Global patterns of intraspecific leaf trait responses to elevation

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Keywords:	altitude, carbon isotope ratio, meta-analysis, leaf nutrient content, intraspecific variability, phenotypic variability, plant functional traits, environmental gradient
Abstract:	<p>Elevational gradients are often used to quantify how traits of plant species respond to abiotic and biotic environmental variation. Yet, such analyses are frequently restricted spatially and applied along single slopes or mountain ranges. Since we know little on the response of intraspecific leaf traits to elevation across the globe, we here perform a global meta-analysis of leaf traits in 104 plant species from 71 studies and four continents published between 1983 and 2017. We quantified the intraspecific change of seven morpho-ecophysiological leaf traits along global elevational gradients: specific leaf area (SLA); leaf mass per area (LMA); leaf area (LA); nitrogen concentration per unit of area (Narea); nitrogen concentration per unit mass (Nmass); phosphorous concentration per unit mass (Pmass) and carbon isotope composition ($\delta^{13}\text{C}$). We found LMA, Narea, Nmass and $\delta^{13}\text{C}$ to significantly increase and SLA to decrease with increasing elevation. Conversely, LA and Pmass showed no significant pattern with elevation worldwide. Furthermore, we found significantly larger increase in Narea, Nmass, Pmass and $\delta^{13}\text{C}$ with elevation in warmer regions. Overall, larger responses to elevation were discernible for SLA, but not for the other traits, of herbaceous compared to woody species. Finally, we also detected strong covariation across morphological and physiological traits within the same elevational gradient. In sum, we demonstrate that there are common cross-species patterns of intraspecific leaf trait variation across elevational gradients worldwide. Irrespective of whether such variation is genetically determined via local adaption or attributed to phenotypic plasticity, the leaf trait patterns quantified here suggest that plant species have different individuals adapted to live on a range of temperature conditions. Since the distribution of mountain biota is predominantly shifting upslope in response to climate change, our results</p>

	are important to further our understanding of how plants species adapt to elevation in a warming climate.

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20 **Abstract**

21 Elevational gradients are often used to quantify how traits of plant species respond to abiotic and
22 biotic environmental variation. Yet, such analyses are frequently restricted spatially and applied along
23 single slopes or mountain ranges. Since we know little on the response of intraspecific leaf traits to
24 elevation across the globe, we here perform a global meta-analysis of leaf traits in 104 plant species
25 from 71 studies and four continents published between 1983 and 2017. We quantified the intraspecific
26 change of seven morpho-ecophysiological leaf traits along global elevational gradients: specific leaf
27 area (SLA); leaf mass per area (LMA); leaf area (LA); nitrogen concentration per unit of area (*Narea*);
28 nitrogen concentration per unit mass (*Nmass*); phosphorous concentration per unit mass (*Pmass*) and
29 carbon isotope composition ($\delta^{13}\text{C}$). We found LMA, *Narea*, *Nmass* and $\delta^{13}\text{C}$ to significantly increase
30 and SLA to decrease with increasing elevation. Conversely, LA and *Pmass* showed no significant
31 pattern with elevation worldwide. Furthermore, we found significantly larger increase in *Narea*,
32 *Nmass*, *Pmass* and $\delta^{13}\text{C}$ with elevation in warmer regions. Overall, larger responses to elevation were
33 discernible for SLA, but not for the other traits, of herbaceous compared to woody species. Finally,
34 we also detected strong covariation across morphological and physiological traits within the same
35 elevational gradient. In sum, we demonstrate that there are common cross-species patterns of
36 intraspecific leaf trait variation across elevational gradients worldwide. Irrespective of whether such
37 variation is genetically determined via local adaption or attributed to phenotypic plasticity, the leaf
38 trait patterns quantified here suggest that plant species have different individuals adapted to live on a
39 range of temperature conditions. Since the distribution of mountain biota is predominantly shifting
40 upslope in response to climate change, our results are important to further our understanding of how
41 plants species adapt to elevation in a warming climate.

42 **Keywords:** altitude, carbon isotope ratio, meta-analysis, leaf nutrient content, intraspecific
43 variability, phenotypic variability, plant functional traits, environmental gradient

44 **Introduction**

45 Mountain ecosystems are characterized by a rapid change of climatic conditions along elevational
46 gradients (Jump, Matyas, & Penuelas, 2009; Körner, 2007). Spatial heterogeneity in site conditions
47 is further increased by small-scale variation in topography, inclination, exposition and substrate.
48 Steep climate gradients along short horizontal distances and pronounced small scale heterogeneity in
49 site conditions make mountain environments unparalleled natural laboratories to study how plants
50 respond to changes in environmental conditions (Graae et al., 2012; Jump et al., 2009; Sides et al.,
51 2014). The environment selects for the optimal phenotype adapted to a given range of resources and
52 conditions, for instance, temperature, soil moisture, nutrient availability and disturbance in each
53 population (Keddy, 1992; Violle et al., 2007; Wellstein & Kuss, 2011). Thus, various abiotic and
54 biotic environmental conditions occurring across elevations tend to select for different ecological
55 strategies among individuals of the same species. Such strategies are reflected in species-specific
56 patterns of changes in physiological and morphological traits along elevation (Sides et al., 2014),
57 which in turn are expected to greatly affect higher levels of organization (Violle et al., 2007).

58 There is a growing body of evidence showing that intraspecific trait variation (ITV) - due to
59 phenotypic plasticity or local adaptation - has a significant and non-negligible effect on species
60 properties and ecosystem function; for instance, to understand phenotypic plasticity, plant community
61 assembly and ecosystem processes (Burton, Perakis, McKenzie, Lawrence, & Puettmann, 2017;
62 Helsen et al., 2017; Kichenin, Wardle, Peltzer, Morse, & Freschet, 2013). Specifically, changes in
63 leaf morpho-physiological traits have been widely adopted as an indicator of plant's trade-off between
64 growth rate and resource conservation (Reich, Walters, & Ellsworth, 1992; Wright et al., 2004). In
65 addition, leaf traits such as specific leaf area (SLA) and nutrient content are linked to various
66 ecosystem services, such as soil fertility (Ordoñez et al., 2009), litter decomposition (Cornwell et al.,
67 2008) and carbon sequestration (De Deyn, Cornelissen, & Bardgett, 2008).

68 Studies comparing plant species individuals located at different elevations (i.e. along elevational
69 gradients or transects) on single slopes or mountain ranges provide relevant insights on the patterns

of the adaptation of the leaf in response to biotic and abiotic variation in the environment (Birmann & Körner, 2009; Seguí, Lázaro, Traveset, Salgado-Luarte, & Gianoli, 2018; Woodward, 1983). Changes in leaf morpho-physiology observed along elevation is not due to elevation per se, but they depend on a set of abiotic and biotic environmental factors that typically change with elevation (Körner, 2007; Read, Moorhead, Swenson, Bailey, & Sanders, 2014). As elevation increases, temperature and atmospheric pressure tend to decrease, and solar radiation under clear-sky conditions to increase (Körner, 2007). Especially air temperature has been described as a key factor that strongly covaries with elevation worldwide and determines vegetation distribution in mountain environments (Jump et al., 2009; Körner, 2007; Körner & Paulsen, 2004). Among other factors affecting plant growth, increased elevation usually implies changes in precipitation, solar radiation, wind velocity, soil fertility and disturbances by land use (Fisher et al., 2013; Lembrechts et al., 2016; Macek, Macková, & Bello, 2009), but the direction and strength of the relationship between elevation and these factors may vary strongly across the globe (Körner, 2007). Although there is no clear elevation-climate relationship in mountain environments worldwide, lower elevation conditions tend to favour resource-acquisitive strategies that help individuals to face higher competition due to higher temperature and resource availability (Callaway et al., 2002; Read et al., 2014). Conversely, higher elevations tend to be characterized by lower temperature and lower competition, pushing individuals to invest more energy in the conservation of resources (Callaway et al., 2002; Callis-Duehl, Vittoz, Defosse, & Rasmann, 2017; Pfennigwerth, Bailey, & Schweitzer, 2017). In general, individuals of the same species growing at higher elevation are expected to show lower leaf area and higher SLA due to increased density of leaf tissues as morphological adaptation to colder conditions (Körner, 2003; Poorter, Niinemets, Poorter, Wright, & Villar, 2009). Given the strong relationship between different leaf traits (Poorter & Bongers, 2006; Reich et al., 1992), concentrations of essential macronutrients such as nitrogen and phosphorus in the leaf can also be expected to decrease with elevation together with increasing leaf carbon isotope composition ($\delta^{13}\text{C}$) (Birmann & Körner, 2009; Hultine & Marshall, 2000). In addition, decreasing temperature with elevation can also be

96 accompanied by decreasing herbivory pressure (Rasman, Pellissier, Defosse, Jactel, & Kunstler,
97 2014), allowing plants to produce morphologically different leaves at higher elevations [i.e. with
98 lower phenolic content, richer in N content and deprived of spines (Callis-Duehl et al., 2017)].

99 Several studies addressed the response of intraspecific leaf trait variation along elevational transects
100 on single slopes or mountain ranges. However, the direction of such changes is expected to vary
101 considerably among studies observed [see e.g. different SLA responses in Seguí et al. (2018), Macek
102 et al. (2009), Woodward (1983)], as the sources of such heterogeneity are manifold. First, studies
103 often report data for only one or few species that largely differ in their ecology and life form. For
104 example, tree species perceive clearly different micro-climatic conditions compared to shorter herbs
105 (Frey et al., 2016; Körner, 2007). Furthermore, woody species are expected to show larger
106 heterogeneity in trait variation due to broader ontogenetic plasticity (Borges, 2009; Siefert et al.,
107 2015). Another source of heterogeneity is the divergent response of precipitation to elevation
108 worldwide (Körner, 2007) because precipitation considerably determines moisture supply and might
109 affect the trait-elevation relationship (Martin & Asner, 2009). Moreover, it has been shown that
110 intraspecific leaf-trait response to aridity can differ between functional groups and between
111 biogeographic species pools, emphasizing the relevance of the evolutionary differences in species
112 strategies (Wellstein et al., 2017). However, applying meta-analytical approaches based on multi-
113 level mixed effect models allows us to compare results from different contexts by controlling for
114 species-specific responses and for the climatic conditions of each gradient (see e.g. Benítez-López et
115 al., 2017; Midolo et al., 2019).

116 Here, we tested the overarching hypothesis that intraspecific leaf trait adjustment appears in the same
117 direction showing a common trait-elevation relationship across different plant species worldwide.
118 Therefore, we summarize with a meta-analysis the intraspecific leaf-trait variation along 92
119 elevational gradients worldwide in order to (i) reveal the overall effects of elevation on leaf-trait
120 variation and (ii) test whether average environmental conditions of each gradient (mean temperature
121 of the growing season, mean annual radiation, aridity and mean absolute elevation of the lowest site),

122 and (iii) the plant functional types considered (herbaceous vs woody) have an effect on the overall
123 trait-elevation relationship. Finally, given the covariation of traits along elevational gradients (Hultine
124 & Marshall, 2000; Zhu, Siegwolf, Durka, & Körner, 2010), we (iv) explore whether variation of
125 single leaf traits along elevation follow consistent patterns with other traits reported in the same study.
126

127 **Materials and Methods**

128 ***Trait selection***

129 We analyzed intraspecific variation of seven leaf functional traits in our analysis: specific leaf area
130 (SLA); leaf mass per area (LMA); leaf area (LA); nitrogen content (N_{mass}); nitrogen content per unit
131 of area (N_{area}); phosphorous content (P_{mass}) and carbon stable isotope composition ($\delta^{13}C$). We
132 focused our analysis on leaf traits given their influence on plant growth, development and ecological
133 performance (Poorter & Bongers, 2006; Wright et al., 2004). Our choice was also based on data
134 availability; since the chosen leaf traits are relatively easy to measure, for instance compared to many
135 belowground root traits (Pérez-Harguindeguy et al., 2013), they are consequently more frequently
136 reported in the literature.

137 The SLA is the inverse of LMA and both indicate the ratio between leaf size and leaf dry weight.
138 Since elevational gradients normally report either the mean SLA or the mean LMA, and very rarely
139 the values for every leaf sampled in a given site, it was not possible to convert SLA in LMA, or vice
140 versa. Thus, to maximize the information in the meta-analysis, we included both SLA and LMA and
141 analyzed the traits in two separate datasets. In rare cases that original publications reported data of
142 leaf dry weight and leaf area for every sample or both the SLA and LMA means, we included mean
143 SLA and LMA in both datasets for that study (10.5 % of the studies).

144 ***Study selection***

145 We searched for studies on Web of Science combining keywords related to elevational gradients and
146 reflecting different dimensions of leaf traits, for example: (“altitude*” OR “elevation”) AND
147 (“specific leaf area” OR “leaf traits” OR “leaf nutrients”) (see Appendix S1 for complete search

148 string). This resulted in a sample of 659 studies published up to March 2018, of which we scanned
149 the titles and abstracts and then selected 71 studies that fitted to our criteria. Studies eligible for
150 inclusion reported means of traits of individuals of the same species sampled across at least two
151 different elevations within a single slope or mountain range. We only included studies explicitly
152 quantifying trait response along elevational gradients in their experimental design. We excluded
153 studies that investigated trait variation across a large geographical range such as latitudinal gradient
154 studies without clear focus on the effect of elevation only.

155 Studies selected in the meta-analysis reported 92 elevational gradients published between 1983 and
156 2017 across the globe (Figure 1) and focused on intraspecific leaf traits variation of 104 seed plant
157 species in total (see summary list in Table S2.1; Appendix S2). Each study reported data for one or
158 more of the leaf traits selected for the meta-analysis.

159 ***Data collection***

160 We extracted the mean, standard error and sample size of each trait reported in each site sampled
161 along the elevational gradient and the absolute elevation (m a.s.l.) of the sites sampled. We used
162 WebPlotDigitizer (Rohatgi, 2018) to extract the data if available only in graphs or retrieved data
163 directly from tables or the main text. In addition, we extracted the name of each species analyzed and
164 the geographical coordinates of the location where the study was performed. Species names were
165 checked across the datasets to standardize species synonyms.

166 We calculated elevation (m) [sensu McVicar and Körner (2013)] as the vertical distance between
167 sites sampled along the gradient and the lowest site sampled as main predictor in the analysis (see the
168 graphical framework in Figure S3.1; Appendix S3). In addition, we collected six other predictors
169 from each elevational gradient that we expected to moderate the response of traits to elevation: 1)
170 absolute elevation of the lowest point sampled (m a.s.l.); 2) mean growing season temperature (°C);
171 3) aridity index (the ratio between mean annual precipitation and mean annual potential
172 evapotranspiration); 4) mean annual solar radiation (W m^{-2}); average latitude of site sampled along
173 the gradient; and 5) plant functional type (herbaceous or woody).

Climatic data (temperature, aridity and solar radiation) were estimated using the mean coordinates of all sites of the gradient, or by using the generic coordinates for the study area in case geographical coordinates of sites sampled within each gradient were not provided. We extracted the mean growing season temperature as the three warmest months of the year from the WorldClim database ('BIO10') [version 2.0; www.worldclim.org, Fick and Hijmans (2017)]. Similarly, we also calculated the average solar radiation and the aridity index from the CliMond database (Kriticos et al., 2012) and from the CGIAR-CSI GeoPortal (Trabucco & Zomer, 2010), respectively. The aridity index was calculated as the ratio of annual precipitation (estimated by WorldClim database) to potential evapotranspiration, meaning that higher aridity index values indicate lower aridity. Climatic data were extracted with the 'raster' function of the R package *raster* (Hijmans, 2017) with 30 seconds ($0.93 \times 0.93 = 0.86 \text{ km}^2$) resolution at the equator.

185 **Data analysis**

We calculated log-response ratios (lnRR) for each trait along environmental gradients and species and used those as the effect size in our meta-analysis (Hedges, Gurevitch, & Curtis, 1999). Response ratios were calculated as follows:

$$\ln\text{RR}_i = \ln(\bar{T}_{A_i}) - \ln(\bar{T}_B)$$

where T_A is the mean value of a trait measured at the higher elevational level i compared to the mean of the same trait measured on the same species at a lowest elevation present along the gradient (T_B) (see the graphical framework in Figure S3.1; Appendix S3). Since the selected studies normally reported data for multiple elevational levels i along the gradient, we calculated lnRR for each elevation compared with the lowest. This made the effect sizes non-independent within each study (i.e. multiple mean values compared to one mean value) (see Nakagawa, Noble, Senior, & Lagisz, 2017). Thus, we accounted for such correlation by computing the variance-covariance matrix proposed by Lajeunesse (2011) when modeling the data. For non-negative trait values (i.e. all traits considered except for $\delta^{13}\text{C}$), a positive value of lnRR indicates that the mean of a certain trait for a

198 given species increases relatively to the mean sampled at the lowest elevational level. Thus, for $\delta^{13}\text{C}$
199 a negative value of $\ln\text{RR}$ means that leaf $\delta^{13}\text{C}$ is increasing with elevation, and vice versa.

200 We calculated the sampling variance for each pairwise comparison following Hedges et al. (1999) by
201 using the standard deviations of the means and the number of replicates extracted from the papers
202 and used it to weight each effect size in the meta-analysis. We imputed missing standard deviations
203 values using the coefficient of variation from all complete cases using the ‘impute_SD’ function of
204 the R package *metagear* (Lajeunesse, 2016).

205 We analyzed the data with multilevel linear mixed-effect models using the ‘rma.mv’ function of the
206 R package *metafor* (Viechtbauer, 2010). These models weight the precision of each effect size by the
207 inverse of its sampling variance and allows to control for non-independence in the data due to multiple
208 effect sizes per study and species (Nakagawa & Santos, 2012). Models were fitted with a crossed
209 random effect structure including both the identity of each elevational gradients and the species as
210 non-nested random components (Benítez-López et al., 2017; Midolo et al., 2019). Following
211 Konstantopoulos (2011), we nested each observation within the grouping-level in the random
212 structure of the models to account for the possibility that the underlying true effects within each
213 elevational gradient are not homogeneous.

214 To quantify overall relative changes of traits for increasing elevation, we first fitted single meta-
215 regression models using elevation only as moderator. Secondly, starting from the full model including
216 each predictor, we performed a multi-model inference analysis quantifying the importance of
217 predictors to moderate traits variation. For each response variable (i.e. each leaf trait), we ranked a
218 set of models according to the small-sample-size corrected Akaike information criterion (AICc) using
219 the ‘glmulti’ function of the R package *glmulti* (Calcagno, 2013). The relative importance of each
220 predictor was evaluated with the AICc weight ($\text{AICc}W$), calculated the sum of the weights for the
221 models in which the predictor appeared (Burnham & Anderson, 2010). For each response variable
222 we used a confidence set of models by selecting the smallest subset of models that have a cumulative
223 sum of $\text{AICc}W \geq 0.95$ (Johnson & Omland, 2004). We calculated the unconditional estimates of the

224 predictors over the confidence set of models averaged. The relative importance of each predictor in
225 the confidence set was calculated as the sum of the Akaike weights over all the models in which the
226 predictor appeared. Continuous variables were log-transformed (when showing a positive skewness)
227 and then scaled prior to modelling. We checked for collinearity among continuous predictors prior to
228 modelling and found no correlation among predictors (Spearman's $\rho < 0.7$) in any of the datasets
229 analyzed. We checked for potential overparameterization for each of the lowest AICc models selected
230 by plotting the profile of the (restricted) log-likelihood over all the variance and the correlation
231 components of the models (Viechtbauer, 2010).

232 To test how traits covaried along elevational gradients, we estimated the relationship between traits
233 by fitting a model with the lnRR of the first trait as response and the second trait as predictor
234 (Vanneste et al., 2018). These models were estimated on a subset of data retaining only the studies
235 reporting both traits. We did not apply multivariate meta-analytic models to estimate the relationship
236 among our response variables, as the correlation among leaf traits reported by the individual studies
237 were not available and they are necessary to compute the variance-covariance matrix in meta-analysis
238 with multiple correlated outcomes (Berkey, Hoaglin, Antczak-Bouckoms, Mosteller, & Colditz,
239 1998; Lajeunesse, 2011).

240 We checked for publication bias by using the 'funnel' function of the R package *metafor*
241 (Viechtbauer, 2010) and used the modification of the Egger's test proposed by Nakagawa and Santos
242 (2012) to assess funnel plots' asymmetry of the null-models' residuals. We found no evidence for
243 funnel plot asymmetry for all traits analyzed except for *Pmass*. The funnel plot of *Pmass* was found
244 to be asymmetric due to the large heterogeneity of the true effect, which was not reduced significantly
245 by the inclusion of any predictor in the model. However, the asymmetry of the residuals did not
246 support evidence of publication bias for *Pmass* when analyzed in a contour-enhanced funnel plot
247 (Nakagawa & Santos, 2012) (see Appendix S4).

248 All the analyses were performed in R version 3.4.4 (R Core Team, 2018).

249

250 **Figure 1:** Geographical distribution of the 92 elevational gradients included in the meta-analysis. For each gradient, point size depict the number of
 251 leaf traits available and the colours depict the total elevation (i.e the vertical distance between the highest site sampled along the gradient and the
 252 lowest site sampled).

253 Results

254 Despite large variation, we found clear evidence that intraspecific leaf traits of plants follow
255 convergent patterns of change in response to increasing elevation worldwide. Both sets of models
256 analysing the effect of elevation alone (Figure 2) and the multi-model analysis (Figure 3), indicated
257 that SLA and $\delta^{13}\text{C}$ response ratios (lnRRs) linearly decreased with increasing elevation, while LMA,
258 *Narea* and *Nmass* lnRRs increased with elevation. Elevation showed the highest relative importance
259 (AICc_w) across candidate models (Figure 3) for SLA, LMA, *Narea*, *Nmass* and $\delta^{13}\text{C}$. Conversely,
260 we found no significant evidence of a consistent trend in LA and *Pmass*. Leaf area (LA) had a
261 regression slope estimate close to zero, while *Pmass* overall increased with elevation, but with a large
262 confidence interval of the slope estimate (Figure 2).

263 Among relevant predictors moderating overall trait-elevation relationship, the multi-model analysis
264 indicated that only *Nmass* was significantly influenced by mean growing season temperature (MGST)
265 (Figure 3). However, the lowest AICc models of *Narea*, *Nmass*, *Pmass* and $\delta^{13}\text{C}$ retained MGST as
266 significant predictor, indicating that overall larger positive trait variation occurred along elevational
267 gradients with relatively warmer growing seasons (Figure 4). The meta-regression analysis also
268 indicated that both herbaceous and woody species tended to have leaf traits changing with the same
269 direction along elevation. However, we found response ratios of SLA to decrease more strongly in
270 herbaceous species than woody species (Figure 3), although the same pattern was not observed in the
271 dataset of LMA. We found no significant evidence for the absolute elevation at the lowest site, mean
272 annual solar radiation, latitude and aridity index to significantly affect the trait variation of any of the
273 leaf functional traits analysed.

274 The comparisons of response ratios of different traits reported in the same study and species revealed
275 that leaf traits tend to co-vary along elevational gradients (Figure 5). We found $\delta^{13}\text{C}$ to increase with
276 decreasing SLA and LA, and with increasing LMA and *Narea*; while *Nmass* and *Pmass* variations
277 were not correlated with $\delta^{13}\text{C}$ variation. In addition, we found that changes in *Pmass* do not change

278 consistently with any other traits except *Nmass* indicating that two traits positively covary along
279 elevation.

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280

281 **Figure 2:** Intraspecific leaf traits variation (lnRRs) in response to elevation (i.e. the vertical distance between two sites sampled along a gradient; log-
282 transformed) across gradients included in the meta-analysis. Solid lines represent the slope estimate of the model (and 95% confidence intervals) with
283 elevation as predictor only. Significance levels (** $P < 0.01$; *** $P < 0.001$) are provided for the slope estimates. A positive value of lnRR indicates
284 that the mean of a certain trait for a given species increases relatively to the mean sampled at the lowest elevational level, except for carbon isotope
285 composition ($\delta^{13}\text{C}$) (see ‘Materials & Methods’). The dashed lines indicate no change compared to the site sampled at the lowest elevation. Point size
286 depicts the observation weight (weighted by $1/\text{SE}$).

Figure 3: Unconditional estimates and 95% confidence intervals (CI) for fixed effects included in the confidence set of models explaining response ratios (lnRR) of intraspecific leaf trait variation observed across elevational gradients. Continuous predictors are scaled to standardize the magnitudes of the estimates. The relative importance (AICcW) is the sum of AICc weights of models in which a given predictor is retained. ELE = elevation range; AELE = absolute elevation of the lowest site sampled in a gradient; MGST = mean growing season temperature; PT = plant functional types mean pooled effect size (woody or herbaceous); LAT = latitude; AI = aridity index; SRAD = mean annual solar radiation.

296

297 **Figure 4:** Intraspecific leaf trait variation along elevational gradients (lnRRs) of *Narea*, *Nmass*,
298 *Pmass* and $\delta^{13}\text{C}$ in response to the average mean growing season temperature (MGST) (°C, three
299 warmest months) of each elevational gradient. Solid lines represent the slope estimate (and 95%
300 confidence intervals) obtained from the lowest AICc model in which MGST was retained as
301 moderator. Significance level (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) is provided for the slope
302 estimate. A positive value of lnRR indicates that the mean of a certain trait for a given species
303 increases relatively to the mean sampled at the lowest elevational level, except for carbon isotope
304 composition ($\delta^{13}\text{C}$) (see ‘Materials & Methods’). The dashed lines indicate no change compared to
305 the site sampled at the lowest elevation. Point size depicts the observation weight (weighted by 1/SE).

306

307

308 **Figure 5:** Heatmap of the slope estimates of models obtained from the regression between response
 309 ratios (lnRR) of the leaf traits included in the meta-analysis. Models are obtained from a subset of
 310 studies reporting data for both traits used in the regression. Values are slope estimates obtained by
 311 fitting a model with the lnRR of the first trait as response (y-axis) and the second trait as predictor (x-
 312 axis). Significance levels of slope estimates are given (* $P < .05$; ** $P < .01$; *** $P < .001$) for each
 313 combination. A negative estimate of leaf carbon isotope composition response ratio ($\delta^{13}\text{C}$) indicate
 314 that $\delta^{13}\text{C}$ is increasing positively correlated with a given trait (see 'Materials & Methods').

315

316 Discussion

317 *Leaf traits response to elevation*

318 Our results corroborate that intraspecific leaf traits follow general patterns of change in response to
319 elevation and that such patterns occur across different plant species and mountain ranges worldwide.
320 This is consistent with previous meta-analyses which found plant traits convergence across multiple
321 elevational (Read et al., 2014) and latitudinal (De Frenne et al., 2013) gradient studies. Despite large
322 variation, we found that elevation significantly affected all leaf traits analyzed (SLA/LMA, *Narea*,
323 *Nmass* and $\delta^{13}\text{C}$) except LA and *Pmass*. Overall, we showed that with increasing elevation, plants of
324 the same species thus produce leaves adapted to abiotic stress (low temperature). At the same time,
325 leaves exhibit lowered competitive abilities (e.g. lower SLA) and decreasing susceptibility to
326 herbivore pressure (i.e. higher *Narea* and *Nmass*) with increasing elevation (Callis-Duehl et al.,
327 2017). It remains uncertain whether trait variation across elevations is genetically fixed via local
328 adaption or can be attributed to phenotypic plasticity (Bresson, Vitasse, Kremer, & Delzon, 2011;
329 Morecroft & Woodward, 1996; Pfennigwerth et al., 2017). With a review of common garden
330 experiments, Read et al. (2014) found that genetic differentiation often explains a significant amount
331 of intraspecific variation of LMA, *Nmass* and *Narea* among populations at different elevations and
332 latitudes.

333 The detected changes in SLA and LMA along elevational gradients indicate that the leaves tend to be
334 thicker and the tissues denser in response to decreasing temperature and increasing irradiance (Poorter
335 et al., 2009). Although plants species growing at high elevations worldwide tend to produce small
336 leaves (Wright et al., 2017), our results indicated that interspecific LA variation is not affected by
337 increasing elevation. Our findings suggest that individuals may need to maintain large LA to capture
338 light while compensating the lower photosynthetic efficiency driven by the increased leaf tissue
339 density as expressed in SLA and LMA (Poorter et al., 2009).

340 The *Narea*, *Nmass* and *Pmass* are traits representing the amount of proteins and nucleic acids stored
341 in the leaf that can be invested for photosynthesis and growth. We found evidence that leaf nutrient

content generally tends to increase with elevation. In addition, we showed that there is a strong positive covariation between P_{mass} and N_{mass} across elevational gradient studies reporting data on both traits (Figure 5). A positive response of N_{area} was expected based on the results of a previous meta-analysis on elevational gradients (Read et al., 2014), on the positive correlation between N_{area} and LMA (Wright et al., 2004), and because leaf size was expected to decrease with elevation, resulting in N dilution per unit of leaf area (Weih & Karlsson, 2001). Similarly, we were expecting N_{mass} to decrease because of the LMA increase and given their negative correlation in the leaf economic spectrum (Wright et al., 2004). In addition, a previous meta-analysis of Read et al. (2014) showed no specific pattern of N_{mass} with elevation across the globe, possibly because of the strong interdependence of N_{mass} with soil fertility, which does not covary with elevation (Körner, 2007). For the same reasons, we were not expecting P_{mass} to increase across elevations either. Thus, the positive trends we found for N_{mass} and P_{mass} were in contrast with the hypothesis that leaf traits shows resource-conservative strategies with increasing elevation (Pfennigwerth et al., 2017; Read et al., 2014). However, the changes in leaf nutrient content we observed are consistent with Körner (1989) and with the increase in leaf N and P concentration toward the poles as average temperature decreases (Reich & Oleksyn, 2004). The increasing leaf nutrient content at lower temperatures might reflect the acclimation of optimal N use-efficiency and decreased N dilution due to the reduced aboveground biomass growth rate (Weih & Karlsson, 1999; Weih & Karlsson, 2001). Furthermore, N_{area} , N_{mass} and P_{mass} positive variation along elevation might be interpreted as a physiological compensation to the decreasing photosynthetic efficiency caused by decreasing SLA, as higher tissue density enhances the leaf internal resistance to water and CO₂ (see Figure 4) (Morecroft, Woodward, & Marris, 1992).

Increasing $\delta^{13}C$ indicated a decreasing ratio of CO₂ partial pressure inside the leaf to that in the atmosphere. Such trend is consistent with the conclusion that plants at higher elevation show higher leaf $\delta^{13}C$ worldwide (Körner, Farquhar, & Roksandic, 1988; Wang et al., 2013). Environmental factors influencing the positive trend of $\delta^{13}C$ along elevational gradients are not fully clear in the

literature (Körner, Farquhar, & Wong, 1991; Morecroft et al., 1992), but evidence suggest that such trend is linked to both decreasing temperature and atmospheric pressure (Cernusak et al., 2013). Lower oxygen partial pressure increases the carboxylation efficiency of the Rubisco enzyme (Farquhar & Wong, 1984) and the decreasing temperature slows down the transport of water in the plant, resulting in reduced leaf CO₂ diffusion rates (Cernusak et al., 2013). Additionally, the leaf $\delta^{13}\text{C}$ response may not depend on environmental factors directly but on the morpho-physiological adjustment of the leaf to elevation (Gerdol, Iacumin, & Tonin, 2018; Hultine & Marshall, 2000; Vitousek, Field, & Matson, 1990; Zhu et al., 2010). Indeed, our results indicate that $\delta^{13}\text{C}$ increase when leaf area is reduced (Figure 5), possibly because the plant adapts the level of water use efficiency to transpiration rate and light interception, which both depend on the leaf area (Wright et al., 2017). Finally, similarly to what we suggested for N_{mass} and P_{mass} , the covariation we found for $\delta^{13}\text{C}$ with SLA and LMA (Figure 5) in both datasets might reflect leaf physiological compensation to decreasing carboxylation efficiency induced by increasing leaf tissue density (Vitousek et al., 1990).

Biogeographic factors influencing the response of leaf traits to elevation

Our analysis revealed that the directions of trait-elevation relationships do generally not differ across different climates mountain ranges worldwide. However, our multi-model inference analysis revealed that the magnitude of the intraspecific leaf trait variation along elevational gradients is affected by the mean temperature of the growing season. The overall responses of N_{area} , N_{mass} , P_{mass} and $\delta^{13}\text{C}$ to increasing elevation tended to be stronger along elevational gradients located in warmer macroclimates (Figure 4). This finding shows that plants growing on warmer mountain ranges tend to store larger amounts of N per unit of mass in response to increasing elevation compared to plant species located in mountain ranges where the growing season is characterized by lower temperatures. This is probably linked to the overall higher N concentration in the leaf of plants in cold climates (Körner, 1989), which smoothens the effect of increasing N-content with decreasing temperatures along elevational gradients compared to generally warmer climatic conditions. From an ecological point of view, this also suggests that species growing on warmer mountains tends to become

394 potentially more competitive and resource-acquisitive with elevation compared to species located in
395 colder mountain areas. In addition, the current climate warming trends enhance species richness and
396 abundance along elevational gradients and might thus favor plant species that are adapted to live in
397 N-rich conditions, because these species are expected to show higher nutrient content and resource
398 investment in response to increasing competition (Rumpf et al., 2018).

399 Finally, we found that the response to increased elevation does not depend on the elevation of the
400 lowest site sampled, indicating that leaf traits of plants growing both at higher and lower elevation
401 respond with the same magnitude of change to increasing elevational range. This suggests that
402 although plants growing at higher absolute elevation levels show e.g. higher LMA and nutrient
403 concentration (Körner, 1989; Körner, 2003), their relative change to increasing elevational range is
404 expected to be proportionally equal to the one of species with lower LMA and nutrient concentration
405 at lower elevation, possibly because absolute elevation alone does not significantly impact plant
406 growth on a large geographical extent (Körner, 2007).

407 ***Plant functional types responses***

408 We found significant differences in magnitude of variation in SLA across woody and herbaceous
409 species. Our results indicated a larger negative response in herbaceous species in intraspecific SLA
410 with increasing elevation compared to woody species. These findings support the general hypothesis
411 that fast-growing herbaceous plants characterized by lower costs to plasticity are better able to adapt
412 to environmental filtering compared to woody species (Maire et al., 2013). Although there is evidence
413 of leaf trait variation along climatic gradients differs among plant functional types (e.g. C_3 / C_4 dicots
414 and monocots; Ivanova, Yudina, Ronzhina, Ivanov, & Hölzel, 2018), differences across functional
415 groups found in SLA were not reflected in the LMA dataset nor in any of the other traits analyzed
416 here, possibly because herbaceous and woody functional types overlap substantially in their leaf trait
417 characteristics (Wright et al., 2004). In addition, the overall low differences between plant functional
418 groups response to elevation reflected the results of Siefert et al. (2015) who compared the relative

419 extent of intraspecific trait variation in plant communities and found no significant difference in the
420 magnitude of variation between woody and herbaceous species.

421 ***From elevation range to temperature changes***

422 The relationships between elevational range and leaf traits we explored here showed strong similarity
423 with the trait syndrome characterizing plants adapting to colder environments along spatial
424 temperature gradients (Reich & Oleksyn, 2004; Tian, Yu, He, & Hou, 2016). Indeed, temperature is
425 the main factor affecting plant growth that covaries consistently with elevation range in different
426 mountains worldwide (Jump et al., 2009; Körner, 2007). Similarly to latitudinal gradients (De Frenne
427 et al., 2013), elevational gradients offer a promising tool to infer plant responses to temperature
428 change. Combining information obtained from elevational gradients and latitudinal gradients, is an
429 exciting challenge to elaborate a space-for-time substitution providing insights into plant traits
430 response to global warming (Fukami & Wardle, 2005; Read et al., 2014). However, predicting the
431 potential effects of temperature change based on multiple elevational and latitudinal gradients suffers
432 from various weaknesses. Besides the heterogeneity caused by different experimental designs, study
433 aims and biogeographical contexts, which can be accounted by using meta-analytical approaches (see
434 e.g. Midolo et al., 2019), studies analyzed often do not report data on temperature change along each
435 gradient (Graae et al., 2012) as well as other relevant factors such as humidity, soil fertility and land-
436 use. As a result, both elevational and latitudinal gradients are still currently underused to study climate
437 change on a large geographical extent (De Frenne et al., 2013).

438 However, considering possible further unexplored impacts of climatic variation, it is even more
439 surprising that our findings suggest the same direction of SLA/LMA response to elevation while they
440 had opposing directions of SLA response to drought depending on functional groups and
441 biogeographic history (Wellstein et al., 2017). According to our findings, species across
442 biogeographical groups evolved strategically the same way of key leaf-trait response to temperature
443 (across elevations) while already only within Europe they are differentiated in their strategy to deal
444 with aridity (Wellstein et al., 2017). This means that direction of plant functional responses to

445 temperature changes could be predicted more easily while plant functional response to changes in
446 water availability has to be tested against the evolutionary background of the species. Moreover, in
447 line with De Frenne et al. (2013), Read et al. (2014) and Wellstein et al. (2017) our findings further
448 reinforce the importance of intraspecific variation as important driver of functional plant response to
449 climate changes.

450 ***Concluding remarks***

451 We here combined results of multiple elevational gradients studies focusing on leaf traits variation
452 with a meta-analysis to reveal patterns of intraspecific morpho-physiological traits adjustment to
453 high-elevation conditions worldwide. Summarizing evidence from a variety of elevational gradients
454 helps us to understand the potential implications of climate change on individual species in the
455 coming years when focusing on temperature changes taking into account other changes across
456 elevations (Körner, 2007). Irrespective of the mechanisms behind traits variation along elevational
457 transects, we clearly showed that populations located along elevational gradients exhibit individuals
458 adapted to different abiotic conditions. Since the distribution of mountain biota is shifting upslope in
459 response to climate change (Lenoir, Gégout, Marquet, Ruffray, & Brisse, 2008; Rumpf et al., 2018;
460 Steinbauer et al., 2018), our results are particularly important to understand how plants adapt to such
461 elevation shift in a warming planet. Within the considered temperature range, our findings indicate
462 that future upward migrating species most likely will lower their SLA and increase their nitrogen
463 content and $\delta^{13}\text{C}$. However, plant-life beyond the here analyzed realized niche, i.e. the emergence of
464 new parts of the realized niche based on the fundamental one has rarely been tested so far (Violle and
465 Jiang, 2009). In this context, experimental research would enable new knowledge on the hidden part
466 of plant variability.

467 Finally, the magnitude of intraspecific trait variation we observed is expected to scale up to higher
468 levels of biological organization (Violle et al., 2007). Thus, when analyzing functional trait response
469 to elevation at the community level, intraspecific variability is expected to significantly contribute to
470 the variation observed among plant assemblages at different elevation levels.

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477

478 **Conflict of interest**

479 The authors have no conflicts of interest to declare.

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Supporting Information

APPENDIX S1: Search string for primary studies collection

APPENDIX S2: List of primary studies and species analysed in the meta-analysis

APPENDIX S3: Details on the calculation of the effect size

APPENDIX S4: Null models, funnel plots and analysis of publication bias

For Review Only