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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

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

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

The (sub)alpine tropics differ from temperate-arctic (sub)alpine regions in their relationships between elevation and climate, the growth forms encountered and the biogeographic origin of species. (Sub)alpine ecosystems worldwide are characterized by low nitrogen availability in soils, low minimum temperatures, and large differences between daily minimum and maximum temperatures (Körner 2003). However, one major climatic distinction between tropical and temperate-arctic alpine regions is the less-pronounced seasonality in the tropics, that causes very frequent freeze-thaw cycles and the absence of seasonal snowpack compared to temperate regions (Körner 2003; Vuille et al. 2018). Another distinction is that the amount of precipitation in tropical mountains regularly decreases above 2500-3000 m, thereby creating tropical alpine deserts at some places, in which plants experience increased constraints in temperature and water availability in comparison with lower elevation (Leuschner 2000; Rada et al. 2019). The combination of colder temperatures, freeze-thaw cycles all year round and lower annual precipitation at higher elevations in the (sub)alpine tropics is expected to generate the functional convergence of species toward more conservative traits in these regions, a hypothesis that has not been tested so far in these regions.

The climate of the tropical (sub)alpine regions has allowed for the development of growth forms highly variable in shape and size. For example, the (sub)alpine tropics can abound with large tussock grasses, giant cushion-forming species, erect shrubs and even trees and giant rosettes exceeding 7 m in height, which are absent or much less abundant outside the (sub)alpine tropics (Hedberg and Hedberg 1979; Ramsay and Oxley 1997). In Venezuela, specific growth forms either develop a high tolerance or use ways to avoid cold and aridity, for example. The high stem density of tussock grasses makes them

especially tolerant to the combination of cold, freeze-thaw cycles and drought (Rada et al. 2019). Similarly, erect shrubs in the Venezuelan paramo develop resistance to drought at high elevations (Sandoval et al. 2019). In Bolivia above 5000 m, the woody *Polylepis tarapacana* Phil. can resist frost and remain vegetatively active at air temperatures below -19°C because of its supercooling capacity (Rada et al. 2001). Given the strong influence of growth forms on the plant functional responses to abiotic constraints, it is possible that the variability of growth forms in the (sub)alpine tropics generates interspecific variations in the functional acclimatation of plants to elevation.

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

In this study, we aimed to characterize the acclimatation of plants from different species and various growth forms to elevation in a tropical (sub)alpine region. We investigated five aboveground functional traits that are known to vary along temperate elevation gradients: specific leaf area (SLA), leaf dry-matter content (LDMC), leaf thickness, leaf area, and plant height, and related this to variations in elevation and the soil microclimate. Our study was performed on 11 plant species present at several elevations along a 1000-m elevation (sub)alpine gradient (3400 - 4400 m a.s.l.), in Mexico's Pico de Orizaba National Park. We tested two hypotheses: (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 that occur in the (sub)alpine tropics may influence the interspecific trait variation along the gradient.

2. MATERIALS AND METHODS

2.1. Study area and sites

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

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

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

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

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

The treeline at Pico de Orizaba is one of the world's highest, at approximately 4000 m a.s.l and it is mainly constituted by Pine forests of *Pinus hartwegii* Lindl. Along the elevation gradient (3400-4400), tussock grasses (termed “zacatonal” or “paramo”, locally) are the dominant growth form, as observed in most tropical alpine environments (Hedberg and Hedberg 1979; Monteiro et al. 2011). The most abundant tussock species along the gradient are *Festuca tolucensis* Kunth, *Calamagrostis tolucensis* (Kunth) Trin. ex Steud., and *Muhlenbergia macroura* (Humb., Bonpl. & Kunth) Hitchc. (Cházaro Basáñez 1992; G. Peyre pers. obs.). Other abundant growth forms in the study area were rosettes, erect shrubs, trees and forbs. In contrast, the cushion growth form, which is characteristic of tropical alpine environments, were present but not abundant or diverse (*Arenaria bryoides* Willd. ex Schldl.).

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

2.2. Sampling

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

2.3. Selection of plant species

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

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 gentianoides* (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)

2.4. Microclimatic measurements

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

2.5. Trait measurements

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

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

In the laboratory, we rehydrated the leaves in water overnight in a cool, dark environment. Then, leaf traits were measured according to the protocol described by Cornelissen et al 2003. Within 24 hours 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

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 content (SWC) decreased significantly with elevation (41.6% and 9.9% at elevations 3400 and 4400, respectively; $R^2_m = 0.80$, Fig. 3e). The random variable “plot” did not influence the observed relationships because R^2_m and R^2_c were similar for each microclimatic variable.

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 ** = $p < 0.01$

3.2. Variation of traits with elevation

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

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

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

Fig. 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

3.3. Effects of growth forms and the biogeographic origin

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

With regard to the biogeographic origin of species, none of the traits of the Holarctic rosettes varied significantly along the elevation gradient (Fig. 7 and Appendix Table S4). In contrast, tropical rosettes had significantly increased LDMC and leaf thickness and reduced height at higher elevations ($P < 0.01$).

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

4. DISCUSSION

4.1. Trait convergence at higher elevations

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

precipitation is particularly low. However, precipitation still increases with elevation in these regions (e.g., from 445 mm at 2500 m to 943 mm at 3200 m in Chile). Accordingly, the microclimatic data we measured in Pico de Orizaba are representative of the specific (micro)climate of tropical alpine regions. They strengthen the pertinence of our first hypothesis (H1) that the combination of increasing microclimatic constraints at higher elevations in our tropical area may lead to the functional convergence of plants toward more conservative traits.

In line with H1, at the species mixture level, we found significant changes in functional traits at higher elevations, that would lead to improved resource conservation. A decrease in SLA was associated to an increase in leaf thickness and greater LDMC, as an acclimatation to colder temperatures, higher temperature amplitude and a drier environment. These variations have already been found in plants acclimating to colder and/or drier environments (Perez-Harguindeguy et al. 2013). Similarly, decreases in allometric traits such as plant height and leaf area reduce exposure to cold air at higher elevations (Körner 2003). Although such adjustments were expected along at the species level, such a clear trend at the species mixture level was not straightforward because the effects of elevation on plant traits can be outweighed by the effects of topography (Albert et al. 2010; Pescador et al. 2015), snowpack distribution (Choler 2018), or interactions among plants (Almeida et al. 2013; Kichenin et al. 2013). In the French Alps, for example, Weemstra et al. (2021) found that species identity, microclimate and soil properties better explained trait variability than the 1000 m altitudinal gradient they studied, and as a result, found highly idiosyncratic root trait patterns among species. In Pico de Orizaba, the trend towards improved resource conservation at higher elevations may have occurred because the combination of lower temperatures, higher temperature range and increased, that is not compensated by increased precipitation or the presence of a snowpack. The effects of these climatic conditions that are specific to tropical (sub)alpine regions on plants (see also Morales et al. 2004) require more attention to better characterize the current and future acclimation of plants to elevation changes.

Considering each species individually (intraspecific level) confirms the trend towards better conservation of resources, with the majority of species having adjusted aboveground traits toward improved resource conservation with increasing elevation, as also found by Albert et al. (2010) studying (sub)alpine plant species at their upper altitudinal distribution. Nevertheless, the fact that SLA decreased for six species, was stable for four species and increased for one species at higher elevations indicates that elevation is not the only factor affecting trait variation (see also Kichenin et al. 2018 and Weemstra et al. 2021 outside the tropics). So, although our first hypothesis was confirmed at the species mixture level, and largely within species, there may be species-specific responses to environmental or elevation gradients, as found already in temperate climates (Albert et al. 2010; Kichenin et al. 2013; Weemstra et al. 2021).

4.2. Divergent responses of growth forms

Characterizing the underlying drivers of the species-specific effects was the core of our second hypothesis (H2): we hypothesized first that the variability of growth forms in tropical alpine environments would influence the outcome of the trait-elevation relationships. Our result that the functional traits of

forbs and rosettes were more sensitive to the elevation gradient than those of erect shrubs and tussock grasses therefore corroborates H2.

Forbs develop erect shapes that are observed in most of the terrestrial environments worldwide but they are not specifically adapted to extreme environments (Ramsay and Oxley 1997). Therefore, having more conservative traits at higher elevations in Pico de Orizaba was expected, reflecting an acclimation to increasing microclimatic constraints (Lavorel et al. 2007). In contrast, rosettes are common in all alpine environments. At extra-tropical latitudes, part of their success is explained by the fact that leaves are mostly located at ground level, thus partly avoiding the effects of cold night air, probably because they benefit from ground-heat output (Ramsay 2001). Therefore, our results, which showed that rosette traits were highly sensitive to the elevation gradient (lower SLA, higher LDMC and leaf thickness, reduced plant height and leaf area), are counter-intuitive in the sense that rosette should be already small and, thus, protected from cold and not requiring more conservative traits. Part of the explanation for this result may lie in the fact that even smaller rosettes close to the ground should develop more conservative traits to acclimate to a drier microclimate at higher elevation in Pico de Orizaba (see Perez-Harguindeguy et al. 2013). Second, in tropical (sub)alpine environments, rosette dimensions are very variable and can reach much larger sizes than outside the tropics (giant rosettes; Hedberg and Hedberg 1979). In Pico de Orizaba, rosettes do not reach giant sizes as observed in the Andes or in East Africa. Nevertheless, we observed that the vegetative height of some rosette individuals exceeded 30 cm (*Cirsium jorullense*, *Pseudognaphalium liebmannii*, *Senecio roseus*), which is particularly uncommon in temperate and arctic alpine environments. As a consequence, their leaves are farther from the ground level, making them more vulnerable to cold and dry air during the night, which may translate into lower SLA and higher LDMC in *P. liebmannii* and in *S. roseus* (see Fig. 5).

The low functional sensitivity of erect shrubs to elevation may be because they invest more in woody material than the other growth forms, which inhibits freezing by presenting a structural barrier to ice intrusion (Rada et al. 2019). Therefore, shrubs are able to cope with leaf damage caused by cold, frost and/or drought with less severe consequences to survival, and so do not need to improve resource conservation with lower SLA, higher LDMC and higher leaf thickness at higher elevations (Díaz et al. 2016; Midolo et al. 2019).

Tussock grasses were the least sensitive to the elevation gradient, with only height and leaf area reduced at higher elevations. These results support the view that tussock grasses in tropical (sub)alpine regions are more tolerant to cold and desiccation than other growth forms (Rada et al. 2019) and therefore do not need to acclimate to colder, drier temperatures at high elevations. The explanation for greater tolerance to cold and drought and, thus, their ability to develop tall individuals (*F. toluensis* up to 1 m above the treeline) with larger SLA than other growth forms, may be because that leaf abscission decreases with elevation in this growth form (McGlone et al. 2014). As a result, living culms at the centres of tussocks at higher elevations are protected from cold and desiccation by the high density of dead culms. The dead culms do reduce the amount of solar radiation received by the living culms, but tussock grasses compensate by having higher values of SLA compared to other growth forms.

Interestingly, tussock grass and erect shrub, the two growth forms that showed divergent functional responses to the elevation gradient in comparison with the species mixture were, to some extent, characteristic of the (sub-)alpine tropics. Large tussock grasses are much more abundant in the tropical than in the extra-tropical alpine environments (Monteiro et al. 2011; Ramsay and Oxley 1997) whereas erect shrubs above the treeline are characteristic of these regions because they are not constrained by the effects of snowpack (Arzac et al. 2019; Ramsay and Oxley 1997). Accordingly, the presence of growth forms characteristic of the (sub)alpine tropics in Pico de Orizaba (tussocks, erect shrubs, taller rosettes) contributes to the divergent functional acclimation to higher elevations among species, in line with H2. However, this divergence was not sufficient to alter the trait convergence towards improved resource conservation at higher elevations (see H1).

4.3. Biogeography influences functional traits?

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

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

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

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

consideration in these scenarios (Peyre et al.). Confirmations of these exploratory results can be provided by replicating data in more sites in the tropical Andes, East Africa, and tropical alpine islands worldwide, and by identifying explicitly the sources of the functional trait variation (plasticity or genetic differentiation), (Gonzalo-Turpin and Hazard 2009; Henn et al. 2018). Our results are a promising avenue to better understand and predict the responses of (sub)alpine plants to global changes in the tropics.

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Data availability statement

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

Author contributions All authors discussed the results and implications and commented on the manuscript at all stages.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical approval This study does not involve research on human participants or animals.

Informed consent Informed consent was obtained from all individual participants included in the study.

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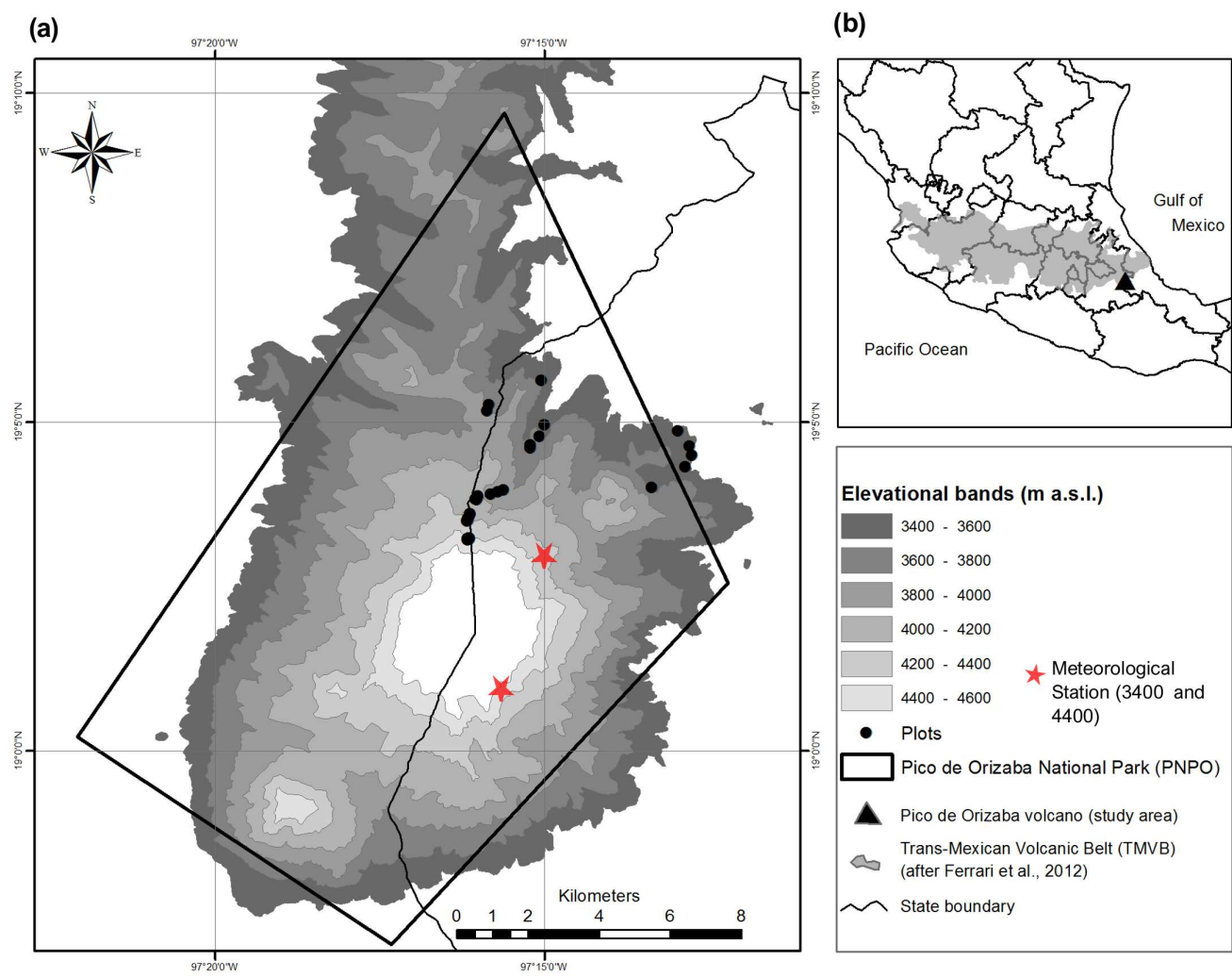
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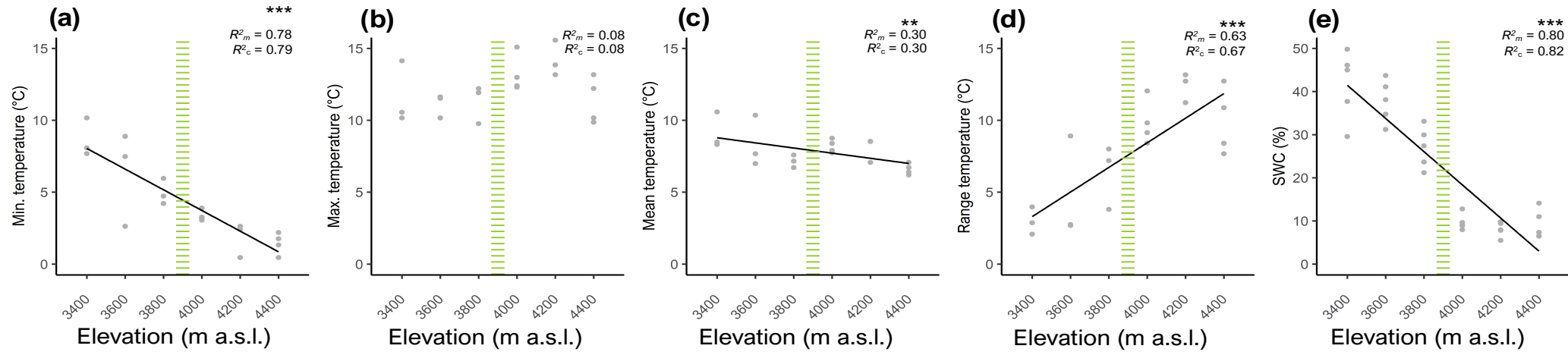
Fig. 1



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

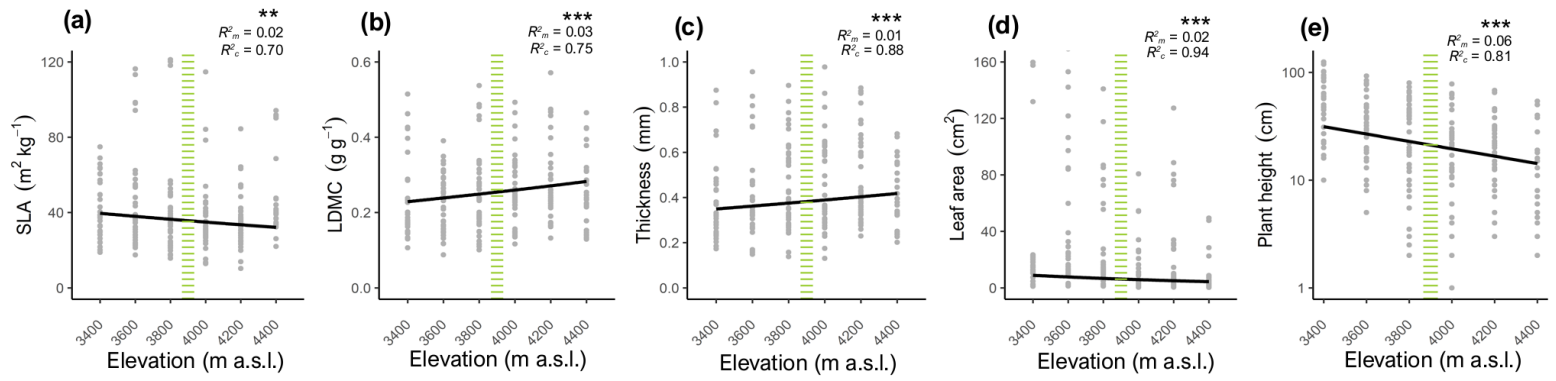
The 11 species selected for sampling along the elevation gradient on Pico de Orizaba volcano (Mexico), and their respective growth forms (in brackets): **(a)** *Chionolaena lavandulifolia* (erect shrub), **(b)** *Cirsium jorullense* (rosette), **(c)** *Draba jorullensis* (rosette), **(d)** *Festuca tolucensis* (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 ** = $p < 0.01$

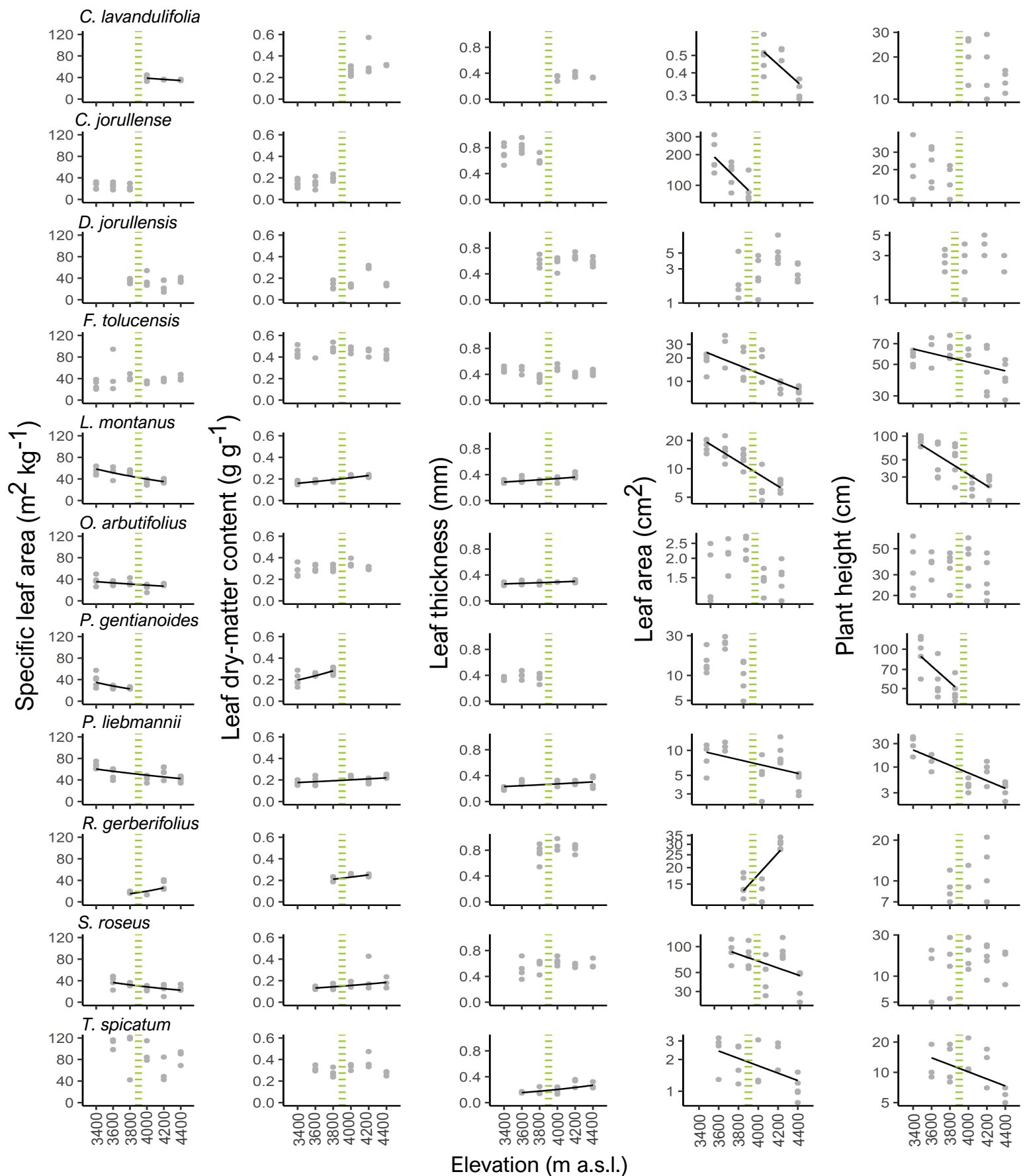
Figure 4

Fig. 4



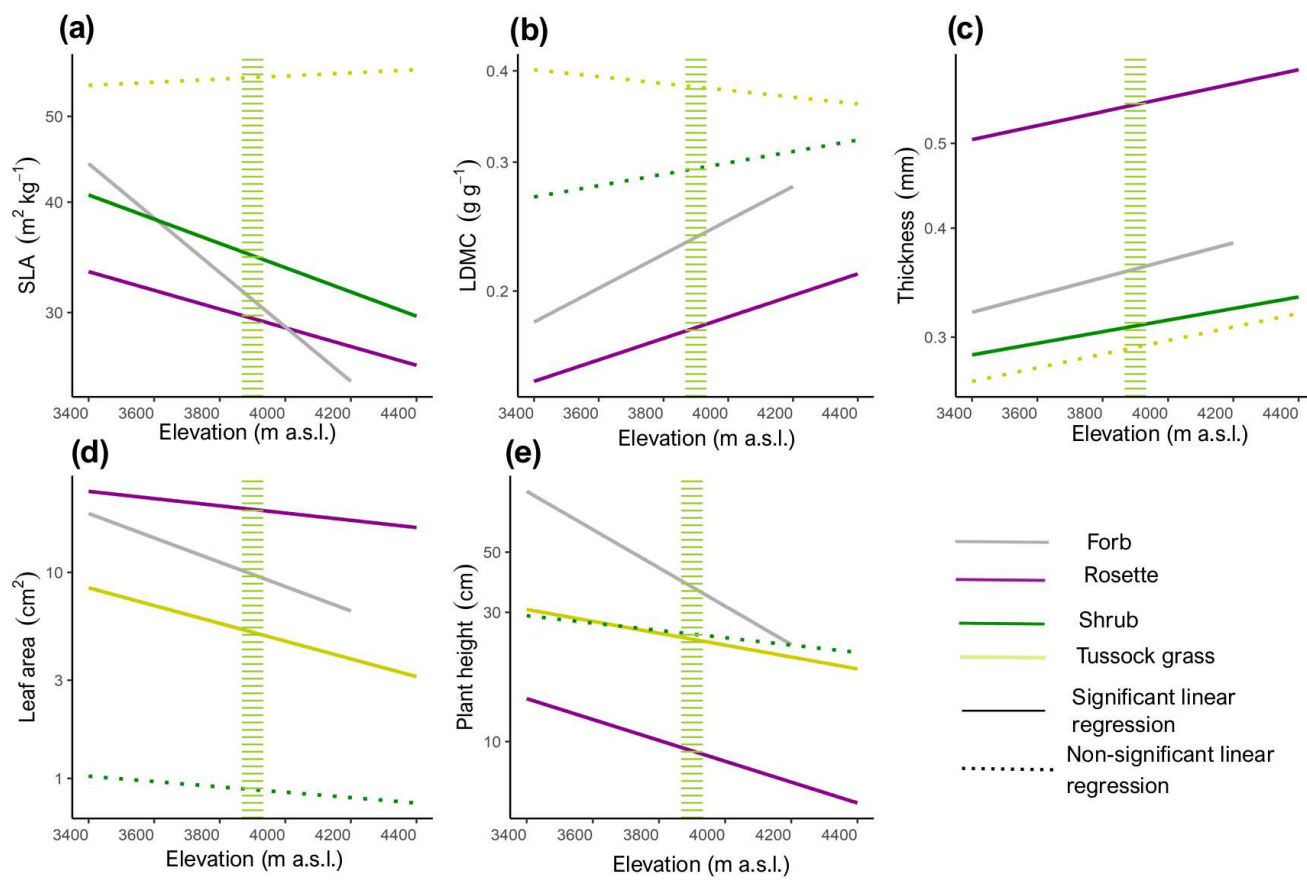
Variation in five plant traits along the elevation gradient: **(a)** SLA: specific leaf area; **(b)** leaf dry-matter content (LDMC, g g^{-1}), **(c)** leaf thickness (mm), **(d)** leaf area (cm^2), and **(e)** plant height (cm). Green dashed lines show the elevation of the treeline. Gray dots: individual 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

Fig. 5



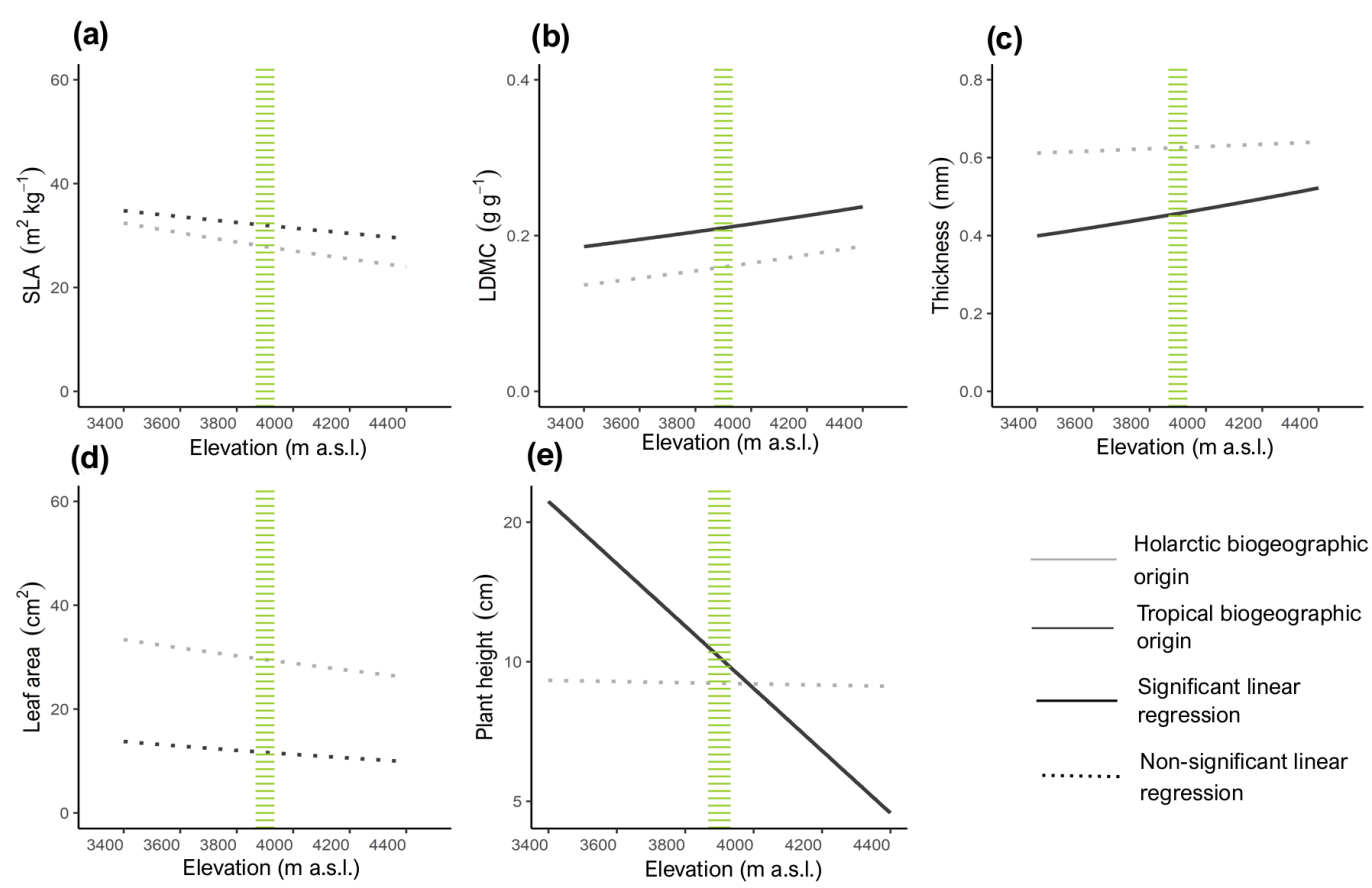
Variation in plant traits along the elevation gradient within 11 species. Gray dots are individual observations. The traits are SLA (specific leaf area, $\text{m}^2 \text{kg}^{-1}$); LDMC (leaf dry-matter content, g g^{-1}); leaf thickness (mm); LA (leaf area, cm^2); and plant height (cm). Dashed lines 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

Figure6
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

Figure7
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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