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Aboveground-trait variations in 11 (sub)alpine plants along a 1000-m elevation gradient in tropical Mexico --Manuscript Draft--

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Aboveground-trait variations in 11 (sub)alpine plants along a 1000-m elevation gradient in tropical Mexico

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Abstract

With the aim to explore how plants acclimate to elevation changes in the understudied (sub)alpine tropics we tested two hypotheses along a 1000-m elevation gradient in Mexico: (H1) due to a severe increase in abiotic constraints at higher elevations, the functional traits of the plant species will converge toward more resource conservation, and (H2) the specific growth forms and biogeographic origins present in the (sub)alpine tropics may influence the interspecific trait variation along the gradient. We measured five aboveground functional traits: specific leaf area (SLA), leaf dry-matter content (LDMC), leaf thickness, leaf area and plant height, of 11 species representing four growth forms: rosette, tussock grass, shrub and tree the soil microclimate. Microclimatic data revealed a steep decrease in soil water content at higher elevations. Across all species and all individuals, SLA, plant height and leaf area decreased with elevation whereas LDMC and leaf thickness increased, all of which revealing adjustments towards resource conservation in line with H1. Consistently with H2, the functional traits of the growth forms that were characteristic of tropical alpine regions (tussock grasses and erect shrubs) were less sensitive to changes in elevation compared to more generalist growth forms such as forbs. Also, within the growth form “rosette” the functional traits of species of tropical biogeographic origin changed with elevation whereas those of Holarctic origin did not. Our Mexican data indicate a convergence of plant traits toward improved resource conservation at higher elevations, which may be influenced partially by the growth form and the biogeographical origin of plant species.

Keywords: biogeographic origin, growth form, leaf functional traits, Pico de Orizaba, plant height, tropical alpine systems

INTRODUCTION

Characterizing the capacities of plants to acclimate to the changing environments that are observed along elevation gradients is useful for improving scenarios on the future of plant diversity under the effects of global change. Because temperatures display monotonous variations along elevation gradients, current variations in plant diversity and in functional traits along gradients reflect, among

43 others, the capacity of individual plant species to respond to short-term changes in temperature (Körner
1 44 2007). In particular, the variations in a species' functional traits should indicate the degree to which
2
3 45 species can acclimate to changes in environmental parameters (Lavorel et al. 2007).
4

5 46 A large body of literature has reported changes in functional traits for plants to acclimate to stronger
6
7 47 abiotic constraints at higher elevations in (sub)alpine environments (Albert et al. 2010; Kichenin et al.
8 48 2013; Pescador et al. 2015). Common adjustments in aboveground functional traits at higher elevations
9
10 49 include a reduction in plant height and leaf area, which decreases the surface area exposed to cold air
11 50 (Körner 2003; Scheepens et al. 2010). Higher elevations also tend to favour more conservative species
12
13 51 that invest more carbon in thick, dense leaves, with low specific leaf area (SLA) associated with high
14 52 leaf dry matter content (LDMC), as morphological adaptations to colder conditions (Körner 2003; Poorter
15
16 53 et al. 2009) . However, not all studies along elevation gradients have found such consistent patterns
17 54 (Midolo et al. 2019; Read et al. 2014), which indicates that elevation alone may not be the only driver of
18
19 55 trait variability and that the abiotic and the biotic (micro)environments can also influence trait variation
20 56 (Almeida et al. 2013; Weemstra et al. 2021). Patterns in leaf functional traits along elevation gradients
21
22 57 have rarely been described explicitly in the (sub)alpine tropics, where ecological studies remain scarce
23 58 (Anthelme and Lavergne 2018; Llambí and Rada 2019). In this study we aimed to examine patterns in
24
25 59 plant traits along a (sub)alpine tropical elevation gradient, which is of particular interest because it
26 60 strongly differs from other gradients worldwide and is understudied.
27

28
29 61 The (sub)alpine tropics differ from temperate-arctic (sub)alpine regions in their relationships between
30 62 elevation and climate, the growth forms encountered and the biogeographic origin of species.
31
32 63 (Sub)alpine ecosystems worldwide are characterized by low nitrogen availability in soils, low minimum
33 64 temperatures, and large differences between daily minimum and maximum temperatures (Körner 2003).
34
35 65 However, one major climatic distinction between tropical and temperate-arctic alpine regions is the less-
36 66 pronounced seasonality in the tropics, that causes very frequent freeze-thaw cycles and the absence of
37
38 67 seasonal snowpack compared to temperate regions (Körner 2003; Vuille et al. 2018). Another distinction
39
40 68 is that the amount of precipitation in tropical mountains regularly decreases above 2500-3000 m, thereby
41 69 creating tropical alpine deserts at some places, in which plants experience increased constraints in
42
43 70 temperature and water availability in comparison with lower elevation (Leuschner 2000; Rada et al.
44 71 2019). The combination of colder temperatures, freeze-thaw cycles all year round and lower annual
45
46 72 precipitation at higher elevations in the (sub)alpine tropics is expected to generate the functional
47
48 73 convergence of species toward more conservative traits in these regions, a hypothesis that has not been
49 74 tested so far in these regions.
50

51 75 The climate of the tropical (sub)alpine regions has allowed for the development of growth forms highly
52 76 variable in shape and size. For example, the (sub)alpine tropics can abound with large tussock grasses,
53 77 giant cushion-forming species, erect shrubs and even trees and giant rosettes exceeding 7 m in height,
54
55 78 which are absent or much less abundant outside the (sub)alpine tropics (Hedberg and Hedberg 1979;
56 79 Ramsay and Oxley 1997). In Venezuela, specific growth forms either develop a high tolerance or use
57
58 80 ways to avoid cold and aridity, for example. The high stem density of tussock grasses makes them
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81 especially tolerant to the combination of cold, freeze-thaw cycles and drought (Rada et al. 2019).
1 82 Similarly, erect shrubs in the Venezuelan paramo develop resistance to drought at high elevations
2 83 (Sandoval et al. 2019). In Bolivia above 5000 m, the woody *Polylepis tarapacana* Phil. can resist frost
3 84 and remain vegetatively active at air temperatures below -19°C because of its supercooling capacity
4 85 (Rada et al. 2001). Given the strong influence of growth forms on the plant functional responses to
5 86 abiotic constraints, it is possible that the variability of growth forms in the (sub)alpine tropics generates
6 87 interspecific variations in the functional acclimatation of plants to elevation.

10 88 Another unique characteristic of the (sub)alpine tropics is that many locations contain species of
11 89 Holarctic biogeographic origin in addition to species that originated in the tropics (Sklenář et al. 2011).
12 90 Therefore, despite belonging to the same growth form, two species of different biogeographic origins
13 91 may display different adaptations to cold and drought (Sklenář et al. 2012). That difference might, in
14 92 turn, translate into interspecific variations in the functional responses of plants to elevation, as expected
15 93 for growth forms.

20 94 In this study, we aimed to characterize the acclimatation of plants from different species and various
21 95 growth forms to elevation in a tropical (sub)alpine region. We investigated five aboveground functional
22 96 traits that are known to vary along temperate elevation gradients: specific leaf area (SLA), leaf dry-
23 97 matter content (LDMC), leaf thickness, leaf area, and plant height, and related this to variations in
24 98 elevation and the soil microclimate. Our study was performed on 11 plant species present at several
25 99 elevations along a 1000-m elevation (sub)alpine gradient (3400 - 4400 m a.s.l.), in Mexico's Pico de
26 100 Orizaba National Park. We tested two hypotheses: (H1) due to a severe increase in abiotic constraints
27 101 at higher elevations, the functional traits of the plant species will converge toward more resource
28 102 conservation, and (H2) the specific growth forms and biogeographic origins that occur in the (sub)alpine
29 103 tropics may influence the interspecific trait variation along the gradient.

37 104

38 105 **2. MATERIALS AND METHODS**

39 106 **2.1. Study area and sites**

40 107 The study area lies between 18° 54' 08" and 19° 40' 29" N and 96° 57' 19" and 97° 34' 41" W in the
41 108 Trans-Mexican Volcanic Belt (TMVB), a tropical volcanic mountain range which hosts Mexico's highest
42 109 peaks and last remaining glaciers (Fig. 1). The study sites were located at the extreme east of the TMVB,
43 110 on the north-eastern slope of Mexico's highest peak: Pico de Orizaba stratovolcano (5636 m; Fig. 1).
44 111 They were distributed along an elevation gradient ranging from 3400 to 4400 m.

50 112 Pico de Orizaba is not active since 1687 (Sapper 1917). It belongs to the "Orizaba Dacite sequence"
51 113 (Alcalá-Reygosa et al. 2018). Recent ash and older pyroclastic material (pumice clasts from the last
52 114 Plinian eruptive sequence, dated at 8500 to 9500 y BP; Rossotti and Carrasco-Núñez, 2004) can be
53 115 found on extensive fan deposits that also include remobilized volcanoclastic material. Chemical
54 116 compositions of the lavas and pyroclastic material in the study area vary from andesite to dacite, plus
55 117 more-evolved products such as localized rhyolitic deposits (B. Marin-Castro, unpublished data). The

118 study area is affected by repeated historical glacier activity, shown by the presence of striated rocks, as
119 well as by deposits of morrainal material (till). The regolith —especially the fan or ramp deposits above
120 the treeline—is modified constantly by wind erosion, physical meteorization, and processes related to
121 mass wasting (e.g., rock falls and debris flows). Soils in the study sites are andosols developed on
122 sediments that are mostly of volcanic origin, but also contain sand. All soils are acidic and extensively
123 podzolized. Below the treeline, the total carbon content of the upper 15 cm of soil reaches 10.15 g kg⁻¹,
124 versus only 1.6 g kg⁻¹ above the treeline (B. Marin-Castro, unpublished data).

125 The climate of the study area is semi-dry and cold between 3500 and 4000 m a.s.l, which corresponds
126 roughly to the tropical subalpine vegetation belt. Above 4000 m (i.e., above the treeline), the climate is
127 dry, tropical alpine (Cházaro Basáñez 1992). Meteorological measurements made between years 2018
128 and 2020 in the vicinity of the elevation gradient selected in this study (Fig. 1a) reveal that the average
129 annual air temperature decreased from 8.8 °C at 3110 m to 3.8 °C at 4410 m, indicating a temperature
130 decrease of approx. 0.4°C each 100m. Annual precipitation during the period 2018-2020 reached 1300
131 mm at 3110 m and 800 mm at 4410 m. The highest monthly precipitation amounts were observed in
132 March and October (unpublished data; Davis Vantage Pro meteorological stations).

133 Pico de Orizaba is a biogeographic crossroad, combining the presence of taxa that migrated from North
134 America (Holarctic origin) and from the tropics (tropical origin; Morrone 2010). Its high prominence in
135 comparison with the surrounding landscape has contributed to isolating it from other alpine areas in
136 central America. It is part of the Mesoamerican biodiversity hotspot (Myers et al. 2000) and a unique
137 refuge for the Mexican (sub)alpine plants that will be subjected to an increase in temperature over the
138 next decades (Ramírez-Amezcuca et al. 2016; Steinmann et al. 2021).

139 The treeline at Pico de Orizaba is one of the world's highest, at approximately 4000 m a.s.l and it is
140 mainly constituted by Pine forests of *Pinus hartwegii* Lindl. Along the elevation gradient (3400-4400),
141 tussock grasses (termed “zacatonal” or “paramo”, locally) are the dominant growth form, as observed in
142 most tropical alpine environments (Hedberg and Hedberg 1979; Monteiro et al. 2011). The most
143 abundant tussock species along the gradient are *Festuca tolucensis* Kunth, *Calamagrostis tolucensis*
144 (Kunth) Trin. ex Steud., and *Muhlenbergia macroura* (Humb., Bonpl. & Kunth) Hitchc. (Cházaro Basáñez
145 1992; G. Peyre pers. obs.). Other abundant growth forms in the study area were rosettes, erect shrubs,
146 trees and forbs. In contrast, the cushion growth form, which is characteristic of tropical alpine
147 environments, were present but nor abundant or diverse (*Arenaria bryoides* Willd. ex Schldl.).

148
149 **Fig. 1** Locations of the study area and the plots from which species were sampled. **(a)** Distribution of
150 plots (black dots) along the 3400-4400 elevation gradient on the NE side of Pico de Orizaba, Mexico
151 (several plots at higher elevations are superimposed); **(b)** Pico de Orizaba location (triangle) within the
152 Trans-Mexican Volcanic Belt
153

154 2.2. Sampling

155 In October 2018, we established five 20 m x 20 m plots on the north-eastern side of Pico de Orizaba at
1 156 each of the following elevations (m): 3400, 3600, 3800, 4000, 4200, and 4400 (N = 30 plots; Fig. 1).
2 157 Each plot was as representative as possible of the vegetation and abiotic environment at each elevation.
3
4 158 In order to reduce the effects of spatial autocorrelation, the minimum distance between plots was 50 m
5
6 159 except at the 4400 m a.s.l elevation, where the minimum distance was 15 m because available space
7 160 was limited. To minimize the effects of variables that were not related to elevation, each plot had a
8
9 161 moderate, N-NE-facing slope (10-20°) and we avoided selecting plots with complex topography.
10 162 However, we acknowledge that the significant cover of trees (*P. hartwegii*) in plots at elevations 3400,
11 163 3600 and 3800 was a significant driver of plant species distribution and functioning.

14 164 2.3. Selection of plant species

16 165 We aimed at sampling a mixture of species representative of the local vegetation and that allowed us to
17 166 infer the effects of elevation on plant traits. For this purpose, we first analysed bibliographic data in order
18 167 to identify the most abundant species in the study area (Cházaro Basáñez 1992; Rzedowski 2001).
19 168 Second, we conducted an exploratory fieldwork in October 2017 and checked which of the preselected
20 169 species (1) were present at least at three consecutive elevations (Appendix Table S1), (2) occupied at
21 170 least 25% of the soil cover within each plot as estimated empirically from visual observations, and (3)
22 171 were representative of the four most-abundant growth forms in the study area (see section 2.1.). We
23 172 used these pieces of information to select 11 species, segregated into two erect shrubs (*Chionolaena*
24 173 *lavandulifolia* and *Oxylobus arbutifolius*), two tussock grasses (*Festuca tolucensis* and *Trisetum*
25 174 *spicatum*), five rosettes (*Cirsium jorullense*, *Draba jorullensis*, *Pseudognaphalium liebmannii*,
26 175 *Robinsonecio gerberifolius* and *Senecio roseus*) and two forbs (*Lupinus montanus* and *Penstemon*
27 176 *gentianoides*; Fig. 2). Eight of the species were of Holarctic biogeographic origin, and four were of
28 177 tropical origin. The rosette was the only growth form combining both species of tropical (*P. liebmannii*
29 178 and *R. gerberifolius*) and Holarctic origins (*C. jorullense*, *D. jorullensis* and *S. roseus*). Appendix Table
30 179 S1 contains detailed description of each of the 11 sampled species, including taxonomic, morphological,
31 180 ecological data (Cházaro Basáñez 1992; Straw 1962) and biogeographical data (Rzedowski 2001;
32 181 Sklenář et al. 2011).

33 182
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35 183
36
37 184
38 185 **Fig. 2** The 11 species selected for sampling along the elevation gradient on Pico de Orizaba volcano
39 186 (Mexico), and their respective growth forms (in brackets): **(a)** *Chionolaena lavandulifolia* (erect shrub),
40 187 **(b)** *Cirsium jorullense* (rosette), **(c)** *Draba jorullensis* (rosette), **(d)** *Festuca tolucensis* (tussock grass),
41 188 **(e)** *Lupinus montanus* (forbs), **(f)** *Oxylobus arbutifolius* (erect shrub), **(g)** *Penstemon gentianoides*
42 189 (forbs), **(h)** *Pseudognaphalium liebmannii* (rosette), **(i)** *Robinsonecio gerberifolius* (rosette), **(j)** *Senecio*
43 190 *roseus* (rosette) and **(k)** *Trisetum spicatum* (tussock grass). Detailed characteristics of the selected plant
44 191 species are given in Appendix 1. Photos: F. Anthelme (a-d, f-k) and N. Cruz (e)

59 193 2.4. Microclimatic measurements

194 To complement existing air temperature data from the two meteorological stations present in the study
195 area (Fig. 1a), we made belowground microclimatic measurements at the plot level with the aim to
196 characterize soil temperature and humidity along the elevation gradient. At the centre of each plot in
197 each elevation, we installed a temperature data logger (HOBO Pendant® data logger 8K) 5 cm
198 belowground, in open areas dominated by herbaceous vegetation (N = 21 after the loss or damage of 9
199 loggers). Measurements were made every two hours between 22 October 2018 and the 2 October 2019.
200 From this dataset, we calculated the average minimum and maximum daily temperature and the average
201 daily temperature range, calculated as the difference between the two former measurements. At the
202 same location as each logger, we measured the soil volumetric water content (SWC, %, values ranging
203 from zero to the total porosity) in each plot with a soil moisture meter TDR100 (6440FS, Fieldscout,
204 Spectrum technologies). We made five snapshot measurements in a 1*1m subplot surrounding the
205 logger, each at 5-10 cm belowground. Measurements were made between the 20th and the 24th of
206 October 2018 between 9:00 AM and 16:40 AM, during a wet period with rain events occurring each night
207 and cloudy days.

2.5. Trait measurements

210 We measured five aboveground functional traits. Two allometric traits were related to the size of
211 individuals (plant height and leaf area), the three others were associated with the leaf economic
212 spectrum of plants (SLA, LDMC, leaf thickness; Wright et al. 2004). Such a combination of traits is
213 expected to capture a representative portion of the functional acclimatation of plants along
214 environmental gradients (Díaz et al. 2016)

215 For each species at each elevation, we selected five mature, healthy individuals for trait measurements,
216 one in each plot at every elevation. Maturity was evaluated by selecting individuals with the presence of
217 reproductive organs (flower and/or fruits). They were collected outside the crown of trees and, more
218 generally, outside the canopy of potential benefactor plants to avoid nurse effects, which are expected
219 to be strong in these environments (Anthelme and Dangles 2012). We measured the vegetative plant
220 height of each individual from the stem base to the top of the main vegetative canopy. For leaf trait
221 measurements, we collected between 3 and 25 leaves (mature, but not senescent or damaged) from
222 each individual's vegetative organs (not on floral stems). The number of leaves collected from a given
223 individual depended upon the average area of the plant's leaves. For leaf area > 1 cm², we collected
224 five leaves (or three, for *C. jorullense*); for area < 1 cm² we collected 10 leaves; and for area < 0.5 cm²
225 we collected 20-25 leaves. To obtain sufficient leaf tissue for analysis from species with few and small
226 leaves, we pooled leaves from multiple, neighbouring individuals. The pooled plants were treated as
227 one individual. Leaves from tussock grasses were collected at the centre of tussocks to avoid collecting
228 dead material. We wrapped the leaves or branches of each individual in a wet paper towel, which we
229 then stored in a plastic bag, in a portable cooler during fieldwork.

230 In the laboratory, we rehydrated the leaves in water overnight in a cool, dark environment. Then, leaf
231 traits were measured according to the protocol described by Cornelissen et al 2003. Within 24 hours
232 after collection, leaf thickness was measured with a digital micrometre (QuantuMike IP65 Mitutoyo 0-25

mm) at three locations (where no veins were present) on five representative leaves per plant and averaged per individual plant. Next, we removed petioles and determined the fresh mass of selected leaves; the leaves were then flattened and scanned at 300 dpi with a flatbed scanner (Cannon MG3010 PIXMA). The scans were analysed with ImageJ software (Schneider et al. 2012) to determine leaf area. Then, the leaves were oven-dried for 72 h at 60° C to determine leaf dry weight. Specific leaf area (SLA) was calculated by dividing the area by the dry weight of the scanned leaves. Average leaf area was calculated by dividing the leaf area by the number of leaves scanned for each plant. Leaf dry-matter content (LDMC) was calculated by dividing the leaf dry weight by the leaf fresh weight.

2.6. Data analysis

We used the statistical software R version 3.6.0 (R Core Team 2019) for all graphs and statistical analyses using *lme4* (Bates et al. 2015), *MuMIn* (Barton 2019), *lmerTest* (Kuznetsova et al. 2017) and *ggplot2* (Wickham et al. 2016). Normality and homoscedasticity of standardized residuals were assessed before running statistical tests. Log10 transformations were made when necessary, to reduce deviation from normality. To facilitate visualization of the results, individual observations were back transformed for SLA, LDMC and leaf thickness (Fig. 4 and 5). For leaf area and plant height we used the log-scale y-axis (Fig. 4 and 5) to avoid curves on the model predictions line.

We first applied linear mixed models to determine the effects of elevation on the microclimatic variables (mean, minimum, maximum and range soil temperatures as well as SWC). We used the microclimatic variables as the dependent factor, “elevation” as the fixed effect and “plot” as the random effect (Nakagawa and Schielzeth 2013).

To test the first hypothesis related to trait-elevation relationship across the 11 species, we applied a linear mixed model, using plant trait as the dependent factor, “elevation” as the fixed effect (R^2_m , marginal R^2 values, *i.e.*, variance explained by fixed factor only) and species nested in growth form as the random effect (R^2_c , conditional R^2 values, *i.e.*, variance explained by fixed and random factors). Then, plant trait responses to elevation were tested for each species. We applied a linear mixed model to assess the contribution of each species and growth form to the mixture of species using plant trait as the dependent factor, “elevation” as the fixed effect and as the random effect “plot” for each species.

To test the second hypothesis on the effects of the variables “growth form” and “biogeographic origin”, we applied a linear mixed model using plant trait as the dependent factor, “elevation” as the fixed effect and “species nested in “plot” as the random effect.

3. RESULTS

3.1. Microclimatic data along the elevation gradient

Minimum daily soil temperature decreased along the gradient, from 7.4°C at 3400 m to 2.4°C at 4400 m, corresponding to an average decrease of 0.5°C every 100 m ($R^2_m= 0.78$; Fig. 3a). Mean daily soil temperatures followed the same trend ($R^2_m= 0.30$; Fig. 3c) whereas maximum daily temperatures were not linearly correlated with elevation (Fig. 3c). Daily temperature range increased significantly

270 with elevation, from 2.8°C at 3400 m to 12.4°C at 4200 m ($R^2_m= 0.63$; Fig. 3d). Soil volumetric water
271 content (SWC) decreased significantly with elevation (41.6% and 9.9% at elevations 3400 and 4400,
272 respectively; $R^2_m= 0.80$, Fig. 3e). The random variable “plot” did not influence the observed
273 relationships because R^2_m and R^2_c were similar for each microclimatic variable.

274 **Fig. 3** Daily variations in the soil microclimate along the elevation gradient. **(a)** Minimum temperature,
275 **(b)** Maximum temperature, **(c)** Mean temperature, **(d)** Range temperature and **(e)** soil volumetric water
276 content (SWC). Data were measured at a depth of 5-10 cm beneath the soil surface. Mean daily
277 temperature was measured in each plot between October 2018 and October 2019. SWC, snapshot
278 measurements in October 2018. Green dashed lines show the elevation of the treeline. Gray dots:
279 individual observations. Black lines: significant regressions between elevation and plant traits obtained
280 from the linear mixed model predictions; *** = $p < 0.001$ and ** = $p < 0.01$

3.2. Variation of traits with elevation

283 Across all individuals of all species, each of the five traits were significantly correlated with elevation:
284 SLA, leaf area and plant height decreased whereas LDMC and leaf thickness increased at higher
285 elevations (linear mixed model: $P < 0.01$; R^2_m between 0.02 and 0.06; Fig. 4). The predictions of the
286 linear mixed model increased as soon as we took into account the effect of the random variable “species
287 nested within growth form” (R^2_c between 0.70 and 0.94; $P < 0.001$).

288 With regard to species-specific trait-elevation patterns, SLA decreased linearly with elevation for six of
289 the 11 species but it increased for one species (*R. gerberifolius*) and was stable for four species (*C.*
290 *zorullense*, *D. zorullensis*, *F. toluensis* and *T. spicatum*; Fig. 5). Among the six species experiencing
291 decreasing SLA along the gradient, *L. montanus*, *P. gentianoides*, *P. liebmanni* and *S. roseus* increased
292 their LDMC (stable leaf thickness) whereas *L. montanus*, *P. liebmanni* and *O. arbutifolius* increased
293 their leaf thickness (LDMC stable). Leaf area decreased linearly at higher elevations for seven species,
294 was stable for three species, and increased for one species (*R. gerberifolius*). The plant height of five
295 species decreased at higher elevations but was constant for the other six species.

297 **Fig. 4** Variation in five plant traits along the elevation gradient: **(a)** SLA: specific leaf area; **(b)** leaf dry-
298 matter content (LDMC, $g\ g^{-1}$), **(c)** leaf thickness (mm), **(d)** leaf area (cm^2), and **(e)** plant height (cm).
299 Green dashed lines show the elevation of the treeline. Gray dots: individual observations. Black lines:
300 significant linear regressions between elevation and plant traits; *** = $p < 0.001$ and ** = $p < 0.01$. Note
301 the log-scale y-axis in Fig. 4e

303 **Fig. 5** Variation in plant traits along the elevation gradient within 11 species. Gray dots are individual
304 observations. The traits are SLA (specific leaf area, $m^2\ kg^{-1}$); LDMC (leaf dry-matter content, $g\ g^{-1}$); leaf
305 thickness (mm); LA (leaf area, cm^2); and plant height (cm). Dashed lines show the elevation of the
306 treeline. Black lines represent significant linear regressions between elevation and plant traits within
307 each species; R^2 values of the regressions are shown only when significant ($P < 0.05$). Note the log-
308 scale y-axis for leaf area and plant height. See appendix Table S2 for detailed statistics

310 **Fig. 6** Variation in plant traits along the elevation gradient for four growth forms: rosette (5 species), forb
311 (2), erect shrub (2) and tussock grass (2). **(a)** SLA: specific leaf area ($m^2\ kg^{-1}$); **(b)** leaf dry-matter content
312 (LDMC, $g\ g^{-1}$), **(c)** leaf thickness (mm), **(d)** leaf area (cm^2), and **(e)** plant height (cm). Green dashed lines

313 show the elevation of the treeline. Solid and dashed lines represent significant and non-significant linear
314 regressions respectively ($P < 0.05$). Note the log-scale y-axis in the panels in Fig.6d and Fig. 6e. See
315 appendix Table S3 for detailed statistics
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317 **3.3. Effects of growth forms and the biogeographic origin**

318 Among growth forms, the five traits of the forbs and the rosettes varied significantly with elevation (Fig.
319 6; linear mixed models $P < 0.05$; Appendix Table S3). These variations went in the same direction as
320 those observed overall for the 11 species (see Fig. 4). Significant increases in SLA and decreases in
321 leaf thickness were observed in erect shrubs at higher elevations ($P < 0.01$ and $P < 0.05$, respectively)
322 whereas reduced height and leaf area were observed in tussock grasses ($P < 0.001$ and $P < 0.01$,
323 respectively). Within erect shrubs and tussock grasses, the remaining leaf traits did not vary along the
324 elevation gradient.

325 With regard to the biogeographic origin of species, none of the traits of the Holarctic rosettes varied
326 significantly along the elevation gradient (Fig. 7 and Appendix Table S4). In contrast, tropical rosettes
327 had significantly increased LDMC and leaf thickness and reduced height at higher elevations ($P < 0.01$).
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329 **Fig. 7** Variation in plant traits along the elevation gradient for holarctic (in grey) and tropical (in black)
330 rosette species. **(a)** SLA: specific leaf area ($\text{m}^2 \text{kg}^{-1}$); **(b)** leaf dry matter content (LDMC, g g^{-1}), **(c)** leaf
331 thickness (mm), **(d)** leaf area (cm^2), and **(e)** plant height (cm). Green dashed lines show the elevation
332 of the treeline. Solid and dashed lines represent significant and non-significant linear regressions
333 respectively ($P < 0.05$). Note the log-scale y-axis in panel Fig.7d and Fig. 7e. See appendix Table S4
334 for detailed statistics
335

336 **4. DISCUSSION**

337 **4.1. Trait convergence at higher elevations**

338 Our microclimatic data confirmed that the abiotic environment in Pico de Orizaba experienced steep
339 environmental changes along the elevation gradient. Beyond decreases in daily minimum and average
340 temperature as well as an increase in daily temperature range at higher elevations, which is commonly
341 observed in (sub)alpine regions, these steep changes materialized in our study area by a sharp
342 decrease in the soil volumetric water content at higher elevations. This result is consistent with Lauer
343 and Klaus (1975) working in the same region and Leuschner (2000) who showed that in the tropics,
344 precipitation was less at higher elevations. It is also consistent with the absence of regular snowmelt
345 during the growing season in the tropics, as a result of the absence of a seasonal snowpack, which
346 otherwise would considerably increase soil moisture, as observed outside the tropics (Saavedra et al.
347 2017; Vuille et al. 2018). In extra-tropical zones, soil moisture frequently remains constant along
348 elevation gradients (Loffler and Pape 2008; Moyes et al. 2013) or even increases at higher elevations
349 in alpine regions with long-lasting snow cover (Zhang and Li 2017). Some extra-tropical alpine
350 environments may also generate strong hydric and temperature stresses for plants at higher elevations,
351 e.g., in the dry, Mediterranean regions of Chile (Cavieres et al. 2006) or in the subtropical Himalayas
352 (Namgail et al. 2011). There, sun-facing slopes are not covered significantly by snow and annual

353 precipitation is particularly low. However, precipitation still increases with elevation in these regions (e.g.,
1 354 from 445 mm at 2500 m to 943 mm at 3200 m in Chile). Accordingly, the microclimatic data we measured
2 355 in Pico de Orizaba are representative of the specific (micro)climate of tropical alpine regions. They
3 356 strengthen the pertinence of our first hypothesis (H1) that the combination of increasing microclimatic
4 357 constraints at higher elevations in our tropical area may lead to the functional convergence of plants
5 358 toward more conservative traits.
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10 359 In line with H1, at the species mixture level, we found significant changes in functional traits at higher
11 360 elevations, that would lead to improved resource conservation. A decrease in SLA was associated to
12 361 an increase in leaf thickness and greater LDMC, as an acclimation to colder temperatures, higher
13 362 temperature amplitude and a drier environment. These variations have already been found in plants
14 363 acclimating to colder and/or drier environments (Perez-Harguindeguy et al. 2013). Similarly, decreases
15 364 in allometric traits such as plant height and leaf area reduce exposure to cold air at higher elevations
16 365 (Körner 2003). Although such adjustments were expected along at the species level, such a clear trend
17 366 at the species mixture level was not straightforward because the effects of elevation on plant traits can
18 367 be outweighed by the effects of topography (Albert et al. 2010; Pescador et al. 2015), snowpack
19 368 distribution (Choler 2018), or interactions among plants (Almeida et al. 2013; Kichenin et al. 2013). In
20 369 the French Alps, for example, Weemstra et al. (2021) found that species identity, microclimate and soil
21 370 properties better explained trait variability than the 1000 m altitudinal gradient they studied, and as a
22 371 result, found highly idiosyncratic root trait patterns among species. In Pico de Orizaba, the trend towards
23 372 improved resource conservation at higher elevations may have occurred because the combination of
24 373 lower temperatures, higher temperature range and increased, that is not compensated by increased
25 374 precipitation or the presence of a snowpack. The effects of these climatic conditions that are specific to
26 375 tropical (sub)alpine regions on plants (see also Morales et al. 2004) require more attention to better
27 376 characterize the current and future acclimation of plants to elevation changes.
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38 377 Considering each species individually (intraspecific level) confirms the trend towards better conservation
39 378 of resources, with the majority of species having adjusted aboveground traits toward improved resource
40 379 conservation with increasing elevation, as also found by Albert et al. (2010) studying (sub)alpine plant
41 380 species at their upper altitudinal distribution. Nevertheless, the fact that SLA decreased for six species,
42 381 was stable for four species and increased for one species at higher elevations indicates that elevation
43 382 is not the only factor affecting trait variation (see also Kichenin et al. 20188 and Weemstra et al. 2021
44 383 outside the tropics). So, although our first hypothesis was confirmed at the species mixture level, and
45 384 largely within species, there may be species-specific responses to environmental or elevation gradients,
46 385 as found already in temperate climates (Albert et al. 2010; Kichenin et al. 2013; Weemstra et al. 2021).
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52 386 **4.2. Divergent responses of growth forms**

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55 387 Characterizing the underlying drivers of the species-specific effects was the core of our second
56 388 hypothesis (H2): we hypothesized first that the variability of growth forms in tropical alpine environments
57 389 would influence the outcome of the trait-elevation relationships. Our result that the functional traits of
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390 forbs and rosettes were more sensitive to the elevation gradient than those of erect shrubs and tussock
1 391 grasses therefore corroborates H2.
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4 392 Forbs develop erect shapes that are observed in most of the terrestrial environments worldwide but they
5 393 are not specifically adapted to extreme environments (Ramsay and Oxley 1997). Therefore, having more
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7 394 conservative traits at higher elevations in Pico de Orizaba was expected, reflecting an acclimation to
8 395 increasing microclimatic constraints (Lavorel et al. 2007). In contrast, rosettes are common in all alpine
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10 396 environments. At extra-tropical latitudes, part of their success is explained by the fact that leaves are
11 397 mostly located at ground level, thus partly avoiding the effects of cold night air, probably because they
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13 398 benefit from ground-heat output (Ramsay 2001). Therefore, our results, which showed that rosette traits
14 399 were highly sensitive to the elevation gradient (lower SLA, higher LDMC and leaf thickness, reduced
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16 400 plant height and leaf area), are counter-intuitive in the sense that rosette should be already small and,
17 401 thus, protected from cold and not requiring more conservative traits. Part of the explanation for this result
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19 402 may lie in the fact that even smaller rosettes close to the ground should develop more conservative traits
20 403 to acclimate to a drier microclimate at higher elevation in Pico de Orizaba (see Perez-Harguindeguy et
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22 404 al. 2013). Second, in tropical (sub)alpine environments, rosette dimensions are very variable and can
23 405 reach much larger sizes than outside the tropics (giant rosettes; Hedberg and Hedberg 1979). In Pico
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25 406 de Orizaba, rosettes do not reach giant sizes as observed in the Andes or in East Africa. Nevertheless,
26 407 we observed that the vegetative height of some rosette individuals exceeded 30 cm (*Cirsium jorullense*,
27 408 *Pseudognaphalium liebmannii*, *Senecio roseus*), which is particularly uncommon in temperate and arctic
28 409 alpine environments. As a consequence, their leaves are farther from the ground level, making them
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30 410 more vulnerable to cold and dry air during the night, which may translate into lower SLA and higher
31 411 LDMC in *P. liebmannii* and in *S. roseus* (see Fig. 5).
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35 412 The low functional sensitivity of erect shrubs to elevation may be because they invest more in woody
36 413 material than the other growth forms, which inhibits freezing by presenting a structural barrier to ice
37 414 intrusion (Rada et al. 2019). Therefore, shrubs are able to cope with leaf damage caused by cold, frost
38 415 and/or drought with less severe consequences to survival, and so do not need to improve resource
39 416 conservation with lower SLA, higher LDMC and higher leaf thickness at higher elevations (Díaz et al.
40 417 2016; Midolo et al. 2019).
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45 418 Tussock grasses were the least sensitive to the elevation gradient, with only height and leaf area
46 419 reduced at higher elevations. These results support the view that tussock grasses in tropical (sub)alpine
47 420 regions are more tolerant to cold and desiccation than other growth forms (Rada et al. 2019) and
48 421 therefore do not need to acclimate to colder, drier temperatures at high elevations. The explanation for
49 422 greater tolerance to cold and drought and, thus, their ability to develop tall individuals (*F. toluensis* up
50 423 to 1 m above the treeline) with larger SLA than other growth forms, may be because that leaf abscission
51 424 decreases with elevation in this growth form (McGlone et al. 2014). As a result, living culms at the
52 425 centres of tussocks at higher elevations are protected from cold and desiccation by the high density of
53 426 dead culms. The dead culms do reduce the amount of solar radiation received by the living culms, but
54 427 tussock grasses compensate by having higher values of SLA compared to other growth forms.
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428 Interestingly, tussock grass and erect shrub, the two growth forms that showed divergent functional
1 429 responses to the elevation gradient in comparison with the species mixture were, to some extent,
2 430 characteristic of the (sub-)alpine tropics. Large tussock grasses are much more abundant in the tropical
3 431 than in the extra-tropical alpine environments (Monteiro et al. 2011; Ramsay and Oxley 1997) whereas
4 432 erect shrubs above the treeline are characteristic of these regions because they are not constrained by
5 433 the effects of snowpack (Arzac et al. 2019; Ramsay and Oxley 1997). Accordingly, the presence of
6 434 growth forms characteristic of the (sub)alpine tropics in Pico de Orizaba (tussocks, erect shrubs, taller
7 435 rosettes) contributes to the divergent functional acclimation to higher elevations among species, in line
8 436 with H2. However, this divergence was not sufficient to alter the trait convergence towards improved
9 437 resource conservation at higher elevations (see H1).

15 438 **4.3. Biogeography influences functional traits?**

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18 439 To further explore our second hypothesis (H2), we tested to what extent the biogeographic origin of
19 440 species could influence their functional responses along the elevation gradient. The pertinence of this
20 441 hypothesis lies in the fact that Pico de Orizaba is a major biogeographic crossroad between (sub)alpine
21 442 plants from the tropics and those from temperate regions of the Northern hemisphere (Holarctic). Within
22 443 the rosette growth form, our results differentiated between rosettes of tropical and Holarctic origins
23 444 based on their functional trait differences along the elevation gradient, thus further supporting H2.

24 445 Trait adjustments toward improved resource conservation (shorter plants with greater LDMC and thicker
25 446 leaves) occurred in tropical rosettes whereas the traits of Holarctic rosettes varied little along the
26 447 elevation gradient. Our interpretation is that the biogeographic origin of the tropical rosettes from warm,
27 448 tropical environments, where productivity is a priority and not tolerance to abiotic stress, results in a
28 449 poorer evolutive adaptation to cold (Donoghue 2008; Smith and Cleef 1988). This translates into higher
29 450 aboveground trait variation in tropical rosettes than in Holarctic rosettes, which are preadapted to cold
30 451 environments (Sklenář et al. 2012).

31 452 Our results support the idea that taking the biogeographic origins of species into account is a promising
32 453 advance toward better understanding the sensitivity of tropical (sub)alpine plants to a changing climate
33 454 (Wellstein et al. 2017). This biogeographic analysis is, nevertheless, exploratory and requires further
34 455 testing, with a more thorough consideration of phylogenetic signals (Donoghue 2008). Especially, the
35 456 hypothesis that the biogeographic origin of species can drive their response to freezing, i.e., higher
36 457 freezing tolerance in Holarctic species *versus* higher freezing avoidance in tropical species (Sklenář et
37 458 al. 2012), is an area of research to focus on.

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40 459 In conclusion, by focusing here on an isolated tropical (sub)alpine region, we examined the plant
41 460 functional responses to an elevation gradient in an understudied biome which is expected to enrich our
42 461 understanding of the (sub)alpine ecology. The convergence of plant functional traits toward resource
43 462 conservation that we observed at higher elevations, associated with increasing soil water stress and
44 463 lower temperatures, is a trend that deserves being considered in scenarios on the future of the alpine
45 464 biodiversity in the (sub)alpine tropics. As well, the variability in growth forms and, possibly, in
46 465 biogeographic origins, generate divergent trends in plant functional responses that should be taken into

466 consideration in these scenarios (Peyre et al.). Confirmations of these exploratory results can be
1 467 provided by replicating data in more sites in the tropical Andes, East Africa, and tropical alpine islands
2 468 worldwide, and by identifying explicitly the sources of the functional trait variation (plasticity or genetic
3 469 differentiation), (Gonzalo-Turpin and Hazard 2009; Henn et al. 2018). Our results are a promising
4 470 avenue to better understand and predict the responses of (sub)alpine plants to global changes in the
5 471 tropics.
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17 480 Technology).
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24 482 **Data availability statement**

25 483 All relevant data are accessible via the INRAE Repository <https://data.inrae.fr/dataverse/ecopics>
26 484

27 485 **Author contributions** All authors discussed the results and implications and commented on the
28 486 manuscript at all stages.
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37 490 **Compliance with ethical standards**

38 491 **Conflict of Interest** The authors declare that they have no conflict of interest.
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41 492 **Ethical approval** This study does not involve research on human participants or animals.
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43 494 **Informed consent** Informed consent was obtained from all individual participants included in the
44 495 study.
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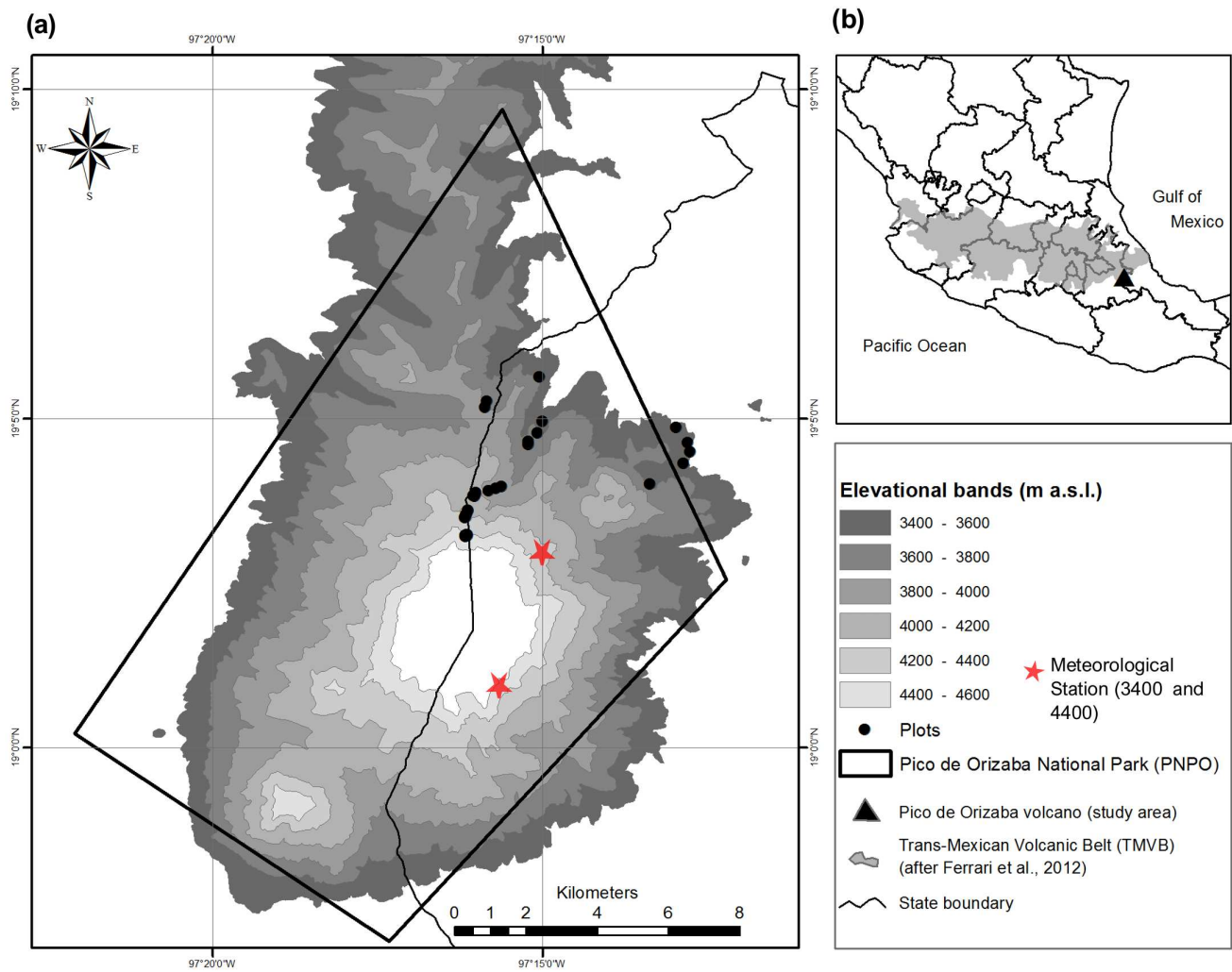
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Fig. 1



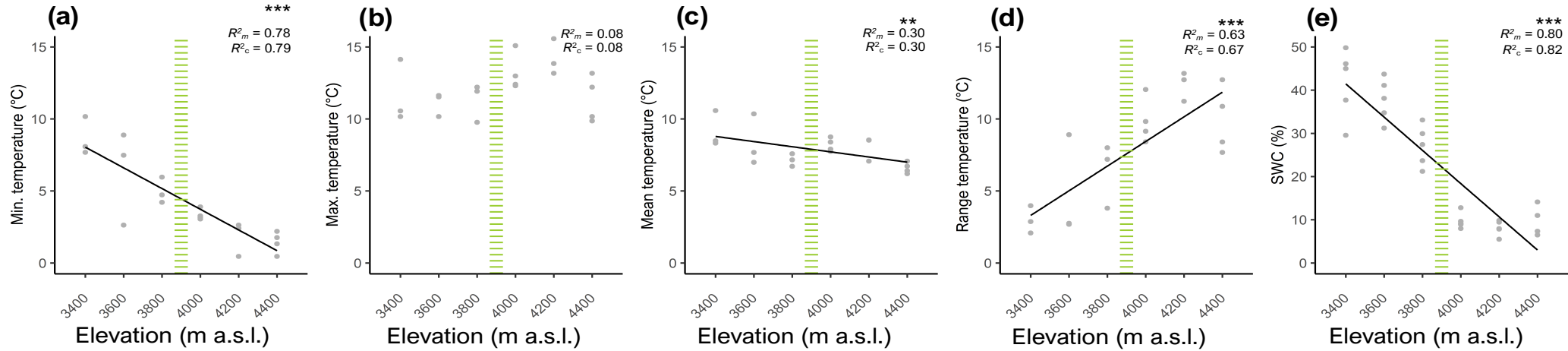
Locations of the study area and the plots from which species were sampled. **(a)** Distribution of plots (black dots) along the 3400-4400 elevation gradient on the NE side of Pico de Orizaba, Mexico (several plots at higher elevations are superimposed); **(b)** Pico de Orizaba location (triangle) within the Trans-Mexican Volcanic Belt

Fig. 2



The 11 species selected for sampling along the elevation gradient on Pico de Orizaba volcano (Mexico), and their respective growth forms (in brackets): **(a)** *Chionolaena lavandulifolia* (erect shrub), **(b)** *Cirsium jorullense* (rosette), **(c)** *Draba jorullensis* (rosette), **(d)** *Festuca tolucensis* (tussock grass), **(e)** *Lupinus montanus* (forbs), **(f)** *Oxylobus arbutifolius* (erect shrub), **(g)** *Penstemon gentia noides* (forbs), **(h)** *Pseudognaphalium liebmannii* (rosette), **(i)** *Robinsonecio gerberifolius* (rosette), **(j)** *Senecio roseus* (rosette) and **(k)** *Trisetum spicatum* (tussock grass). Detailed characteristics of the selected plant species are given in Appendix 1. Photos: F. Anthelme (a -d, f-k) and N. Cruz (e)

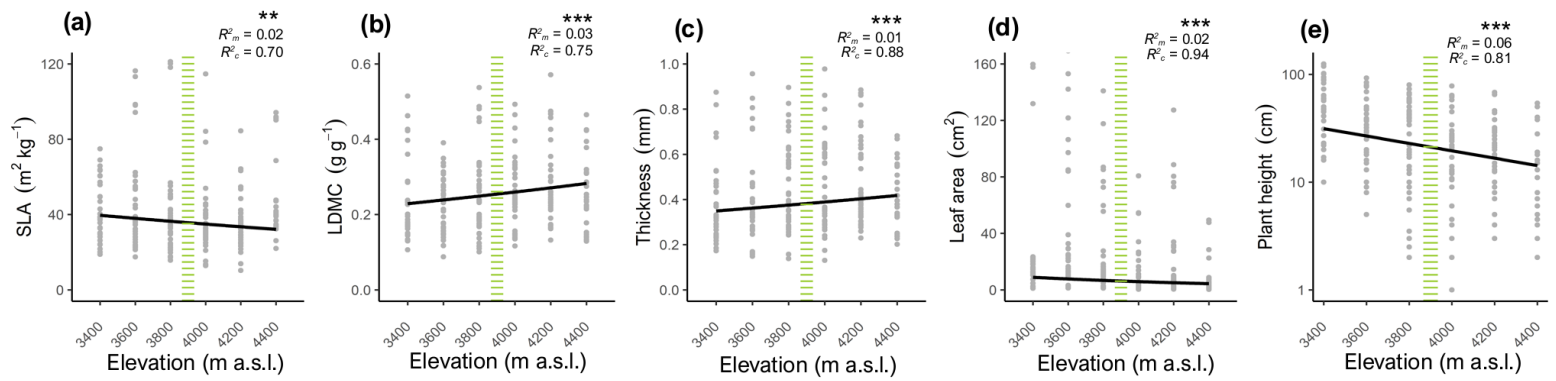
Fig. 3



Daily variations in the soil microclimate along the elevation gradient. **(a)** Minimum temperature, **(b)** Maximum temperature, **(c)** Mean temperature, **(d)** Range temperature and **(e)** soil volumetric water content (SWC). Data were measured at a depth of 5 -10 cm beneath the soil surface. Mean daily temperature was measured in each plot between October 2018 and October 2019. SWC, snapshot measurements in October 2018. Green dashed lines show the elevation of the treeline. Gray dots: individual observations. Black lines: significant regressions between elevation and plant traits obtained from the linear mixed model predictions; *** = $p < 0.001$ and ** = $p < 0.01$

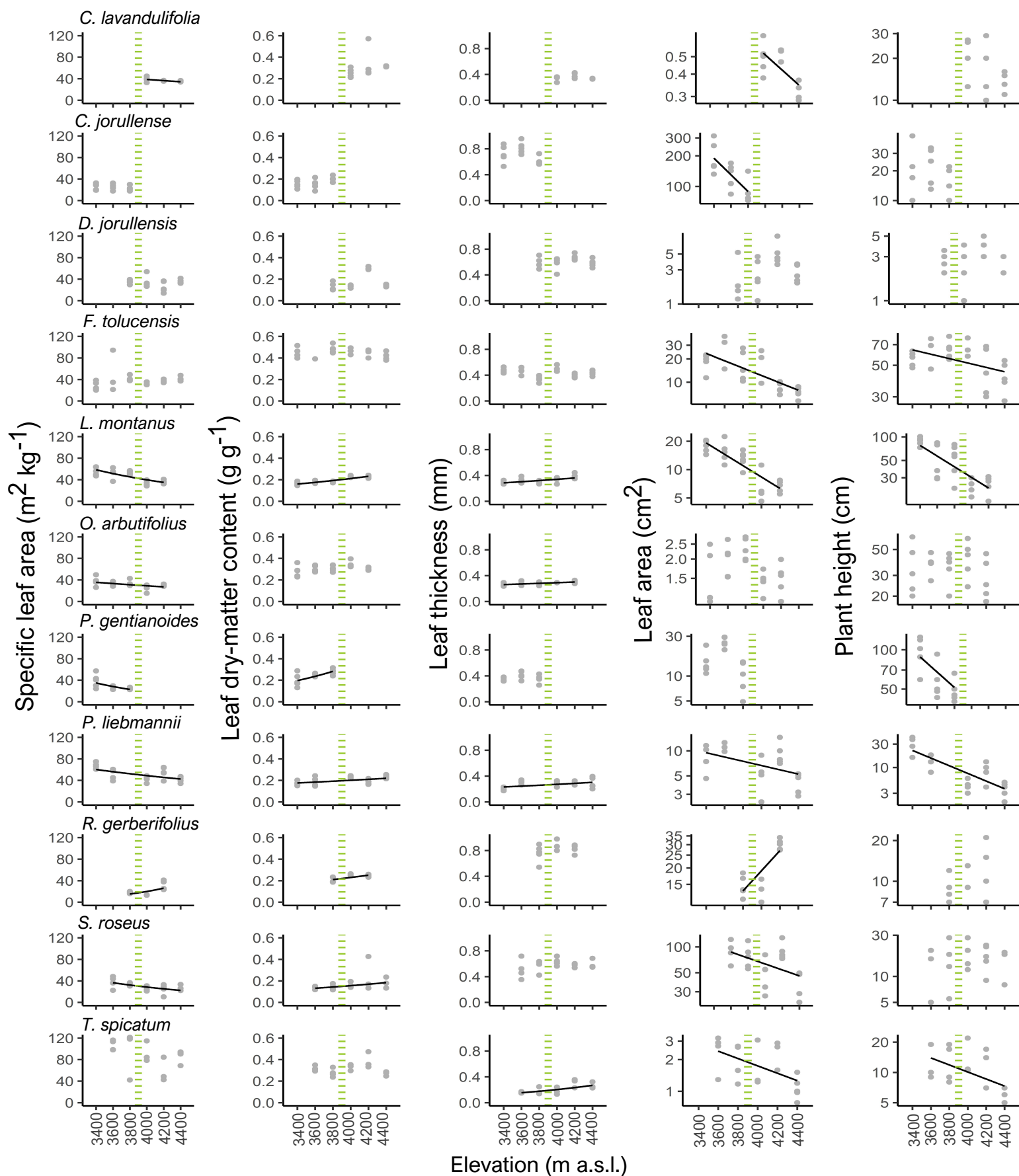
Figure 4

Fig. 4



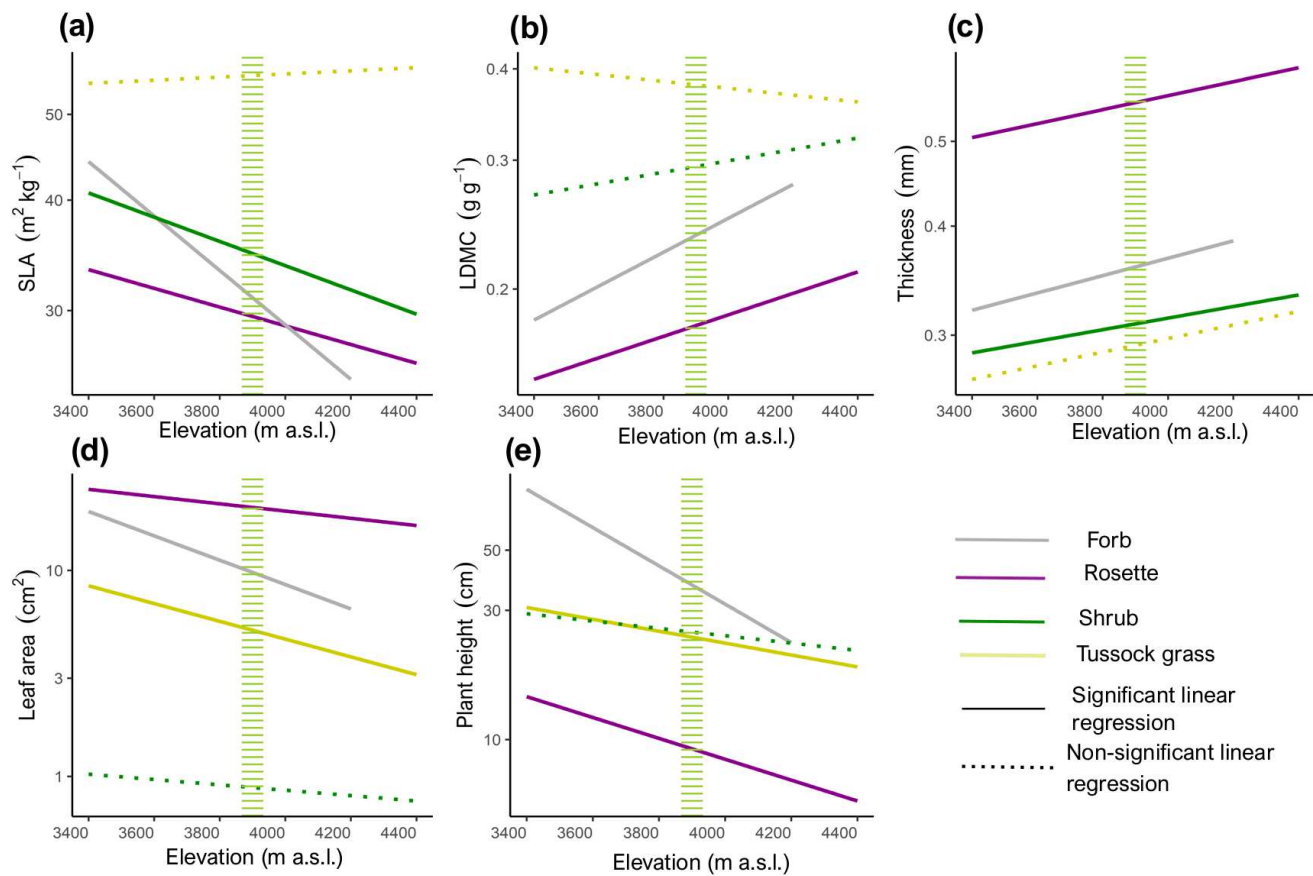
Variation in five plant traits along the elevation gradient: **(a)** SLA: specific leaf area; **(b)** leaf dry-matter content (LDMC, g g^{-1}), **(c)** leaf thickness (mm), **(d)** leaf area (cm^2), and **(e)** plant height (cm). Green dashed lines show the elevation of the treeline. Gray dots: individual observations. Black lines: significant linear regressions between elevation and plant traits; *** = $p < 0.001$ and ** = $p < 0.01$. Note the log-scale y-axis in Fig. 4e

Figure 5



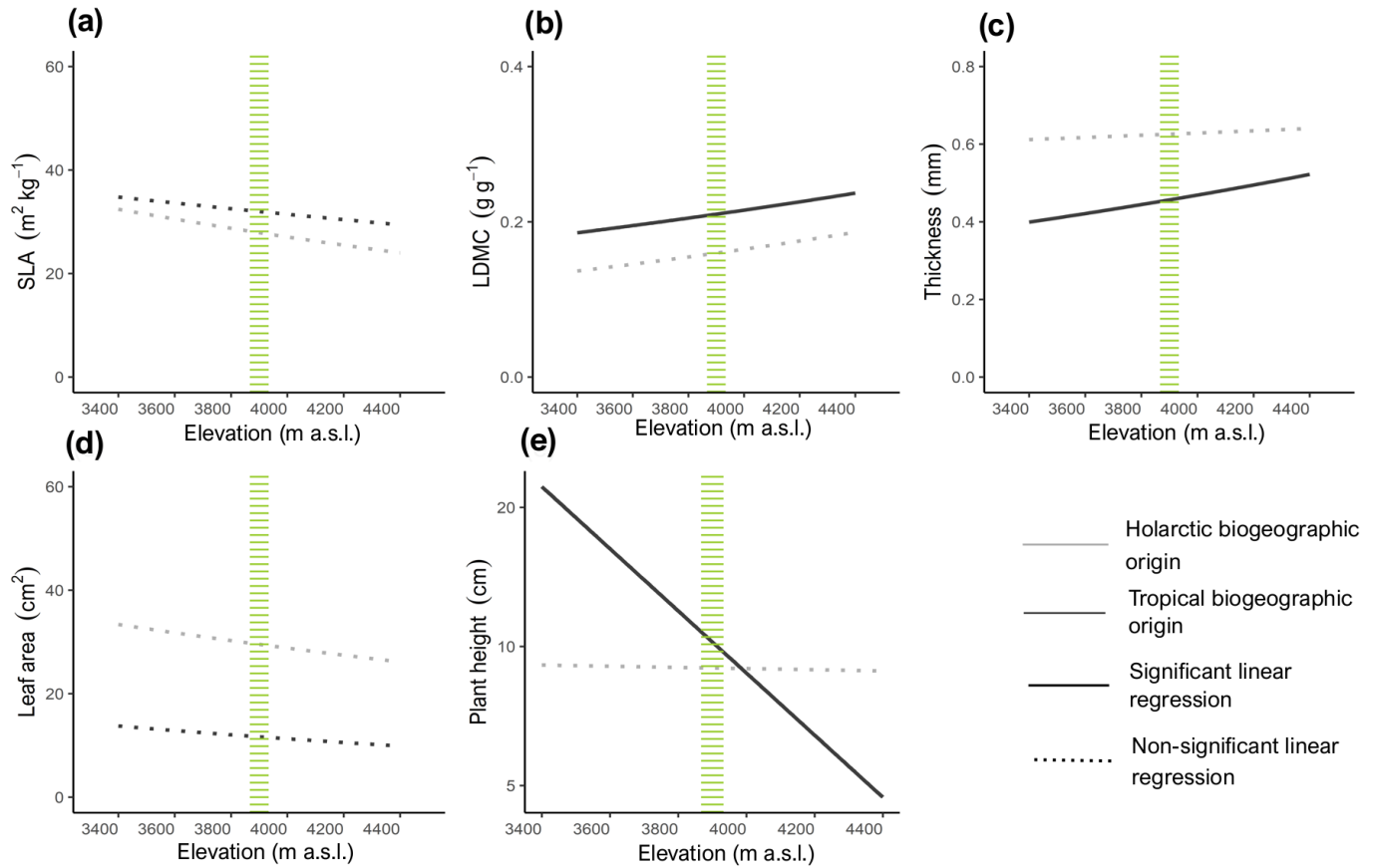
Variation in plant traits along the elevation gradient within 11 species. Gray dots are individual observations. The traits are SLA (specific leaf area, $\text{m}^2 \text{kg}^{-1}$); LDMC (leaf dry-matter content, g g^{-1}); leaf thickness (mm); LA (leaf area, cm^2); and plant height (cm). Dashed lines show the elevation of the treeline. Black lines represent significant linear regressions between elevation and plant traits within each species; R^2 values of the regressions are shown only when significant ($P < 0.05$). Note the log-scale y-axis for leaf area and plant height. See appendix Table S2 for detailed statistics

Fig. 6



Variation in plant traits along the elevation gradient for four growth forms : rosette (5 species), forb (2), erect shrub (2) and tussock grass (2). **(a)** SLA: specific leaf area ($\text{m}^2 \text{kg}^{-1}$); **(b)** leaf dry-matter content (LDMC, g g^{-1}), **(c)** leaf thickness (mm), **(d)** leaf area (cm^2), and **(e)** plant height (cm). Green dashed lines show the elevation of the treeline. Solid and dashed lines represent significant and non-significant linear regressions respectively ($P < 0.05$). Note the log -scale y-axis in the panels in Fig.6d and Fig. 6e . See appendix Table S3 for detailed statistics

Fig. 7



Variation in plant traits along the elevation gradient for holarctic (in grey) and tropical (in black) rosette species. **(a)** SLA: specific leaf area ($\text{m}^2 \text{kg}^{-1}$); **(b)** leaf dry matter content (LDMC, g g^{-1}), **(c)** leaf thickness (mm), **(d)** leaf area (cm^2), and **(e)** plant height (cm). Green dashed lines show the elevation of the treeline. Solid and dashed lines represent significant and non-significant linear regressions respectively ($P < 0.05$). Note the log-scale y-axis in panel Fig.7d and Fig. 7e. See appendix Table S4 for detailed statistics



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