (pCO₂) decrease with altitude whereas solar
54 irradiance, wind speed and incident UV radiation could increase (Barry 1992, Körner 2007). Such
55 changes in climatic variables along altitudinal gradients have induced altitude-linked variations in
56 soil properties by influencing key soil processes such as mineralization and organic matter
57 decomposition (Dieleman et al. 2013). Evolutionary and adaptive responses of plants to altitude-
58 linked gradients in climate and soil variables have led to variations in species composition and
59 diversity in TRFs along altitudinal gradients (Lieberman et al. 1996, Vazquez G and Givnish 1998,
60 Aiba and Kitayama 1999, Givnish 1999). Furthermore, differences in key plant functional
61 traits/leaf traits have been observed across plant species that inhabit altitudinal gradients (Oliveras
62 et al. 2020).

63 Stomata occupy a strategic location in plants to regulate their carbon and water balances via leaf-
64 air exchange of CO₂ and water vapour. Therefore, responses of stomatal density and size to

65 altitude represent a key pathway to optimize carbon and water balances in the face of
66 environmental variation across an altitudinal gradient. Many early work reported increased
67 stomatal density (SD) and stomatal index (SI) with increasing altitude (Korner and Cochrane 1985,
68 Korner et al. 1986, McElwain 2004, Kouwenberg et al. 2007), which was interpreted as
69 confirmation of the decrease of SD and SI with increasing pCO₂, reported by (Woodward 1987,
70 Woodward et al. 2002). However, a broader survey of literature shows a diverse range of stomatal
71 responses to altitude. Accordingly, increased SD and SI with increasing pCO₂ along altitudinal
72 gradients (Hu et al. 2015) and time (Bai et al. 2015) have been reported. Furthermore, increases
73 in SD and SI up to 2800 - 3000 m above mean sea level (asl) have been observed to reverse in
74 some plant species at higher altitudes (Qiang et al. 2003, Li et al. 2006, Luo et al. 2006). In
75 comparison to SD and SI, response of stomatal size, measured as the product between guard cell
76 length (GCL) and closed stomatal width, to environmental variation has been studied less
77 frequently (Lomax et al. 2009). A highly-conserved inverse relationship has been shown between
78 stomatal density and size across different taxonomic groups and environmental variables (Franks
79 and Beerling 2009, Doheny-Adams et al. 2012). However, GCL of *Arabidopsis thaliana* ecotypes
80 from a range of altitudes from 50 to 1260 m amsl showed all possible responses (i.e. increase,
81 decrease and no change) to increased pCO₂ while not showing significant correlations with either
82 SD or SI (Caldera et al. 2017). The above range of stomatal responses illustrates the inter- and
83 intra-specific diversity in this response. Accordingly, in an ecosystem of high species diversity
84 such as the TRFs, a diversity of stomatal responses to altitude could be expected. Long-term
85 evolutionary responses and short-term acclimation to environmental variation across altitudes
86 probably combine to bring about these diverse stomatal responses (Hultine and Marshall 2000,
87 Qiang et al. 2003, Franks and Beerling 2009, Haworth et al. 2015).

88 As stomatal anatomy influences the assimilate supply for construction of leaves, altitudinal
89 variation of leaf structural traits such as the leaf mass per area (LMA) could be linked to the
90 corresponding variation of stomatal traits. Leaf mass per area is a measure of assimilate investment
91 per unit area of photosynthetic surface and is regarded as a key trait of the leaf economic spectrum
92 (Wright et al. 2004). Shi et al. (2015) showed that diverse responses of stomatal traits to altitude
93 were linked to leaf economic strategy and plant growth habit. Nitrogen (N) and phosphorus (P) are
94 the two foremost plant nutrients and are considered most limiting for plant growth (Reich and
95 Oleksyn 2004). Nitrogen is an essential component of enzymes, especially the key photosynthetic
96 enzyme Rubisco, while P is a key part of nucleic acids, cell membranes and energy-carriers such
97 as ATP and NADPH. Leaf N and P concentrations are indicative of their availability in the soil as
98 well as their proportional allocation to leaves to meet the physiological demands of processes that
99 require N (e.g. enzymes) and P (e.g. ATP and nucleic acids). Accordingly, variation in leaf N and
100 P concentrations across an altitudinal gradient, when considered along with corresponding
101 variations in stomatal and leaf structural traits could be part of an integrated ecosystem level
102 response to environmental variation as represented by altitude (Oliveras et al. 2020). The role of
103 genetic variation, based on taxonomic grouping, as opposed to variation caused by local
104 environmental factors is poorly understood for leaf traits in different plant species in complex
105 ecosystems such TRFs.

106 Tropical rainforests of Sri Lanka (TRFSL) are located in the humid tropical climatic zone of Sri
107 Lanka in its south-western plains and the western slope of its central highlands. Lowland
108 rainforests are found in the lower altitudes up to ca. 1000 m amsl while montane forests are found
109 in the higher altitudes up to 2200 m. Species richness and diversity of TRFSL have been shown
110 to decrease with increasing altitude (Sanjeevani et al. 2020) and there are no plant species

111 inhabiting the whole altitudinal range of the TRFSL (Sanjeevani et al. Unpublished). Therefore,
112 genetically-determined variations in stomatal and leaf traits among different species inhabiting
113 different altitudes would confound responses of these traits to environmental gradients across
114 altitudes. Furthermore, proportional contributions from genetic and environmental components to
115 the observed variation of these traits could be different for different species (Zhang et al. 2012).
116 In this study, we examine altitudinal variation of stomatal traits in three plant genera that have
117 different species inhabiting a sufficiently wide altitudinal gradient with the objective of identifying
118 variation patterns of their stomatal traits with altitude and then elucidating their possible
119 environmental and genus-level genetic controls. As a second objective, we examine possible inter-
120 relationships between stomatal and leaf traits with a view to determine possible trait assemblies
121 that underpin the response of these traits to specific climatic variation across altitudes. We asked
122 the following questions: (a) Does the response to altitude by stomatal anatomical traits differ
123 among plant taxa (e.g. genera) in TRFs?; (b) Are the altitudinal responses of key leaf structural
124 traits (LMA) and major leaf nutrient (N and P) concentrations linked to the corresponding variation
125 in stomatal traits in a coordinated response across different taxa (e.g. genera, species)?; (c) How
126 strong is the influence of climate variation across altitudinal gradients on responses of leaf traits
127 to altitude?. Specifically, we tested the following hypotheses: Hypothesis 1: There is significant
128 variation among taxa in the response of their stomatal traits to variation in altitude; Hypothesis 2:
129 Altitudinal responses of key leaf structural traits, major leaf nutrient concentrations and stomatal
130 traits form a coordinated response across different taxa in tropical rainforests; Hypothesis 3:
131 Responses of leaf traits to altitude are strongly influenced by climatic variables that vary across
132 altitudinal gradients.

133

134 **Materials and methods**

135 *Study sites and climate*

136 Collection of leaf samples for the study was carried out in six permanent sampling plots (PSPs) of
137 1 ha (100 m x 100 m) each established along an altitudinal gradient from 117 m to 2080 m asl (Fig.
138 1). All plots were located in the undisturbed areas inside selected TRFSL. Two plots were located
139 in the Kanneliya Forest Reserve. Another two plots were located in the Sinharaja Forest Reserve
140 (Enasalwatte) which is a World Heritage site. The other two plots were located in the Hakgala
141 Strict Nature Reserve and Pidurutalagala Forest Reserve. Long-term (1970-2000) averages of the
142 climate variables of study sites were obtained from the high-resolution (1 km²) global climatic
143 database WorldClim 2 (Fick and Hijmans 2017) (Table 1). Long-term annual averages of the
144 mean, maximum and minimum air temperatures, vapour pressure, wind speed and daily solar
145 irradiance were computed from their monthly means. Long-term average annual precipitation was
146 computed from the respective annual totals.

147 **Table 1.** Study sites and their long-term (1970-2000) climatic variables

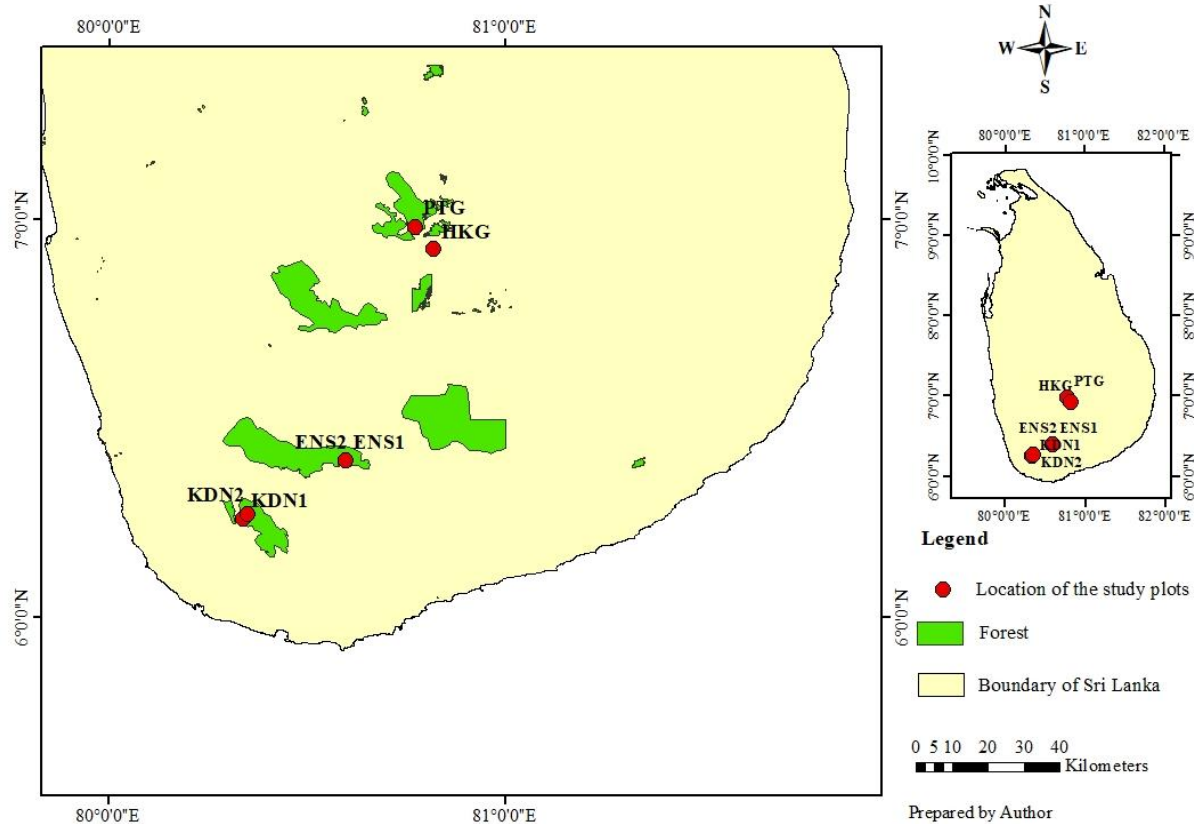
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Location	Latitude (N)	Longitude (E)	Altitude (m)	T _{AV} (°C)	T _{max} (°C)	T _{min} (°C)	R _F (mm y ⁻¹)	Radiation (kJ m ⁻² d ⁻¹)	WVP (kPa)	WS (ms ⁻¹)
KDN 1	6.24749 N	80.34071 E	117	26.3	29.8	23.5	3656.0	18945.3	2.8	2.3
KDN 2	6.26045 N	80.35112 E	174	26.3	29.3	22.8	3788.0	18832.8	2.8	2.3
ENS 2	6.39433 N	80.59709 E	1042	21.3	24.5	18.1	3103.0	17910.3	2.0	2.2
ENS 2	6.39441 N	80.59652 E	1065	21.3	24.5	18.1	3103.0	17910.3	2.0	2.2
HKG	6.92725 N	80.81839 E	1804	17.2	20.3	13.8	2017.0	17688.3	1.8	2.0
PTG	6.98197 N	80.77276 E	2080	15.3	18.4	12.3	1903.0	17460.0	1.2	1.9

149

150 KDN 1 & 2 – Kanneliya Forest Reserve Plots 1 & 2; ENS 1 & 2 – Sinharaja-Enasalwatte Plots 1 & 2; HKG
151 – Hakgala Strict Nature Reserve; PTG – Pidurutalagala Forest Reserve. T_{AV} – Mean annual average
152 temperature; T_{max} - Mean annual maximum temperature; T_{min} - Mean annual minimum temperature; R_F -
153 Mean annual total precipitation; Radiation – Mean daily incident solar radiation; WVP - Mean atmospheric
154 vapour pressure; WS – Mean wind speed (Source: Fick and Hijmans, 2017).

155



156

157 **Figure 1.** Permanent sampling plots of the present study located at different altitudes in tropical rainforests
158 of Sri Lanka. Kanneliya Plot 1 (KDN1-117 m asl), Kanneliya Plot 2 (KDN2-174 m asl), Sinharaja-
159 Enasalwatta Plot 1 (1042 m asl), Sinharaja-Enasalwatta Plot 2 (1065 m asl), Hakgala Plot (1804 m asl) and
160 Piduruthalagala Plot (2080 m asl).

161 *Plant species*

162 A complete census of all plants having a diameter at breast height (1.3 m above ground level or
163 buttress) of 10 cm or greater had been carried out in the PSPs in 2019 (Sanjeevani et al. 2020).

164 Plant species for the study were selected based on the availability of a species across a sufficiently
165 wide altitudinal range. However, except *Semecarpus gardneri*, which was present in plots at 150
166 m and 1050 m, there was no other species inhabiting more than one altitude. At the genus level,
167 there were three genera, viz. *Syzygium*, *Semecarpus* and *Calophyllum* which had different species
168 occupying a wide altitudinal range. Accordingly, 19 species from these genera were selected for
169 the study (Table 2).

170 **Table 2.** Species from selected genera for the study and their locations

171

Location	Altitude (m)	<i>Syzygium</i>	<i>Semecarpus</i>	<i>Calophyllum</i>
Kanneliya	150 [†]	<i>S. alubo</i>	<i>S. walkeri</i>	<i>C. bracteatum</i>
		<i>S. neesianum</i>	<i>S. gardneri</i>	<i>C. cordato-oblongum</i>
		<i>S. firmum</i>	<i>S. subpeltata</i>	
Sinharaja- Enasalwatte	1050 [†]	<i>S. cylindricum</i>	<i>S. gardneri</i>	<i>C. acidus</i>
		<i>S. micranthum</i>	<i>S. parvifolia</i>	
		<i>Syzygium</i> new sp.		
Hakgala	1800	<i>S. paniculatum</i>	<i>S. obovata</i>	
		<i>S. zeylanicum</i>		
Pidurutalagala	2100	<i>S. revolutum</i>		<i>C. walkeri</i>
		<i>S. rotundifolium</i>		

172 [†]Altitude at mid-point between the two plots at Kanneliya and Sinharaja-Enasalwatte was selected.

173

174 The three core-eudicot genera belong to the clade Rosids and are classified under three different
175 orders, viz., *Calophyllum* (Calophyllaceae) in Malpighiales, *Semecarpus* (Anacardiaceae) in
176 Sapindales and *Syzygium* (Myrtaceae) in Myrtales (Angiosperm Phylogeny Group 2009).
177 *Calophyllum* is a pantropical genus consisting of approximately 187 species. In Sri Lanka, 11 out
178 of 13 *Calophyllum* species are endemic and are restricted to rainforests (Kostermans 1980). About
179 50 species of *Semecarpus* species are distributed from tropical Asia (Indo-Malaysian region) to
180 Oceania. All 12 species of *Semecarpus* in Sri Lanka are endemic and mainly distributed in low- to
181 mid-altitude rainforests (Meijer 1983). Approximately 1200 species of *Syzygium* are found in
182 tropical Africa, subtropical to tropical Asia, Australia, New Caledonia, New Zealand and Pacific
183 islands. There are 32 *Syzygium* species among Sri Lankan flora and 25 of them are endemic. The
184 genus has a wide altitudinal range in Sri Lanka with a majority of species in higher-altitude
185 rainforests. There are some introduced species, usually under cultivation while some are
186 naturalized (Ashton 1981).

187 *Sample collection*

188 Samples of mature, healthy leaves were taken from harvested branches of trees of the above 19
189 species. Three leaves per plant from five plants per species were used for stomatal measurements.
190 Species from two PSPs at near equal altitudes (Kanneliya and Enasalwatta) were pooled for
191 measurements and data analysis. All measurements were done on fully-expanded mature leaves
192 of each branch.

193 *Measurement of stomatal traits*

194 Using high precision Polyvinylsiloxane (Muller-Omicron GmbH & Co. KG, Germany) negative
195 impressions were acquired from leaves within 24 hours following harvesting of branches, before
196 leaf wilting occurred. Impressions were taken from a leaf position in the middle between mid-rib
197 and leaf margin and in the middle between the apex and the base of the leaf. A preliminary study
198 on each sampled species revealed that stomata are present only on the abaxial leaf surface.
199 Therefore, the negative impressions were taken only from the abaxial leaf surface.

200 Afterwards transparent cellulose varnish was applied on the negative silicone rubber impressions.
201 The varnish was allowed to dry off and then the positive impressions of the leaf abaxial surfaces
202 were peeled off. Thereafter, they were mounted onto microscopic slides and covered with
203 coverslips which were secured with adhesive tape. Five random fields per leaf sample were
204 selected and the images of these fields were taken under light microscopy (Primo Star, Zeiss,
205 Germany). This was done by a camera coupled with the microscope and Zeiss Zen microscope
206 software. Using these microscopic images (Plates S1-S5), number of stomata and epidermal cells
207 per microscopic field were counted.

208 All sampled species are rainforest tree species. Therefore, the borders of epidermal cells were
209 difficult to differentiate in the leaf imprints. To overcome this difficulty all images were sharpened
210 and the black and white contrast was adjusted using Adobe Photoshop 7.0.1. The multi-point tool
211 of the ImageJ software was used when counting stomata and epidermal cells. Editing of images
212 and use of the multi-point tool enabled precise counts of stomata and epidermal cells.

213 Stomatal density (SD) and epidermal density (ED) were calculated as the number of stomata and
214 epidermal cells per unit leaf area (mm^{-2}). Stomatal index (SI) was computed as the ratio between
215 stomatal number and the number of total epidermal cells, including stomatal guard cells, using the
216 formula, $SI = (SD/[SD + ED] \times 100)$.

217 Guard cell length (GCL) was measured using the slides prepared for determination of stomatal
218 traits. Measurements were taken by the Line tool of the Graphics in UI Automation mode of the
219 Zeiss Zen Microscope Software. This was done for one stomate each from randomly selected five
220 fields of view per leaf sample.

221 Potential conductance index (PCI) was computed as $PCI = [GCL]^2 \times SD \times 10^{-4}$ (Holland and
222 Richardson 2009), where GCL is given in μm and SD in stomata per mm^2 , assuming that the
223 stomatal aperture area is proportional to the GCL^2 . As PCI combines measures of maximum
224 stomatal size (GCL) with stomatal density (SD), it can be considered a measure of maximum
225 stomatal conductance (g_{max}) (Franks and Beerling 2009).

226 *Measurement of leaf structural traits and nutrients*

227 Leaf blade area (LBA) was measured on fully-expanded leaves by tracing the outline of the leaves
228 on a white paper. A printed scale was kept on the white paper and images were taken by a camera.
229 Leaf blade area was measured using these images using ImageJ software. The leaf samples were

230 dried to constant weight at 105°C in an oven (MOU-1125, SANYO, Japan). Each leaf was weighed
231 separately using an electronic balance (AR2130, Ohaus Co., USA). Leaf mass per area was
232 computed as the ratio between leaf dry mass and LBA. Taking PCI as a proxy for photosynthetic
233 capacity (Wong et al. 1979, Farquhar and Sharkey 1982), proxy indices of photosynthetic nitrogen
234 and phosphorus use efficiencies ('PNUE' and 'PPUE' respectively) were computed as the
235 respective ratios of PCI with leaf N and P.

236 *Determination of N and P concentrations of leaf tissues*

237 Leaf N concentration was measured using the modified Kjeldahl method (Motsara and Roy 2008).
238 Leaf P concentration was measured by dry ashing at 450°C followed by digestion in HNO₃.
239 Phosphorus concentration of the ash extract was determined by the spectrophotometric vanadium
240 phosphomolybdate method (Motsara and Roy 2008).

241 *Statistical analysis*

242 An initial exploration of the distributions of measured variables within each species at each altitude
243 was done with box plots. Normality of distributions was tested by the Shapiro-Wilk statistic
244 computed with Proc Univariate in SAS[®] Studio (Version 9.4). The influence of genus and altitude
245 on the measured traits was examined by a linear mixed model (LMM). As the three genera have
246 been selected from among a large number of genera in the PSPs, the genus effect was considered
247 as random. The altitude effect was considered as fixed because the selection of altitudes for leaf
248 sampling was constrained to those where PSPs have been established. This LMM analysis was
249 done for each trait using Proc Mixed in SAS with the residual (restricted) maximum likelihood
250 method. The genus x altitude interaction was considered a random effect.

251 Inter-relationships among stomatal anatomical traits were determined by linear correlation analysis
252 using all replicate measurements (i.e. n=1425). In order to examine inter-relationships among the
253 leaf anatomical and structural traits and leaf nutrients, linear correlation analysis was done on the
254 means of all leaf traits of the 19 plant species at different altitudes. The respective variables were
255 averaged across altitudes, replicate trees, leaves and microscope fields when calculating species
256 means. Influence of individual climatic variables on measured leaf traits were determined by
257 simple linear regression. Data from all species and altitudes were pooled in the regressions
258 between leaf traits and climatic variables. *Semecarpus gardneri* was the only species which was
259 present at more than one altitude (i.e. at 150 and 1050 m). Mean values from *S. gardneri* at the
260 two altitudes were considered as two separate data points in the correlation and regression
261 analyses.

262 A factor analysis (Child 1990) was done to identify underlying factors that cause variation in leaf
263 traits in the 19 plant species belonging to the three genera that are distributed across the altitudinal
264 gradient. In this analysis also, *S. gardneri* at 150 and 1050 m were considered as two observations.
265 The factor analysis was done using the principal axis method with prior communality estimates as
266 one. All factors that showed Eigen values greater than one were extracted and varimax rotation
267 was performed to identify variables that loaded on to extracted factors. Factor scores of the 20
268 observations were plotted in the factor space of the two principal factors (i.e. the two factors having
269 the highest Eigen values) to determine aggregation/separation of the 19 species based on their leaf
270 traits. A cluster analysis using the complete linkage method was performed to determine species
271 clusters among the 19 species, based on their leaf traits. Multivariate analysis of variance
272 (MANOVA) and the Wilks' Lambda Statistic was used to determine the significance of differences
273 between clusters. All statistical analyses were performed using SAS[®] Studio (Version 9.4).

274 Results

275 *Distributions of measured stomatal traits at different altitudes*

276 All stomatal traits showed substantial variation among species at a given altitude (Fig. S1).
277 Species-wise distributions of most stomatal traits showed normality in a majority of species
278 whereas most distributions of stomatal index deviated from normality (Table S1). The few outliers
279 were located on both ends of the distributions.

280 *Influence of genus and altitude on stomatal traits*

281 Analysis of stomatal traits in a linear mixed model showed that the fixed effect of altitude was
282 significant ($p=0.06$) on potential conductance index (PCI), but was non-significant ($p>0.07$) on all
283 other stomatal traits (Table 3). The random effect of the genus contributed a higher percentage
284 (56-64%) of the total variance of guard cell length (GCL), stomatal density (SD) and epidermal
285 density (ED) in comparison to the genus x altitude interaction (10-26%) (Table 4). The genus
286 effect contributed very little to the total variance of stomatal index (SI) and PCI (0-4%).

287 **Table 3.** Results of Type III tests for fixed altitude effect on leaf traits of plant species in tropical
288 rainforests of Sri Lanka along an altitudinal gradient

289

	GCL	SD	ED	SI	PCI	LMA	LBA	N	P	N:P
$F_{3,4}$	1.21	0.50	1.47	1.65	5.84	26.30	17.87	13.72	2.33	0.73
$p>F$	0.414	0.702	0.350	0.313	0.061	0.0043	0.0088	0.0143	0.2161	0.5840

290 GCL – Guard cell length; SD – Stomatal density; ED – Epidermal density; SI – Stomatal index; PCI –
291 Potential conductance index; LMA – Leaf mass per unit area; LBA – Leaf blade area; N – Leaf nitrogen
292 concentration; P – Leaf phosphorus concentration; N:P – Leaf nitrogen: phosphorus ratio.

293

294 *Variation of stomatal traits of different genera with altitude*

295 Guard cell length of *Calophyllum* was greater than that of *Semecarpus* and *Syzygium* at all
296 altitudes (Fig. 2.a). There was no consistent trend with altitude for GCL of any genus, thus

297 confirming the absence of an altitude effect (Table 3). The two notable increases in GCL with
 298 increasing altitude in *Calophyllum* at 1050 m and *Semecarpus* at 1800 m occurred because of a
 299 single species (*Calophyllum acidus* and *Semecarpus obovata*, Fig. S1.a) having a substantially
 300 higher GCL than others within the respective genera.

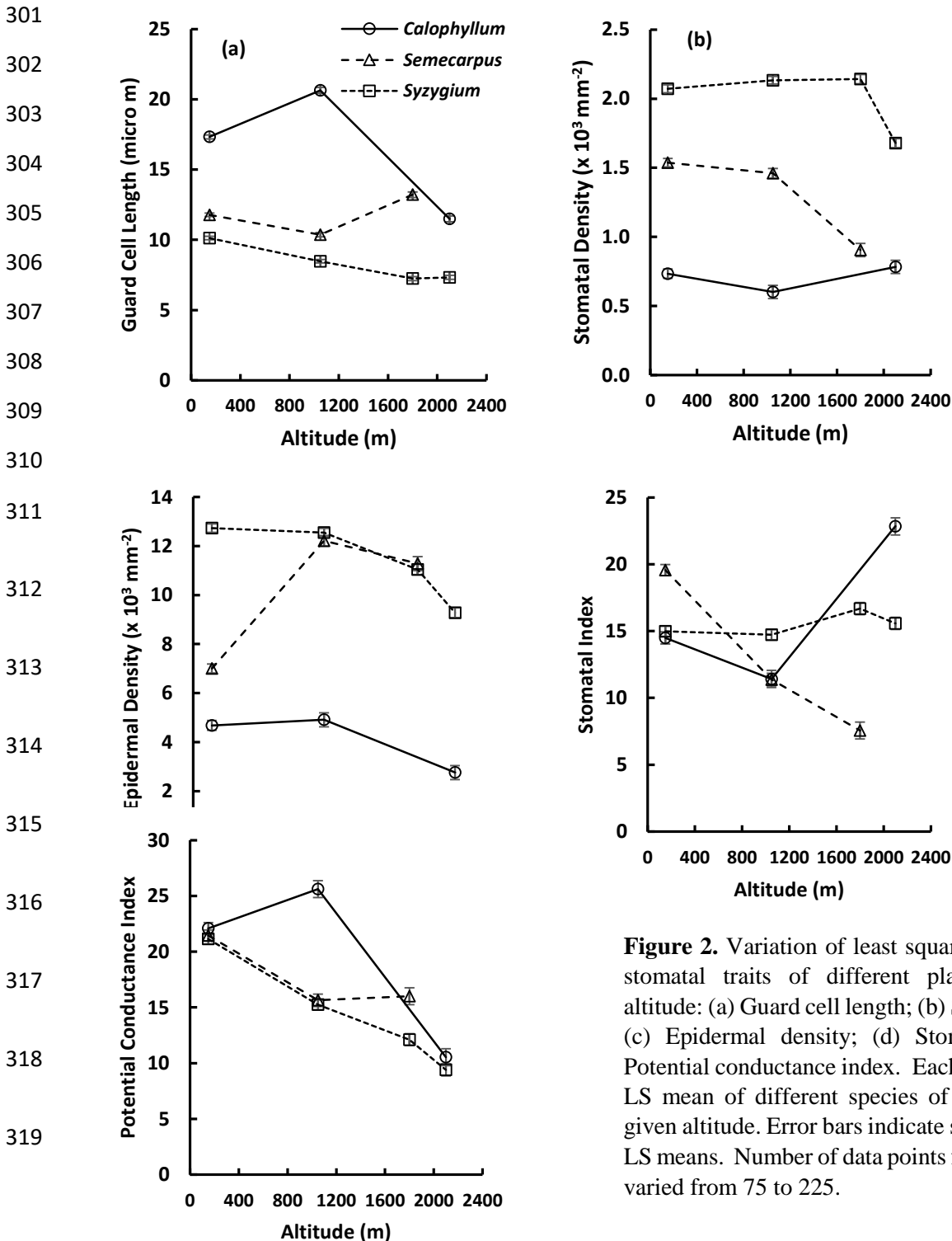


Figure 2. Variation of least square (LS) means of stomatal traits of different plant genera with altitude: (a) Guard cell length; (b) Stomatal density; (c) Epidermal density; (d) Stomatal index; (e) Potential conductance index. Each data point is the LS mean of different species of each genus at a given altitude. Error bars indicate standard errors of LS means. Number of data points for each LS mean varied from 75 to 225.

320 Stomatal density (SD) showed clear separation between the three genera with *Syzygium* having the
321 highest and *Calophyllum* the lowest with *Semecarpus* intermediate (Fig. 2.b). In all genera,
322 variations of SD with altitude were smaller than the variation among genera, thus confirming the
323 higher variance component attributed to genus (Table 4). The two notable reductions of SD at
324 higher altitudes in *Syzygium* and *Semecarpus* had occurred because of species level variation
325 (*Syzygium rotundifolium*, *Syzygium revolutum* and *Semecarpus obovata*) (Fig. S1.b). These
326 reductions contributed to the genus x altitude variance component. There is a highly-significant
327 ($p < 0.0001$) negative correlation between GCL and SD ($r = -0.67$; $n = 1425$) (Table S2). Similar
328 correlations were evident at the genus level as well (*Syzygium*: $r = -0.160$, $p < 0.0001$, $n = 735$;
329 *Calophyllum*: $r = -0.312$, $p < 0.0001$, $n = 300$; *Semecarpus*: $r = -0.335$, $p < 0.0001$; $n = 390$).

330 Epidermal density (ED) of *Calophyllum* was substantially lower than those of *Semecarpus* and
331 *Syzygium* (Fig. 2.c). Even though ED of all genera showed a decreasing trend from 1050 m
332 onwards, the observed reductions were smaller than differences among genera, which confirmed
333 the greater variance component due to the genus effect (Table 3). The lower ED of *Semecarpus*
334 at 150 m, which occurred because of lower ED of all three *Semecarpus* species (i.e. *S. subpeltata*,
335 *S. walkeri* and *S. gardneri*) at this altitude (Table 2), contributed to genus x altitude variance (Table
336 4). Epidermal density showed a positive correlation with SD and a negative correlation with GCL
337 (Table S2).

338 Stomatal index (SI) did not show a consistent variation with altitude or genus (Fig. 2.d).
339 Accordingly, the variance component due to genus was zero (Table 4). Furthermore, the variance
340 component attributed to genus x altitude interaction (32%) was lower than the residual (68%).
341 Stomatal index was negatively-correlated with ED and GCL and positively-correlated with SD
342 (Table S2).

343 **Table 4.** Estimates of variance components for random effects on stomatal traits in plant species of
 344 tropical rainforests in Sri Lanka across an altitudinal gradient
 345

Effect	GCL	%	SD	%	ED	%	SI	%	PCI	%
Genus	16.517	63	428810	64	12190137	56	0	0	2.341	4
Genus x Altitude	6.678	26	68110	10	3178939	15	14.875	32	10.053	18
Residual	2.982	11	169329	25	6212000	29	31.107	68	42.550	77
ML	5646.7		21193.9		26313.4		8965.3		9407.4	
AIC	5652.7		21199.9		26319.4		8969.3		9413.4	
BIC	5650		21197.2		26316.7		8967.5		9410.6	

346 ML: -2Res Maximum Likelihood statistic; AIC: Akaike Information Criterion; BIC: Bayesian
 347 Information Criterion.

348

349 Potential conductance index (PCI) showed a general decreasing trend with altitude (Fig. 2.e) which
 350 confirmed the significance of the fixed effect of altitude (Table 3). Furthermore, PCI did not show
 351 variance due to genus. The high PCI of *Calophyllum* at 1050 m, which was an exception to the
 352 decreasing trend with altitude, occurred because of the high GCL of *C. acidus* (Fig. S1.a). Potential
 353 conductance index was negatively-correlated with GCL (Table S2). PCI also showed a positive
 354 correlation with SI and a negative correlation with ED, but both were very weak ($r < 0.12$).

355 *Variation of leaf structural traits of different genera with altitude*

356 Distributions of leaf mass per area (LMA) and leaf blade area (LBA) showed variation among
 357 different species at each altitude (Fig. S2). A large majority of the species x altitude combinations
 358 showed normal distributions in their LMA and LBA (Table S1) with very few outliers.

359 The fixed effect of altitude on LMA and LBA were highly-significant (Table 3). The random
 360 effect of the genus did not contribute to variance of LMA (Table 5). In contrast, the variance due
 361 to genus contributed 46% to the total variance of LBA. Leaf mass per area showed increasing
 362 trends with altitude in all three genera (Fig. 3.a). In contrast, LBA of all genera showed decreasing

363 trends (Fig. 3.b). There was clear separation between genera with *Semecarpus* having greater LBA
 364 than the other two.

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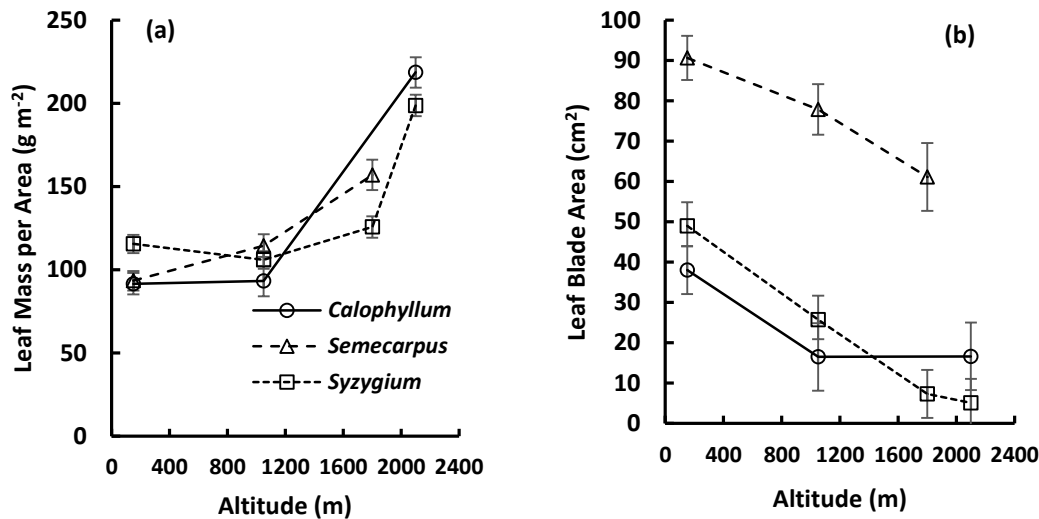


Figure 3. Variation of LS means of leaf structural properties of different plant genera with altitude: (a) Leaf mass per area; (b) Leaf blade area. Number of data points for each LS mean varied from 15 to 45.

Table 5. Estimates of variance components for random effects on leaf structural traits and nutrients in plant species of tropical rainforests in Sri Lanka along an altitudinal gradient

Effect	LMA	%	LBA	%	N	%	P	%	N:P	%
Genus	0	0	892.6	45.9	0	0	8333	23	53.7	28
Genus x Altitude	156.9	11	1.44	0.1	0.574	14	0	0	0	0
Residual	1242.5	89	1049.9	54.0	3.576	86	27304	77	140.9	72
ML	2825.1		2777.6		244.6		731.9		434.8	
AIC	2829.1		2783.6		248.6		735.9		438.8	
BIC	2827.3		2780.9		246.8		734.1		437.0	

383 ML: -2Res Maximum Likelihood statistic; AIC: Akaike Information Criterion; BIC: Bayesian
 384 Information Criterion.

385

386 *Variation of leaf nutrients of different genera with altitude*

387 Distributions of leaf nitrogen (N) and phosphorus (P) concentrations and their ratio (N:P) showed
388 variation among species and altitudes, especially in their spread (Fig. S3). However, all but one
389 (N) or two (N:P) species showed normality in their distributions (Table S1). Because of the lower
390 number of data points at the species level, distributions of leaf nutrients were examined at the
391 genus level as well. The distributions of leaf nutrients of different genera showed a narrower
392 spread, especially for P and N:P (Fig. S4). All but one (N:P) and two (P) distributions were normal
393 (Table S3).

394 The fixed effect of altitude was significant ($p < 0.05$) for leaf N, but non-significant for P and N:P
395 (Table 3). The random effect of genus did not contribute to the variance of leaf N, but contributed
396 23% and 28% respectively to the variances of P and N:P (Table 5). The random effect of genus x
397 altitude did not contribute to the variances of P or N:P while contributing 14% to that of N.

398 All three genera showed increased leaf N from 150 m to 1050 m (Fig. 4), which was followed by
399 decreases in *Calophyllum* and *Semecarpus* at higher altitudes. In *Syzygium*, leaf N continued to
400 increase up to 1800 m, which was followed by a steep decline at 2100 m. Leaf P and N:P did not
401 show consistent trends with altitude. Differences in leaf nutrients among genera were not
402 consistent across altitudes. *Syzygium* had higher leaf N at 1800 m whereas *Semecarpus* had lower
403 leaf P had 150 m. *Calophyllum* had higher leaf N:P at 150 m and 2100 m.

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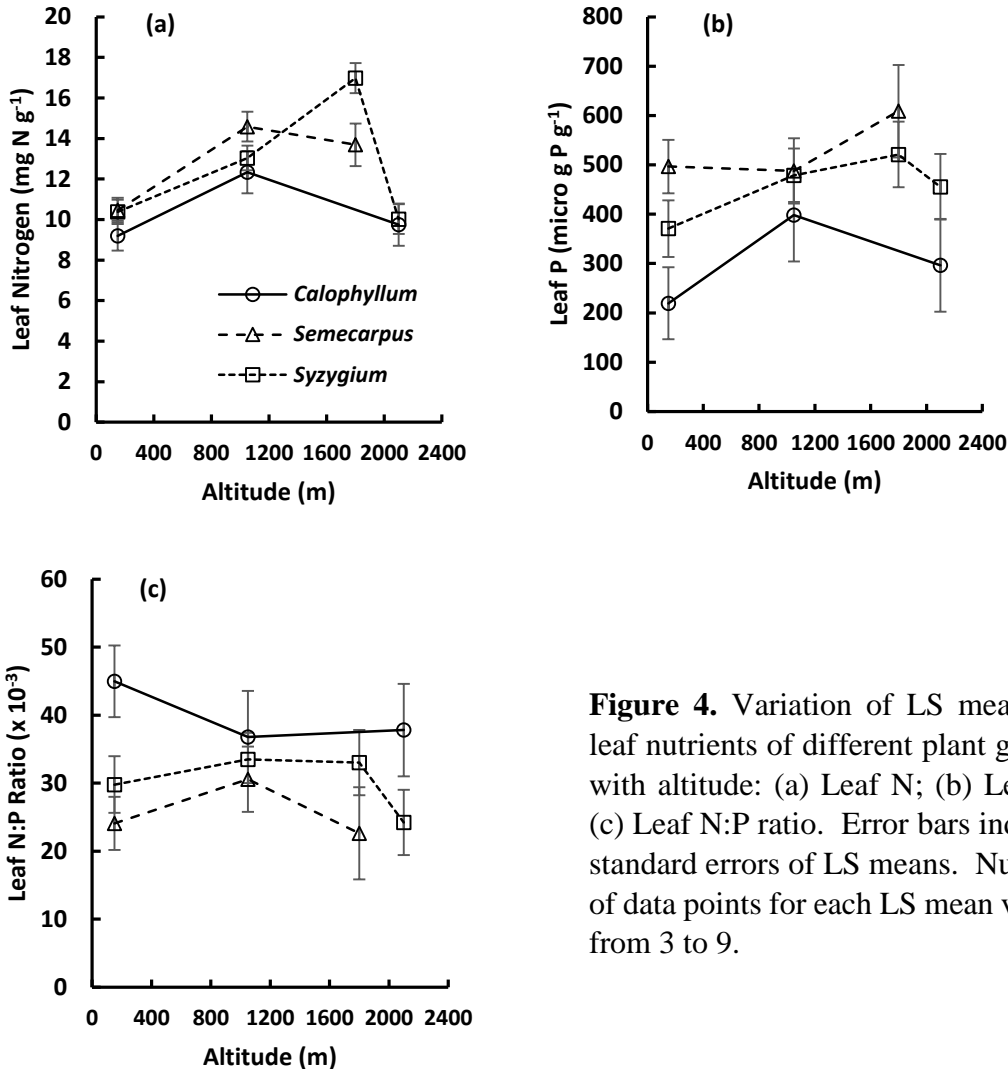


Figure 4. Variation of LS means of leaf nutrients of different plant genera with altitude: (a) Leaf N; (b) Leaf P; (c) Leaf N:P ratio. Error bars indicate standard errors of LS means. Number of data points for each LS mean varied from 3 to 9.

418 *Inter-relationships among measured variables*

419 Table 6 shows the linear correlation matrix among species means of leaf stomatal and structural

420 traits and leaf nutrients. Guard cell length (GCL) showed significant ($p < 0.05$) negative correlations

421 with SD and ED, but had a positive correlation with PCI. Stomatal density (SD) was positively

422 correlated with ED, which in turn was negatively correlated with SI. Except for a positive

423 correlation between ED and leaf N, there were no significant correlations between stomatal

424 anatomical traits and leaf nutrients.

425 **Table 6.** Linear correlation matrix of leaf traits, averaged for different plant species at different
426 altitudes in tropical rainforests across an altitudinal gradient in Sri Lanka

Trait	Pearson Correlation Coefficients (<i>r</i>), n = 20									
	Prob > <i>r</i> under H0: <i>r</i> = 0									
	GCL	SD	ED	SI	PCI	LBA	LMA	N	P	N:P
†GCL	1.00000	-0.792 <.0001‡	-0.542 0.0135	-0.214 ns	0.706 0.0005	0.177 ns	-0.329 ns	-0.371 ns	-0.391 ns	0.068 ns
SD	-	1.000	0.681 0.0009	0.212 ns	-0.211 ns	-0.132 ns	-0.086 ns	0.368 ns	0.258 ns	0.147 ns
ED	-	-	1.000	-0.504 0.0234	-0.114 ns	0.114 ns	-0.056 ns	0.477 0.0333	0.358 ns	-0.114 ns
SI	-	-	-	1.000	-0.062 ns	-0.085 ns	0.087 ns	-0.279 ns	-0.166 ns	0.190 ns
PCI	-	-	-	-	1.000	0.352 ns	-0.566 0.0093	-0.360 ns	-0.291 ns	0.067 ns
LBA	-	-	-	-	-	1.00000	-0.328 ns	-0.103 ns	0.226 ns	-0.357 ns
LMA	-	-	-	-	-	-	1.000	-0.162 ns	-0.065 ns	-0.176 ns
N	-	-	-	-	-	-	-	1.000	0.612 0.0042	-0.007 ns
P	-	-	-	-	-	-	-	-	1.000	-0.582 0.0071
N:P	-	-	-	-	-	-	-	-	-	1.000

427 ‡Significant correlations are shown in bold.

428 †GCL - Guard cell length; SD – Stomatal density; ED – Epidermal density; SI – Stomatal index;
429 PCI – Potential conductance index; LBA – Leaf blade area; LMA – Leaf mass per area; N – Leaf
430 nitrogen concentration; P – Leaf phosphorus concentration; N:P – Leaf N:P ratio.

431

432 There was a highly-significant ($p < 0.01$) negative correlation between LMA and PCI. Among the

433 leaf nutrients, N and P were positively correlated while leaf N:P was negatively correlated to leaf

434 P. Species means of ‘PNUE’ and ‘PPUE’ were negatively correlated to LMA (Fig. 5.a, b).

435 Strength of these correlations increased when species-level variation within each genus was

436 removed by averaging across species at each altitude (Fig. 5.c, d).

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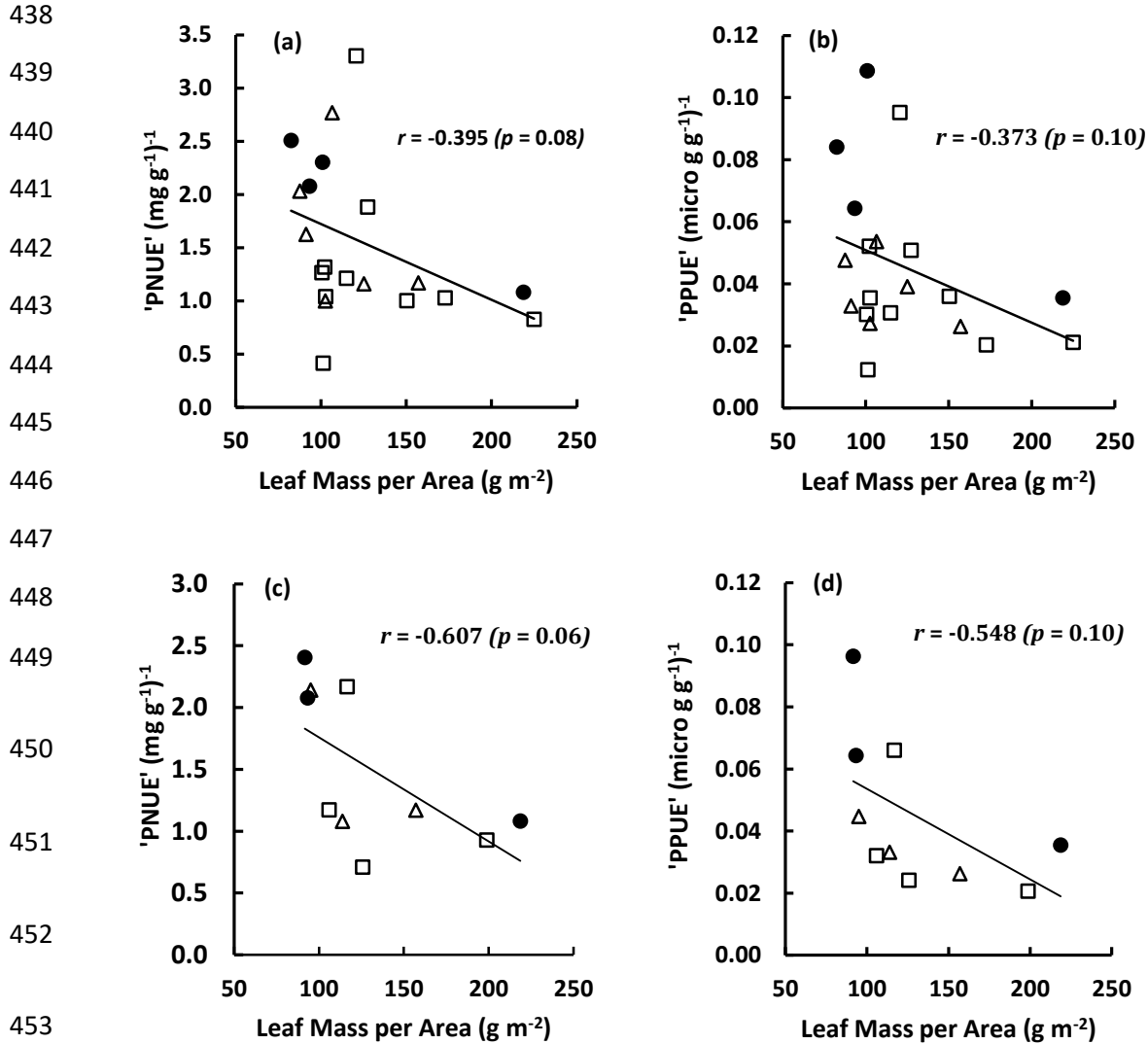


Figure 5. Correlation of proxy indices of photosynthetic nitrogen use efficiency ('PNUE') and phosphorus use efficiency ('PPUE') to leaf mass per area (LMA) of different plant species (a, b) and genera (c, d) in tropical rainforests in Sri Lanka across an altitudinal range from 150 m to 2100 m above sea level. ● - *Calophyllum*; Δ - *Semecarpus*; □ - *Syzygium*. 'PNUE' and 'PPUE' were calculated as the respective ratios of potential conductance index (unitless) and leaf N and P concentrations.

457 Trends with key climatic variables

458 When the data from all species and altitudes were pooled, stomatal density (SD), guard cell length
459 (GCL), epidermal density (ED) and stomatal index (SI) did not show significant ($p > 0.05$) linear
460 relationships with long-term mean air temperature (T_{AV}), annual precipitation (R_F) and daily solar

461 irradiance (S_R). However, potential conductance index (PCI) showed highly-significant ($p < 0.001$)
462 positive linear relationships with T_{AV} , R_F and S_R (Fig. 6.a-c). Leaf mass per area (LMA) showed
463 significant ($p < 0.01$) negative linear relationships with T_{AV} , R_F and S_R (Fig. 6.d-f).
464 Because of the substantial variance component due to the random effect of genus (Table 5),
465 relationships of LBA with climatic variables were examined separately for *Semecarpus* which had
466 substantially higher LBA than *Calophyllum* and *Syzygium* (Fig. 3.b), whose LBA trends were
467 examined together. Mean LBA of different *Semecarpus* species did not show significant trends
468 with any of the climate variables (Fig. S5). However, when LBA of *Semecarpus* species at each
469 altitude were averaged, the genus-level mean LBA of *Semecarpus* showed clear increasing trends
470 with T_{AV} , R_F and S_R (Fig. S6). Pooled species-level and genus-level LBA means of *Syzygium* and
471 *Calophyllum* showed highly-significant ($p < 0.01$) increasing trends with T_{AV} , R_F and S_R .

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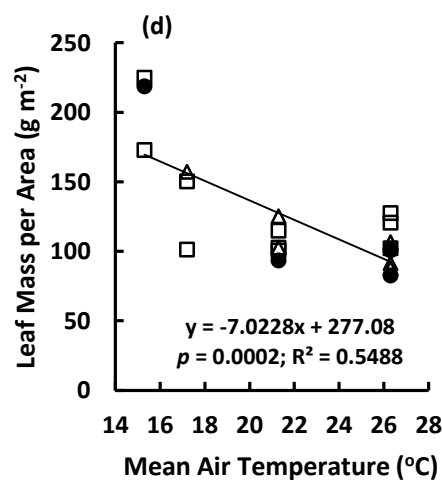
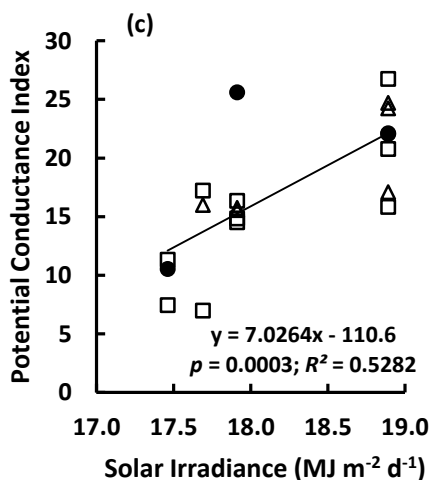
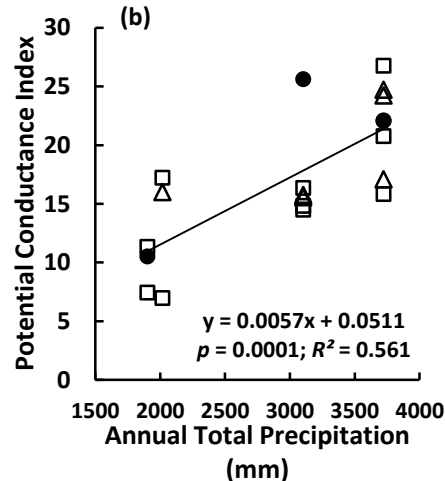
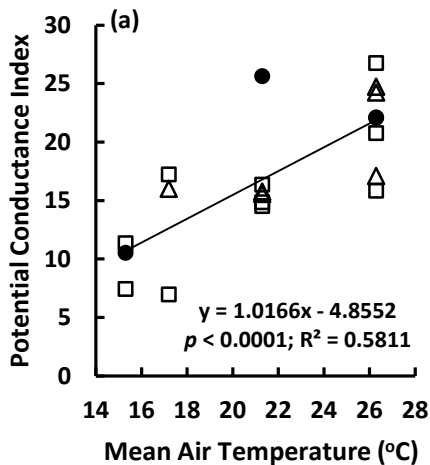
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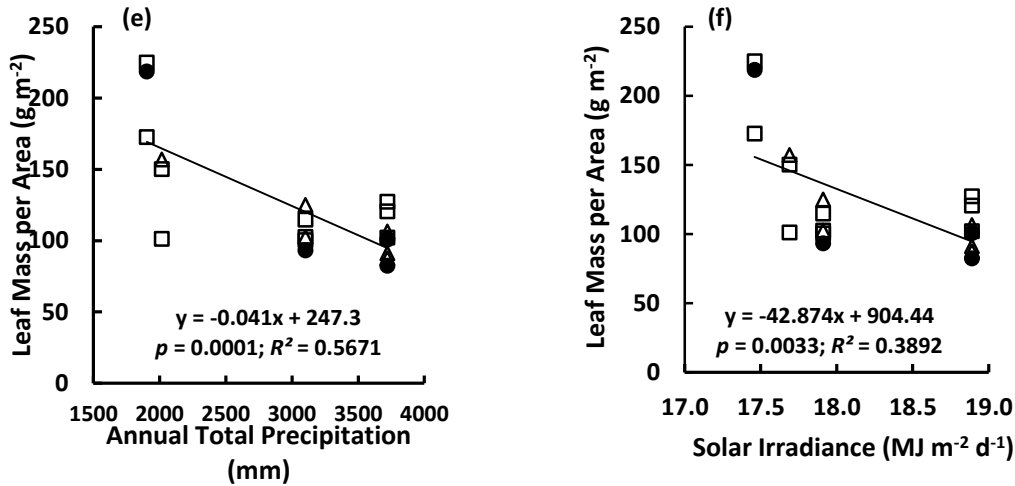
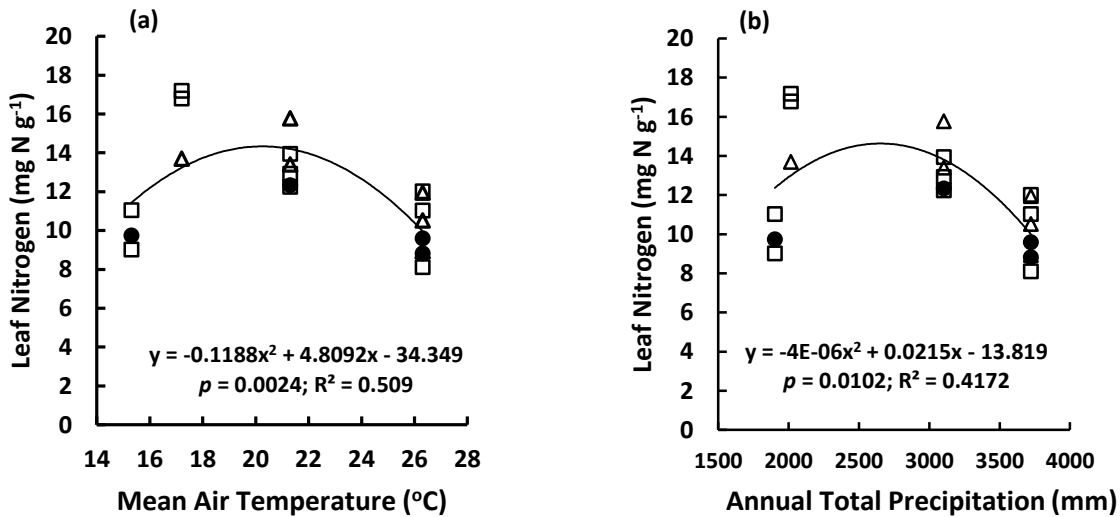
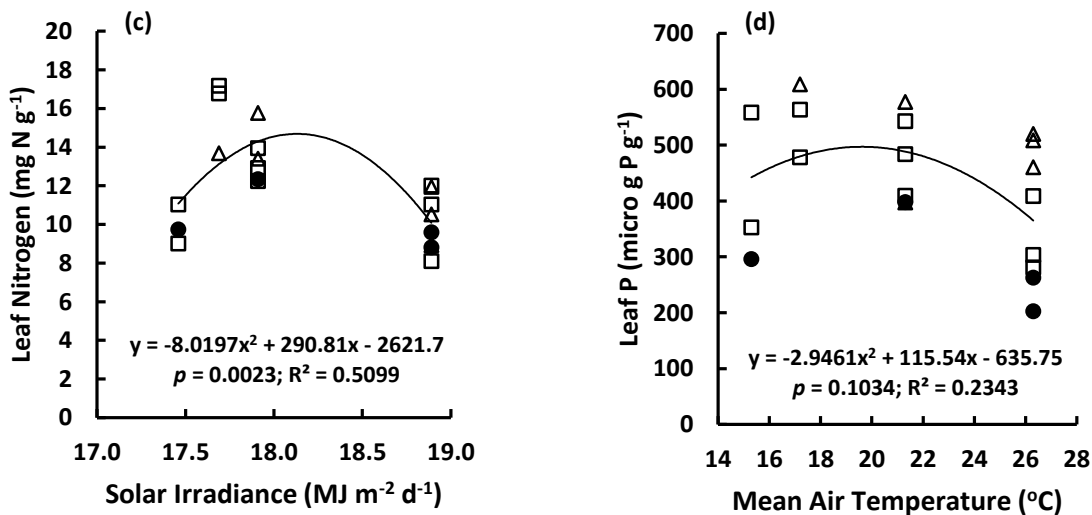


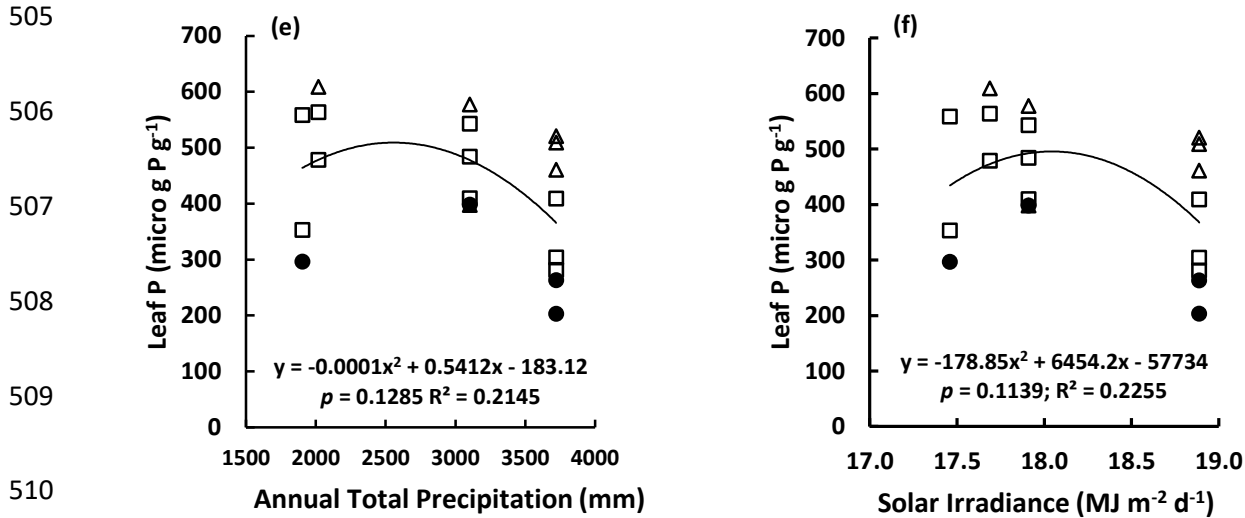
Figure 6. Variation of potential conductance index (a-c) and leaf mass per area (d-f) of different plant species with temperature (a, d), precipitation (b, e) and solar irradiance (c, f) in tropical rainforests in Sri Lanka across an altitudinal range from 150 m to 2100 m above sea level. ● - *Calophyllum*; Δ - *Semecarpus*; □ - *Syzygium*. Each data point is a species-level mean value.

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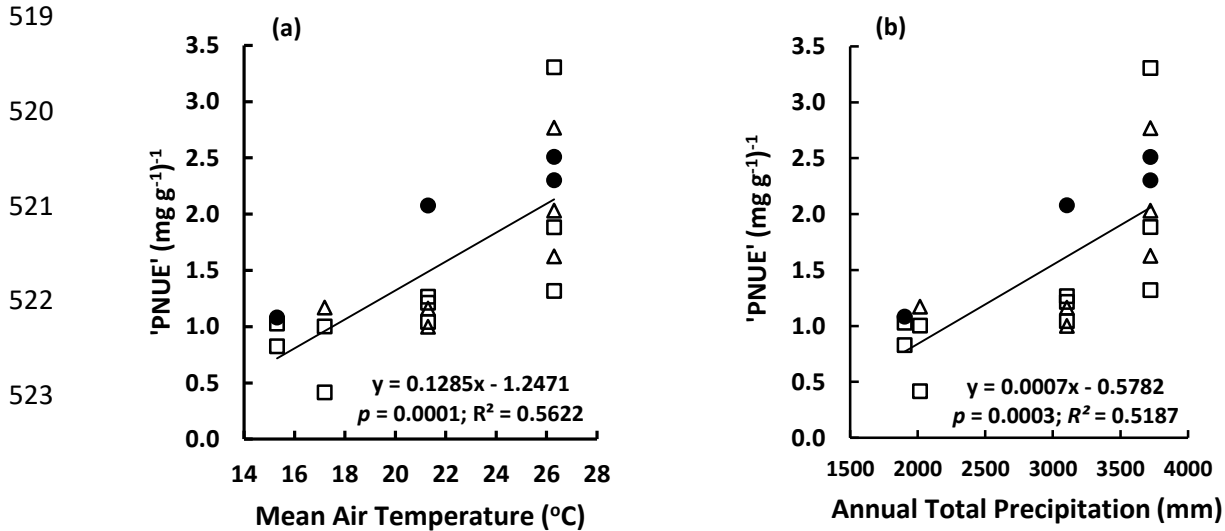


511 **Figure 7.** Variation of leaf nitrogen (a - c) and phosphorus (d - f) concentrations of different
 512 plant species with temperature (a, d), precipitation (b, e) and solar irradiance (c, f) in tropical
 rainforests in Sri Lanka across an altitudinal range from 150 m to 2100 m above sea level.

● - *Calophyllum*; Δ - *Semecarpus*; □ - *Syzygium*. Each data point is a species-level mean value.

513

514 Leaf N and P showed second-order polynomial trends with T_{AV} , R_F and S_R (Fig. 7). Trends for
 515 leaf P showed greater scatter (i.e. lower R^2 values and greater p) than those for leaf N. Leaf N:P
 516 ratio did not show significant trends with any climate variable (data not shown). Proxy indices of
 517 photosynthetic N and P use efficiencies (‘PNUE’ and ‘PPUE’) showed highly-significant positive
 518 trends with T_{AV} , R_F and S_R (Fig. 8).



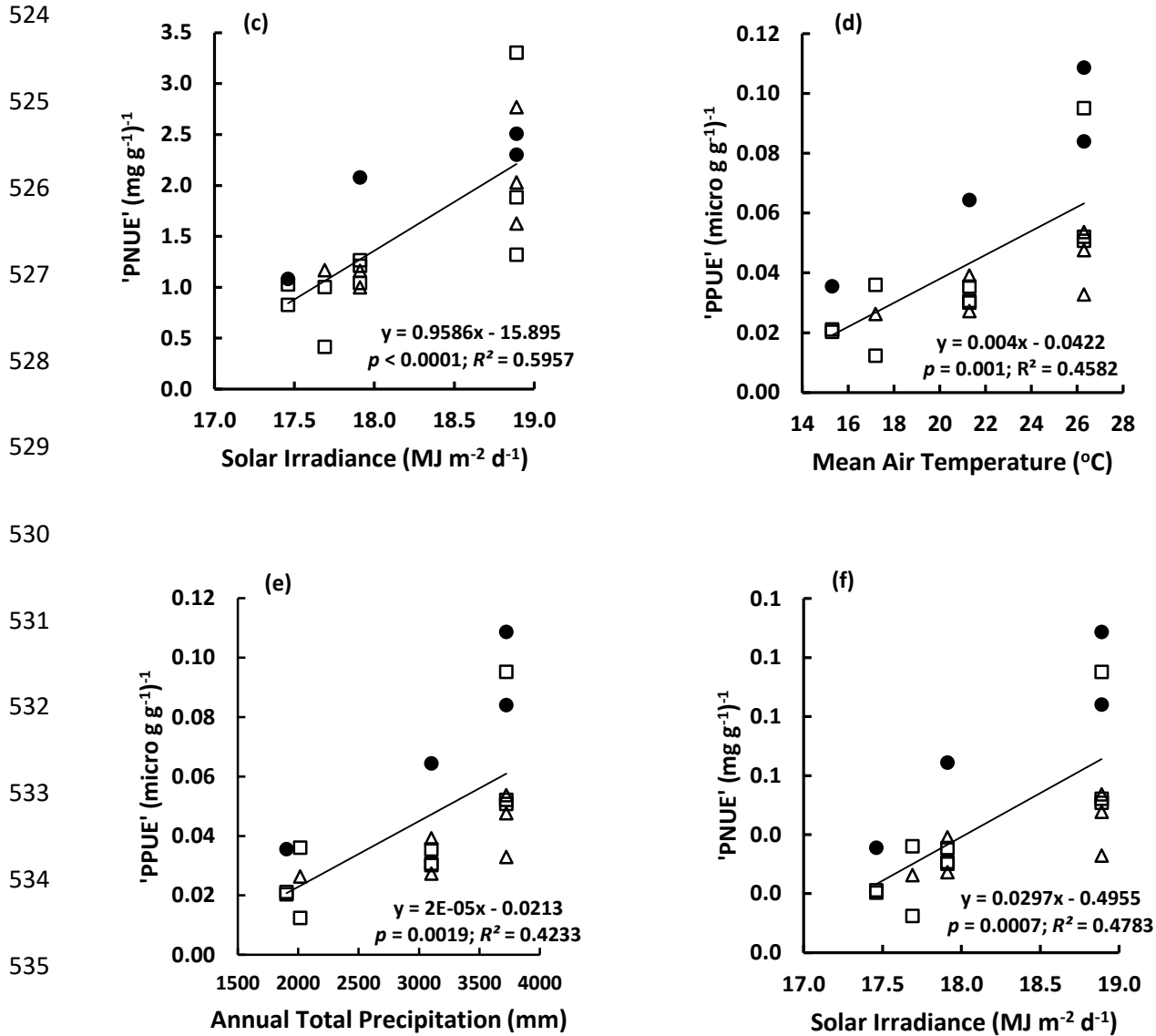
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537 **Figure 8.** Variation of proxy indices of photosynthetic nitrogen use efficiency ('PNUE') and phosphorus
 538 use efficiency ('PPUE') of different plant species with temperature (a, d), precipitation (b, e) and solar
 irradiance (c, f) in tropical rainforests in Sri Lanka across an altitudinal range from 150 m to 2100 m above
 sea level. ● - *Calophyllum*; Δ - *Semecarpus*; □ - *Syzygium*. Each data point is a species-level mean value.

539 *Factor analysis*

540 Four factors that had Eigen values greater than one were extracted initially and these accounted
 541 for 82% of the total variance in leaf traits in different plant species. The rotated factor pattern,
 542 following a varimax rotation, and the variables which loaded to each factor are given in Table 7.

543 A variable was considered to load on a factor if its loading on that factor is greater than 0.8 and if
 544 the same variable does not load on another factor. Accordingly, two variables, namely, SD and
 545 GCL, loaded on Factor 1, which accounted for 33% of the total variance of leaf traits. Leaf mass
 546 per area and PCI loaded on Factor 2 (21% of total variance) while leaf N:P (16%) and SI (12%)
 547 loaded on Factors 3 and 4 respectively.

548

549 **Table 7.** Rotated factor pattern for extracted factors in a factor analysis of leaf traits of plant species
 550 distributed along an altitudinal gradient in tropical rainforests in Sri Lanka

Variable	Loadings [†] of variables to factors			
	Factor1	Factor2	Factor3	Factor4
Stomatal density (SD)	94*	3	11	16
Epidermal density (ED)	73	9	-11	-48
Leaf nitrogen (N)	60	-9	-10	-52
Guard cell length (GCL)	-83*	47	11	-18
Potential conductance index (PCI)	-34	84*	7	2
Leaf mass per area (LMA)	-14	-86*	-6	10
Leaf N:P ratio	10	14	90*	9
Leaf blade area (LBA)	-6	56	-63	9
Leaf phosphorus (P)	45	-9	-72	-24
Stomatal index (SI)	9	-7	10	94*

551 [†]Multiplied by 100; *Loadings greater than 0.8.

552

553 Figure 9 shows the distribution of different tree species in the factor space based on their rotated
 554 factor scores for Factors 1 and 2. It can be observed that the three species found at 2100 m in
 555 Pidurutalagala are located away from the rest (group A). Two of the three species at 1800 m in
 556 Hakgala are located together (group B), but one (*Semecarpus obovata*) is located away from all
 557 species in all locations. Out of the six species found at 1050 m in Sinharaja-Enasalwatte, the three
 558 *Syzygium* species are located close to each other (group C). However, the two *Semecarpus* species

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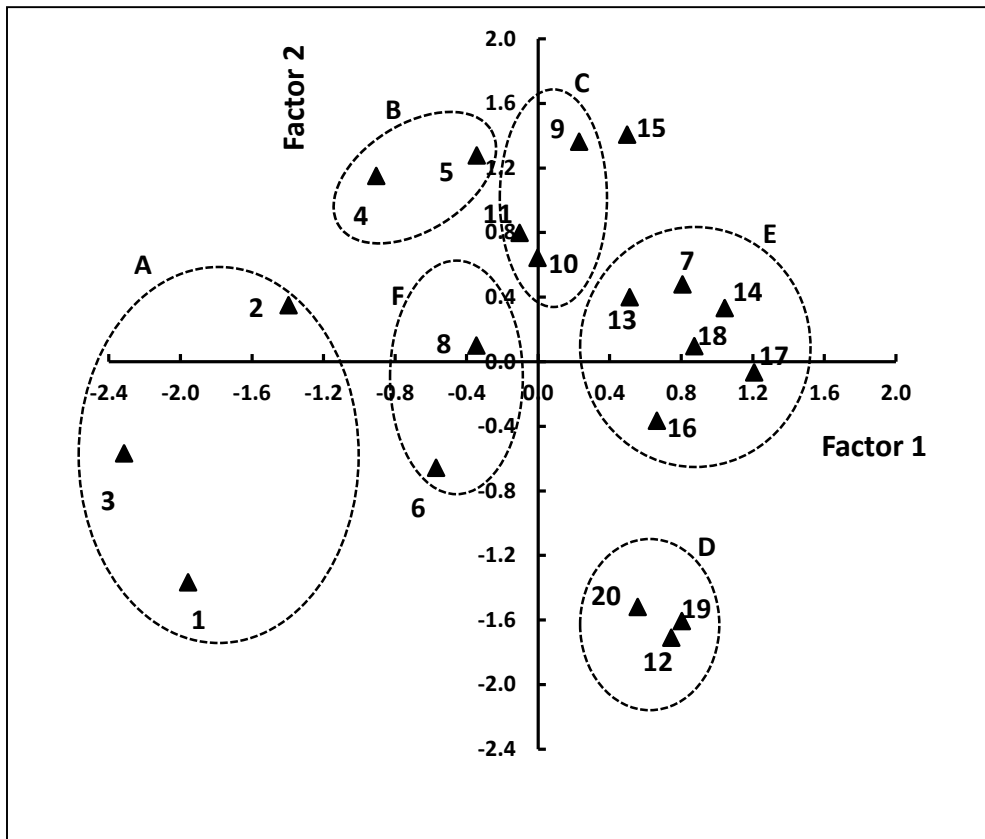


Figure 9. Distribution of plant species in factor space based on factor scores (after rotation) of Factors 1 and 2. 1 – 3: Species at 2100 m in Pidurutalagala; 4 – 6: Species at 1800 m in Hakgala; 7 – 12: Species at 1050 m Sinharaja-Enasalwatte; 13 – 20: Species at 150 m in Kanneliya. 1: *Calophyllum walkeri*; 2: *Syzygium rotundifolium*; 3: *Syzygium revolutum*; 4: *Syzygium zeylanicum*; 5: *Syzygium paniculatum*; 6: *Semecarpus obovata*; 7: *Semecarpus gardneri* (At Sin-Enasalwatte); 8: *Semecarpus parvifolia*; 9: *Syzygium cylindricum*; 10: *Syzygium micranthum*; 11: *Syzygium spp.*; 12: *Calophyllum acidus*; 13: *Syzygium alubo*; 14: *Syzygium firmum*; 15: *Syzygium neesianum*; 16: *Semecarpus gardneri* (At Kanneliya); 17: *Semecarpus subpeltata*; 18: *Semecarpus walkeri*; 19: *Calophyllum bracteatum*; 20: *Calophyllum cordato-oblongum*.

569 and the *Calophyllum* species are located away from the cluster formed by the three *Syzygium*
570 species and from each other. A majority of the eight species found at 150 m in Kanneliya have
571 formed two separate groups. One group (D) is formed by the two *Calophyllum* species (i.e. *C.*
572 *bracteatum* and *C. cordata-oblongum*). Notably, *Calophyllum acidus* from Sinharaja-Enasalwatte
573 also is located within this species group. All three *Semecarpus* species in Kanneliya are located
574 within group E. Interestingly, *Semecarpus gardneri* in Sinharaja-Enasalwatte also is located in

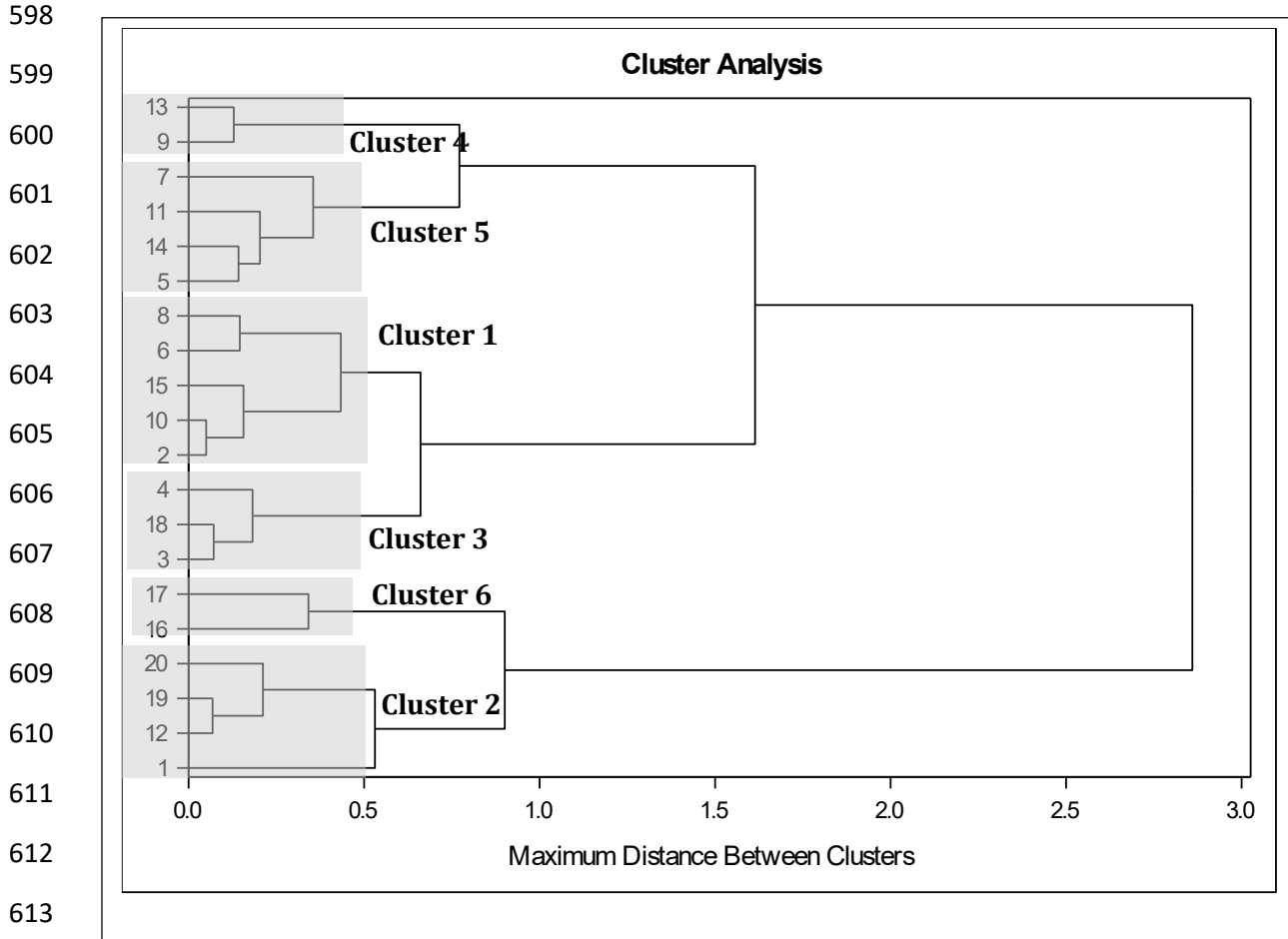
575 group E so that *S. gardneri* in both locations is within the same species group. Two of the three
576 *Syzygium* species in Kanneliya (*S. alubo* and *S. firmum*) are also located within group E along with
577 the three *Semecarpus* species. Notably, the other *Syzygium* species in Kanneliya (*S. neesianum*)
578 is located close to group C, which consists of the three *Syzygium* species in Sinharaja-Enasalwatte.
579 *Semecarpus obovata* (at Hakgala) and *S. parvifolia* (at Sinharaja-Enasalwatte) which are located
580 away from all other species at all locations form a final species group (F).

581 *Cluster analysis*

582 The dendrogram produced by cluster analysis of the leaf traits of different plant species using the
583 complete linkage method is shown in Fig. 10. At a linkage distance of just above 0.5, six species
584 clusters were identified. Multivariate analysis of variance (MANOVA) showed that the six
585 clusters were significantly different (Wilks' Lambda Statistic = 0.00000956, $p < 0.0001$). Notably,
586 all four *Calophyllum* species across the altitudinal range from 150 m to 2100 m grouped in to
587 Cluster 2. Cluster 1 consists of two sub-clusters, one having two *Semecarpus* species and the other
588 having three *Syzygium* species. It is notable that the species in both these sub-clusters come from
589 different altitudes. Cluster 3 has two *Syzygium* species from 2100 m and 1800 m, along with a
590 *Semecarpus* species from 150 m. Cluster 4 consists of two *Syzygium* species, one from 1050 m
591 and the other from 150 m. Cluster 5 has three *Syzygium* species from three different altitudes (i.e.
592 1800 m, 1050 m and 150 m) and one *Semecarpus* species from 1050 m. Lastly, Cluster 6 has two
593 *Semecarpus* species from 150 m. Therefore, while all *Calophyllum* species have grouped into one
594 cluster, there has been mixing between *Syzygium* and *Semecarpus* species during clustering. In all
595 three genera, species from different altitudes have come together in common clusters.

596

597



615 **Figure 10.** Dendrogram of cluster analysis based on leaf traits of plant species from three genera in
616 permanent sampling plots within tropical rainforests along an altitudinal gradient in Sri Lanka. 1 – 3:
617 Species at 2100 m asl in Pidurutalagala; 4 – 6: Species at 1800 m in Hakgala; 7 – 12: Species at 1050 m in
618 Sinharaja-Enasalwatte; 13 -20: Species at 150 m in Kanneliya. 1: *Calophyllum walkeri*; 2: *Syzygium*
619 *rotundifolium*; 3: *Syzygium revolutum*; 4: *Syzygium zeylanicum*; 5: *Syzygium paniculatum*; 6: *Semecarpus*
620 *obovata*; 7: *Semecarpus gardneri* (In Sin-Enasalwatte); 8: *Semecarpus parvifolia*; 9: *Syzygium cylindricum*;
621 10: *Syzygium micranthum*; 11: *Syzygium spp.*; 12: *Calophyllum acidus*; 13: *Syzygium alubo*; 14: *Syzygium*
622 *firmum*; 15: *Syzygium neesianum*; 16: *Semecarpus gardneri* (In Kanneliya); 17: *Semecarpus subpeltata*;
623 18: *Semecarpus walkeri*; 19: *Calophyllum bracteatum*; 20: *Calophyllum cordato-oblongum*. Six clusters
624 are identified by hatched boxes.

625

626 Discussion

627 We discuss the above results in terms of how they have provided answers to the questions that we
628 posed at the beginning of this work.

629 *Do the altitudinal responses of stomatal traits vary for different taxa?*

630 As the genus x altitude interaction did not account for a substantial variance component in any of
631 the stomatal traits (Table 4), our results do not support the hypothesis that the altitudinal variation
632 of individual stomatal anatomical traits is different among different taxa in TRFSL. Out of the
633 stomatal traits, only PCI, a composite trait combining the effects of stomatal size (GCL) and
634 density (SD) in determining leaf gas exchange capacity, and a proxy for photosynthetic capacity
635 (Wong et al. 1979, Farquhar and Sharkey 1982) showed variation with altitude (Table 3). It is
636 notable that neither the genus nor genus x altitude interaction contributed appreciably to the total
637 variance of PCI, thus indicating that the observed altitudinal response of PCI is common for
638 different taxa. Interestingly, the two component traits of PCI (i.e. GCL and SD) show substantial
639 variation among genera (Table 4) while not responding to altitudinal variation. Therefore, our
640 observation of a common altitudinal response of PCI supports a hypothesis that the genetically
641 diverse altitudinal responses of individual stomatal traits are part of an integrated altitudinal
642 response in the leaf gas exchange capacity.

643 *Are the altitudinal responses of different leaf traits linked in an integrated/coordinated response?*

644 Among the other leaf traits, LMA, LBA and leaf N showed significant altitudinal trends (Table 3).
645 Despite LBA showing an appreciable variance component due to genus, the altitudinal trends of
646 the above three traits did not show variation among the three genera (Table 4, Figs. 3, 4). This
647 affirmed the absence of evidence to support Hypothesis 1 with regard to leaf structural traits and
648 key nutrients. However, two of our observations support the hypothesis that the altitudinal
649 responses of different leaf traits are interlinked across different taxa to form a coordinated response
650 across different taxa) (Hypothesis 2). One is the significant negative correlation between PCI and
651 LMA (Table 6). The other is the result from factor analysis where PCI and LMA formed one of

652 the two major variable constructs that explained the observed variance of leaf traits (Table 7). The
653 negative correlation between PCI and LMA is mechanistically plausible. Increasing LMA
654 indicates greater investment of biomass per unit leaf area, especially in structural tissue, thus
655 enabling a longer leaf lifespan. The leaf economic spectrum (Wright et al. 2004, Onoda and
656 Wright 2018) specifies that greater investment in leaf structure is accompanied by lower
657 investment in photosynthetic machinery, which entails a ‘slower return’ in terms of assimilate
658 production. Lower photosynthetic capacity does not require a high CO₂ absorption capacity via a
659 higher stomatal conductance. As PCI can be considered as the maximum possible stomatal
660 conductance (g_{\max}), it is physiologically plausible that photosynthetic capacity and g_{\max} are
661 negatively correlated to greater investment in leaf structural tissue (i.e. higher LMA) and longer
662 leaf lifespan.

663 We find only partial evidence that variation of N and P across altitudes, their associated climatic
664 parameters and genera is part of a coordinated response along with stomatal anatomical and leaf
665 structural traits. For example, we could expect leaf N to be positively correlated to PCI if higher
666 leaf N has resulted in greater investment of N in photosynthetic machinery (e.g. Rubisco).
667 Similarly, a positive correlation could be expected between PCI and leaf P, if higher leaf P
668 indicates greater investment in energy storage molecules such as ATP and NADPH. Across the
669 whole range of altitudes in our study, leaf N and P were not significantly correlated to PCI (Table
670 7). However, both leaf N and P, which had shown increases from low (150 m) to mid (1050 m)
671 altitude, showed decreases from mid- to high (> 1800 m) altitudes (Fig. 4) where parallel decreases
672 have occurred in PCI as well (Fig. 2). Therefore, the reductions in N and P from mid- to high
673 altitudes could be due to down-regulation of photosynthetic capacity, as shown by decreased PCI.
674 Based on global scale studies, leaf N and P were also expected to be negatively correlated to LMA

675 as greater investment in leaf structural tissue in the high-LMA leaves would mean reduced
676 allocation of N and P to leaves. However, across the whole range of altitudes we have not found
677 significant correlations between LMA and leaf nutrients. On the other hand, the expected strong
678 negative correlations are found between LMA and leaf nutrients from mid- to high altitudes.
679 Therefore, our results suggest that leaf nutrients vary as part of integrated/coordinated leaf trait
680 response at lower temperature and rainfall regimes (i.e. mid- to high altitudes), but not at higher
681 temperature and rainfall regimes (i.e. low altitudes). Shifts in trait relationships with variation in
682 climate have been observed by (Wright et al. 2005).

683 Examination of trait relationships and climate responses of nutrient use efficiencies of our data
684 provide evidence for leaf nutrients also being part of a coordinated response in concert with
685 stomatal anatomical and leaf structural traits. Both ‘PNUE’ and ‘PPUE’ showed negative
686 correlations with LMA (Fig. 8). As suggested by Onoda and Wright (2018), allocation of a higher
687 proportion of leaf N and P to structural tissue could have caused these reductions in ‘nutrient use
688 efficiencies’ with LMA. Furthermore, the positive relationships of ‘PNUE’ and ‘PPUE’ with T_{AV} ,
689 R_F and S_R (Fig. 8) are in accordance with a strategy of increased investment of N and P in
690 photosynthetic machinery in higher temperature and precipitation environments where LMA is
691 lower and photosynthetic capacity is higher (Fig. 6).

692 *How strong is the influence of climate variation across altitudinal gradients on responses of leaf*
693 *traits to altitude?*

694 Assessing the influence of climatic variation across the altitudinal gradient on leaf traits and their
695 associations is constrained in our work by the limited number of data points (i.e. four altitudes).
696 However, the strong trends of PCI (positive, Figs. 6.a-c) and LMA (negative, Figs. 6.d-f) with
697 temperature (T_{AV}), precipitation (R_F) and incident solar radiation (S_R) indicate significant

698 environmental control over key leaf traits. The absence of a genus level effect as shown by its
699 very low variance component (Tables 4, 5) show that climatic factors exert an influence that
700 overrides genetic variation. Mechanistic explanations are available for the observed trends
701 between the above leaf traits and climatic factors. Increase of PCI with increasing R_F agrees with
702 the tight control exerted by water availability on leaf gas exchange capacity. Species in sites of
703 higher precipitation have evolved greater gas exchange capacity (i.e. higher PCI), probably to
704 maximize carbon assimilation without being constrained by the possibility of water stress due to
705 excessive transpiration. It is possible that the higher PCI at higher R_F sites is the result of a
706 combination of climatic factors that favour higher assimilation rates. This is because in the present
707 study, higher R_F at lower altitudes (e.g. 150 m) is accompanied by higher T_{AV} , S_R and pCO_2 , as
708 can be assumed with higher WVP, (Table 1), all of which promote photosynthesis. In contrast, as
709 R_F declined progressively with increasing altitude, the lower R_F sites are at higher altitudes where
710 T_{AV} , S_R and pCO_2 also are lower. The lower T_{AV} , T_{min} and T_{max} levels at higher altitudes of the
711 present study are more likely than their R_F to impose limitations on photosynthesis (James et al.
712 1994, Cabrera et al. 1998, Zhang et al. 2005). Therefore, it is possible that the lower PCI at the
713 lower R_F sites have occurred more as a response to decreased photosynthetic potential at lower
714 temperatures than a response to lower R_F .

715 The negative trends of LMA with T_{AV} , R_F and S_R (Fig. 6) indicate increased allocation of leaf
716 biomass per unit leaf area. While the negative trend with R_F agrees with the broader global trend,
717 the decrease of LMA with increasing T_{AV} is opposite to the trend shown with a broader range of
718 plant species and climates (Wright et al. 2004, 2005). However, when the trend for evergreens is
719 separated from the global trend (Wright et al. 2005), it agrees with that observed in our study. It
720 is possible that the increase of LMA with decreasing R_F and T_{AV} is a consequence of restrictions

721 imposed by both these climatic variables on LBA. In all three genera, LBA decreased with
722 decreasing R_F and T_{AV} (Figs. S5, S6) probably because of their effects on leaf cell division (CDR)
723 and expansion rates (CER). There is ample evidence from agricultural crops that in plant tissues
724 growing without water deficits, both leaf CDR and CER increases with increasing temperature
725 above a base temperature (Squire and Ong 1983, Squire 1990, Chapman et al. 1993, Salah and
726 Tardieu 1996, Tardieu et al. 2005). Furthermore, CDR and CER are highly-sensitive to water
727 availability, showing reduction with water deficit (Squire et al. 1983, Ong et al. 1985, Granier and
728 Tardieu 1999, Tardieu et al. 2000, 2005, Chenu et al. 2008). It is highly-likely that these effects
729 on CDR and CER have combined to decrease LBA with decreasing T_{AV} and R_F along the
730 altitudinal gradient in our study (Figs. S5, S6). Such a restriction of LBA would concentrate the
731 biomass allocated to leaves within a smaller area, thus causing the observed increase of LMA with
732 decreasing T_{AV} and R_F . This increase of LMA is in agreement with the observed trend of thicker
733 and leathery leaves in environments with lower R_F and with longer leaf lifespans (LL) in cooler
734 and drier environments (Niinemets 2001, Wright et al. 2001, 2004). This trend is also in
735 accordance with an integrated response, which includes the observed reduction of PCI and
736 photosynthetic capacity with decreasing T_{AV} , R_F and S_R . Even though photosynthesis was not
737 measured in the present study, mass-based photosynthetic capacity has been shown to decline with
738 increasing LMA (Osnas et al. 2013, Onoda and Wright 2018). Therefore, the highly-significant
739 negative correlation between PCI and LMA observed in our study (Table 6) is mechanistically
740 plausible and is in agreement with the global trends.

741 The highly-significant positive correlation between leaf N and P is in agreement with global scale
742 observations (Wright et al. 2004, Reich and Oleksyn 2004, Osnas et al. 2013, Onoda and Wright
743 2018). The second-order polynomial trends of leaf N and P with T_{AV} (Fig. 7) are similar to the

744 global-scale patterns for a wider range of species and climates (Reich and Oleksyn 2004). The
745 absence of a response to climatic factors in leaf N:P ratio indicates that the responses of N and P
746 to climate variation occurs in a way that N:P is conserved across different climates. This is
747 confirmed by N:P being extracted as one of the underlying variable constructs influencing the
748 variation of leaf traits across species, genera and climates.

749 *Evidence from factor analysis for an integrated/coordinated altitudinal response*

750 We preferred to perform a factor analysis instead of a principal component analysis because of our
751 assumption based on previous work (Wright et al. 2004, Onoda and Wright 2018) that there is an
752 underlying structure of trait assemblies (i.e. underlying variable constructs) that causes the
753 observed variation of leaf traits across altitudes, genera and species (Child 1990, Suhr 2005). The
754 four extracted factors, which accounted for 82% of the variance of measured leaf traits, represented
755 physiologically-meaningful trait assemblies. Factor 1, composed of SD and GCL, constituted an
756 underlying composite factor incorporating two properties that determine the abundance of stomata
757 on the leaf surface and their size. Factor 2, being loaded by PCI and LMA, clearly constituted the
758 leaf economic spectrum (Wright et al. 2004). Factor 3 constituted an underlying factor based on
759 leaf nutrient contents, the leaf N:P ratio. Stomatal index, a measure of the fraction of stomata
760 relative to the total cell population on the leaf surface, formed the fourth underlying factor. The
761 distribution of different plant species and genera in the factor space of Factors 1 and 2 did not
762 show a clear separation of species or genera. This confirms that the influence of underlying factors
763 runs across taxonomic boundaries. This is confirmed by the clustering of species in cluster
764 analysis, where except for the four *Calophyllum* species, there was considerable mixing of species
765 of *Syzygium* and *Semecarpus* from different altitudes.

766

767 **Conclusions**

768 We, therefore, conclude that: (a) the response of stomatal anatomy to altitude does not differ
769 among taxa of trees in TRFs; (b) the altitudinal responses of leaf structural traits and major nutrient
770 concentrations are interlinked in a coordinated response across different taxa; and (c) climate
771 exerts a strong influence on the variation of leaf traits to environmental gradients, which spans
772 across taxonomic boundaries of species and genera. We also find evidence that the coordinated
773 response of leaf traits to climate variation is in accordance with the leaf economics spectrum.
774 These findings have important implications on how leaf traits and their responses to climate
775 variations are represented in dynamic vegetation models to predict the response of TRFs to future
776 climate change.

777

778 **Supplementary data**

779 Supplementary data of this article is available separately.

780

781 **Conflict of interest**

782 All authors declare no conflict of interest.

783

784 **Funding**

785 This work was funded by a research grant from the National Science Foundation of Sri Lanka
786 (NTRP/2017/CC&ND/TA-04/P-01/01) in the thematic research program on climate change and
787 natural disasters.

788 **Acknowledgements**

789 We thank the Departments of Forest Conservation and Wildlife Conservation, Sri Lanka for
790 logistical support, Gemunu Wijesooriya for assistance in leaf nutrient analysis and Chameesha
791 Madhumali, Dineth Dhanushka and Suneth Kanishka for field assistance.

792

793 **Authors' contributions**

794 JC, IC and BW conceptualized and designed the work. NS and DS established permanent
795 sampling plots and carried out the plant census, with assistance from HJ and AW in taxonomic
796 identification and description. BW collected leaf samples and carried out all measurements under
797 the supervision of IC. JC, IC and BW did the statistical analysis of data. JC wrote the paper with
798 assistance from IC, AW and BW. IC and BW reviewed the initial draft and IC reviewed the final
799 draft.

800

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983

984 **Figure legends**

985 **Figures in the main text**

986 **Figure 1.** Permanent sampling plots of the present study located at different altitudes in tropical
987 rainforests of Sri Lanka. Kanneliya Plot 1 (KDN1-117 m asl), Kanneliya Plot 2 (KDN2-174 m
988 asl), Sinharaja-Enasalwatta Plot 1 (1042 m asl), Sinharaja-Enasalwatta Plot 2 (1065 m asl),
989 Hakgala Plot (1804 m asl) and Piduruthalagala Plot (2080 m asl).

990 **Figure 2.** Variation of least square (LS) means of stomatal traits of different plant genera with
991 altitude: (a) Guard cell length; (b) Stomatal density; (c) Epidermal density; (d) Stomatal index; (e)
992 Potential conductance index. Each data point is the LS mean of different species of each genus at
993 a given altitude. Error bars indicate standard errors of LS means. Number of data points for each
994 LS mean varied from 75 to 225.

995 **Figure 3.** Variation of LS means of leaf structural properties of different plant genera with altitude:
996 (a) Leaf mass per area; (b) Leaf blade area. Number of data points for each LS mean varied from
997 15 to 45.

998 **Figure 4.** Variation of LS means of leaf nutrients of different plant genera with altitude: (a) Leaf
999 N; (b) Leaf P; (c) Leaf N:P ratio. Error bars indicate standard errors of LS means. Number of data
1000 points for each LS mean varied from 3 to 9.

1001 **Figure 5.** Correlation of proxy indices of photosynthetic nitrogen use efficiency ('PNUE') and
1002 phosphorus use efficiency ('PPUE') to leaf mass per area (LMA) of different plant species (a, b)
1003 and genera (c, d) in tropical rainforests in Sri Lanka across an altitudinal range from 150 m to 2100
1004 m above sea level. ● - *Calophyllum*; Δ - *Semecarpus*; □ - *Syzygium*. 'PNUE' and 'PPUE' were
1005 calculated as the respective ratios of potential conductance index (unitless) and leaf N and P
1006 concentrations.

1007 **Figure 6.** Variation of potential conductance index (a-c) and leaf mass per area (d-f) of different
1008 plant species with temperature (a, d), precipitation (b, e) and solar irradiance (c, f) in tropical
1009 rainforests in Sri Lanka across an altitudinal range from 150 m to 2100 m above sea level. ● -
1010 *Calophyllum*; Δ - *Semecarpus*; □ - *Syzygium*. Each data point is a species-level mean value.

1011 **Figure 7.** Variation of leaf nitrogen (a - c) and phosphorus (d - f) concentrations of different plant
1012 species with temperature (a, d), precipitation (b, e) and solar irradiance (c, f) in tropical rainforests
1013 in Sri Lanka across an altitudinal range from 150 m to 2100 m above sea level. ● - *Calophyllum*;
1014 Δ - *Semecarpus*; □ - *Syzygium*. Each data point is a species-level mean value.

1015 **Figure 8.** Variation of proxy indices of photosynthetic nitrogen use efficiency ('PNUE') and
1016 phosphorus use efficiency ('PPUE') of different plant species with temperature (a, d),
1017 precipitation (b, e) and solar irradiance (c, f) in tropical rainforests in Sri Lanka across an altitudinal
1018 range from 150 m to 2100 m above sea level. ● - *Calophyllum*; Δ - *Semecarpus*; □ - *Syzygium*.
1019 Each data point is a species-level mean value.

1020 **Figure 9.** Distribution of plant species in factor space based on factor scores (after rotation) of
1021 Factors 1 and 2. 1 – 3: Species at 2100 m in Pidurutalagala; 4 – 6: Species at 1800 m in Hakgala;
1022 7 – 12: Species at 1050 m Sinharaja-Enasalwatte; 13 –20: Species at 150 m in Kanneliya. 1:
1023 *Calophyllum walkeri*; 2: *Syzygium rotundifolium*; 3: *Syzygium revolutum*; 4: *Syzygium zeylanicum*;
1024 5: *Syzygium paniculatum*; 6: *Semecarpus obovata*; 7: *Semecarpus gardneri* (At Sin-Enasalwatte);
1025 8: *Semecarpus parvifolia*; 9: *Syzygium cylindricum*; 10: *Syzygium micranthum*; 11: *Syzygium spp.*;
1026 12: *Calophyllum acidus*; 13: *Syzygium alubo*; 14: *Syzygium firmum*; 15: *Syzygium neesianum*; 16:
1027 *Semecarpus gardneri* (At Kanneliya); 17: *Semecarpus subpeltata*; 18: *Semecarpus walkeri*; 19:
1028 *Calophyllum bracteatum*; 20: *Calophyllum cordato-oblongum*.

1029 **Figure 10.** Dendrogram of cluster analysis based on leaf traits of plant species from three genera
1030 in permanent sampling plots within tropical rainforests along an altitudinal gradient in Sri Lanka.
1031 1 – 3: Species at 2100 m asl in Pidurutalagala; 4 – 6: Species at 1800 m in Hakgala; 7 – 12: Species
1032 at 1050 m in Sinharaja-Enasalwatte; 13 –20: Species at 150 m in Kanneliya. 1: *Calophyllum*
1033 *walkeri*; 2: *Syzygium rotundifolium*; 3: *Syzygium revolutum*; 4: *Syzygium zeylanicum*; 5: *Syzygium*
1034 *paniculatum*; 6: *Semecarpus obovata*; 7: *Semecarpus gardneri* (In Sin-Enasalwatte); 8:
1035 *Semecarpus parvifolia*; 9: *Syzygium cylindricum*; 10: *Syzygium micranthum*; 11: *Syzygium sp.*; 12:
1036 *Calophyllum acidus*; 13: *Syzygium alubo*; 14: *Syzygium firmum*; 15: *Syzygium neesianum*; 16:
1037 *Semecarpus gardneri* (In Kanneliya); 17: *Semecarpus subpeltata*; 18: *Semecarpus walkeri*; 19:
1038 *Calophyllum bracteatum*; 20: *Calophyllum cordato-oblongum*. Six clusters are identified by
1039 hatched boxes.

1040

1041 **List of supplementary figures**

1042 **Figure S1.** Box plots of distributions of stomatal traits of plant species of tropical rainforests in
1043 Sri Lanka at different altitudes: (a) guard cell length; (b) stomatal density; (c) epidermal density;
1044 (d) stomatal index; (e) potential conductance index. Abbreviations for species: Calbrac -
1045 *Calophyllum bracteatum*; Calcoobl - *Calophyllum cordato-oblongum*; Semgard – *Semecarpus*
1046 *gardneri* (at 150 and 1050 m); Semsu - *Semecarpus subpeltata*; Semwalk – *Semecarpus walkeri*;
1047 Syzalu - *Syzygium alubo*; Syzferm - *Syzygium firmum*; Syznees - *Syzygium neesianum*; Calcid -
1048 *Calophyllum acidus*; Semparv - *Semecarpus parvifolia*; Syzcyl - *Syzygium cylindricum*; Syzmic -
1049 *Syzygium micranthum*; Syznew - *Syzygium sp.*; Semobo - *Semecarpus obovata*; Syzpan - *Syzygium*
1050 *paniculatum*; Syzzel - *Syzygium zeylanicum*; Calwalk - *Calophyllum walkeri*; Syzrev - *Syzygium*
1051 *revolutum*; Syzrot - *Syzygium rotundifolium*.

1052 **Figure S2.** Box plots of distributions of leaf structural traits of plant species of tropical rainforests
1053 in Sri Lanka at different altitudes: (a) leaf mass per area; (b) leaf blade area. Abbreviations for
1054 species are as in Fig. S.1.

1055 **Figure S3.** Box plots of distributions of leaf nutrients of plant species of tropical rainforests in Sri
1056 Lanka at different altitudes: (a) leaf nitrogen; (b) leaf phosphorus; (c) leaf nitrogen:phosphorus
1057 ratio. Abbreviations for species are as in Fig. S.1.

1058

1059 **Figure S4.** Box plots of distributions of leaf nutrients of plant genera of tropical rainforests in Sri
1060 Lanka at different altitudes: (a) leaf nitrogen; (b) leaf phosphorus; (c) leaf nitrogen:phosphorus
1061 ratio.

1062 **Figure S5.** Species-level variation of leaf blade area (LBA) with climatic variables: (a)
1063 temperature; (b) precipitation; (c) solar irradiance. Regression lines are fitted for *Semecarpus*
1064 species separately and for *Syzygium* and *Calophyllum* species together. Each data point is a species
1065 mean.

1066 **Figure S6.** Genus-level variation of leaf blade area (LBA) with climatic variables: (a) temperature;
1067 (b) precipitation; (c) solar irradiance. Regression lines are fitted for *Semecarpus* separately and
1068 for *Syzygium* and *Calophyllum* together. Each data point is the mean of different species of a given
1069 genus.