



Plant physical and chemical defence variation along elevation gradients: a functional trait-based approach

Alan Kergunteuil¹ · Patrice Descombes^{2,3} · Gaetan Glauser⁴ · Loïc Pellissier^{2,3} · Sergio Rasmann¹ 

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Abstract

Predicting variation in plant functional traits related to anti-herbivore defences remains a major challenge in ecological research, considering that multiple traits have evolved in response to both abiotic and biotic conditions. Therefore, understanding variation in plant anti-herbivore defence traits requires studying their expression along steep environmental gradients, such as along elevation, where multiple biotic and abiotic factors co-vary. We expand on plant defence theory and propose a novel conceptual framework to address the sources of variations of plant resistance traits at the community level. We analysed elevation patterns of within-community trait dissimilarity using the RaoQ index, and the community-weighted-mean (CWM) index, on several plant functional traits: plant height, specific leaf area (SLA), leaf-dry-matter-content (LDMC), silicium content, presence of trichomes, carbon-to-nitrogen ratio (CN) and total secondary metabolite richness. We found that at high elevation, where harsh environmental conditions persist, community functional convergence is dictated by traits relating to plant growth (plant height and SLA), while divergence arises for traits relating resource-use (LDMC). At low elevation, where greater biotic pressure occurs, we found a combination of random (plant height), convergence (metabolite richness) and divergence patterns (silicium content). This framework thus combines community assembly rules of ecological filtering and niche partition with plant defence hypotheses to unravel the relationship between environmental variations, biotic pressure and the average phenotype of plants within a community.

Keywords Environmental filtering · Niche partitioning · Alpine environment · Secondary metabolites · Leaf economic spectrum

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✉ Sergio Rasmann
Sergio.rasmann@unine.ch

- ¹ Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland
- ² Landscape Ecology, Institute of Terrestrial Ecosystems, ETH Zurich, Zurich, Switzerland
- ³ Swiss Federal Research Institute WSL, Birmensdorf, Switzerland
- ⁴ Neuchâtel Platform of Analytical Chemistry, University of Neuchâtel, Avenue de Bellevaux 51, 2000 Neuchâtel, Switzerland

Introduction

The radiation of plant lineages into different environments has required the evolution of a variety of life-history traits and forms to maximize growth and reproduction under the local biotic and abiotic constraints (Díaz et al. 2016). Such stunning diversity of plant forms and functions is similarly reflected in the diversity of ways a plant can defend itself against herbivore attack (Futuyma and Agrawal 2009). Plant defences against herbivores include chemical and mechanical features that either directly deter, hinder or intoxicate the herbivores (Agrawal 2007; Carmona et al. 2011; Mithoefer and Boland 2012), or indirectly reduce herbivore pressure by providing shelter, reward, or informational cues that foster predator recruitment and presence near the plant (Kessler and Heil 2011). Here, we address how to disentangle the sources of variation in plant defence traits, which remains a major challenge in ecology (Coley et al. 2018).

While some phylogenetic conservatism dictates the shape of plant defence expression at least at higher taxonomic levels (Futuyma and Agrawal 2009), strong variability exists at the genus, or even at the within-species level of individuals having colonized specific habitats (Fine et al. 2004). Indeed, habitat-driven variation in plant defences has often resulted in convergence (Agrawal and Fishbein 2006; Becerra 2007; Defosse et al. 2018; Fine et al. 2004, 2006; Kursar and Coley 2003; Travers-Martin and Müller 2008). Given the link between plant defence traits and habitat specialization (Wright et al. 2004), plant defence hypotheses are inherently associated to the plant economic spectrum (Defosse et al. 2018). Hence, exploring the co-variation between plant defensive and functional traits is key for fully explaining plant defence response within the larger context of environmental variation (Agrawal and Fishbein 2006).

The link between plant defences against biotic attack and habitat specialization is particularly important in cases when functional traits serve the double role of defence against herbivores, while aiding plants adapting to different habitat and climatic conditions (Table 1) (Moles et al. 2013; Wright et al. 2004). Examples of traits with adaptive value under both biotic and abiotic stress include specific leaf area (SLA), leaf-dry-matter-content (LDMC), trichomes, silicium (Si) and secondary metabolites. SLA and LDMC generally co-vary with leaf nitrogen, photosynthetic capacity and plant growth rate (Westoby and Wright 2006), but they are also good proxies for physical anti-herbivore defences (Hanley and Sykes 2009; Wilson et al. 1999). Trichomes, while generally considered to be plant defence traits (Dalin et al.

2008; Levin 1973), can also protect plants against drought stress (Fahn 1986; Huttunen et al. 2010; Schreuder et al. 2001) and damaging UV-radiation (Xiao et al. 2017). Moreover, the beneficial effects of silicium have been observed in Si-accumulating plants under various stressful abiotic (e.g., drought, temperature) and biotic (e.g., insect and pathogen attacks) conditions (Debona et al. 2017; Hartley and DeGabriel 2016; Ma 2004). Similarly, secondary metabolites are also recognized to confer plant resistance against a wide array of biotic (Wink 2008) and abiotic stresses (Sampaio et al. 2016). Therefore, comprehending variation in plant defence traits requires studying the co-variation of both abiotic and biotic factors.

To tease apart the relative contribution of different ecological factors on plant functional traits, elevation gradients, in particular, have been proposed to serve as natural experiments, in which steep variation in both biotic and abiotic factors within the same biogeographic zone and phylogeographic history could be used to disentangle their relative effect (Körner 2007; Rasmann et al. 2014). For instance, a general decrease in plant palatability has been associated to a reduction in herbivore pressure at high elevation (Callis-Duehl et al. 2017; Descombes et al. 2016). We address the sources of variation of individual traits and along elevation gradients using a conceptual model that incorporates biotic and abiotic variation along elevation gradient and community-level responses for each trait (Fig. 1). Such model allows the formulation of specific predictions about (1) community-level trait convergence/divergence patterns (the degree to which a trait differs between species within a

Table 1 Plan functional traits used in the conceptual model

Trait	Function		
	Growth	Abiotic stress	Biotic stress
Plant size	Competition for light	Structural resistance (small size is better)	Faster growth facilitate tolerance and/or escape from herbivory
SLA	Potential growth rate, maximum photosynthetic capacity, lower values correlate with long leaf lifespan	Low SLA values correlate with high structural strength, necessary to protect against physical hazard (e.g., wind, hail)	Long leaf life span (low SLA) correlate with high structural strength, necessary to protect against herbivores (hard leaves to chew or pierce)
LDMC	Correlated with leaf life span (LLS), correlated with leaf dry mass and leaf water content	Positively correlated with leaf physical properties	Negatively correlated with dry matter digestibility
C/N	Photosynthetic activity is mediated by the Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which is correlated to nitrogen content in leaves		Plant palatability
Silicium	Structural stability	Tougher tissues	Tougher tissues, defence activation, induction
Secondary metabolites		Protection against UV, temperature	Deter and/or kill herbivores
Trichomes		Protection against drought, UV damage	Deter insect herbivores

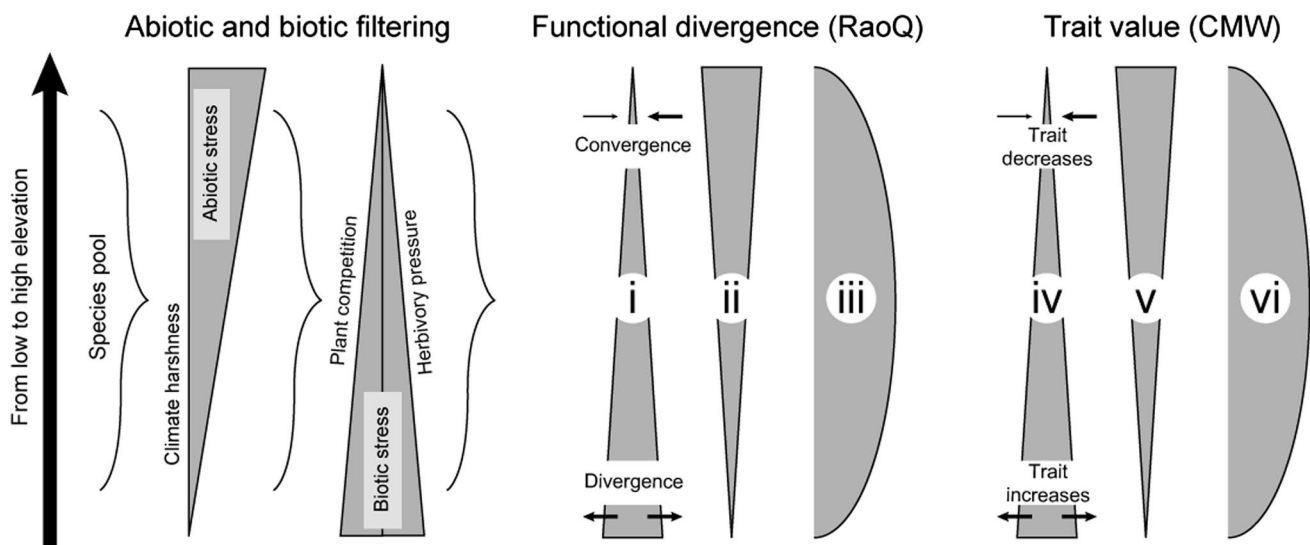


Fig. 1 Theoretical model of functional trait change along elevation gradients. Functional divergence of traits as well as the value of trait change along elevation gradients is based on variation in abiotic (climate) and biotic (herbivore pressure, plant competition) factors. With increasing elevation, plants experience harsher climatic conditions such as colder temperatures and extreme climatic events, but also experience lower direct competition and herbivore pressure. Based on this model: **i** Biotic stress mainly results in functional divergence at low elevation for traits involved in anti-herbivore defence (e.g., silicium, secondary metabolites). **ii** Abiotic stress mainly mediates

trait divergence at high elevation, but only for traits relating to abiotic resistance (e.g., resource-use strategies). **iii** Traits relating to both biotic and abiotic resistance should diverge at mid-elevation (SLA, LDMC, silicium, phenolic metabolites). **iv** Biotic pressure is dominating at low elevation, and traits relating to biotic resistance should increase (e.g., secondary metabolites). **v** Abiotic pressure is dominating at high elevation, and traits relating to abiotic resistance should increase (e.g., leaf physical toughness, plant size). **vi** Traits relating to both biotic and abiotic resistance should be maximal at mid-elevation (SLA, LDMC, silicium, phenolic metabolites)

given community), and (2) community-level trait average. The model stands on the assumption that trait divergence/convergence patterns are dictated by two community assembly rules: ‘environmental filtering’ and ‘niche partitioning’. The ‘environmental filtering’ concept suggests that not all organisms are able to successfully establish and persist in all environmental conditions. Therefore, the ‘environmental filter’ selects for phenotypic similarities among community members and leads to phenotypic convergence in key ecological dimensions (Cornwell and Ackerly 2009; Kraft et al. 2015). The ‘niche partitioning’ hypothesis, on the other hand, states that plant co-existence within local communities relies on trait differentiation (i.e., functional divergence) and ultimately, partial segregation of ecological niches (MacArthur and Levins 1967; Stubbs and Bastow Wilson 2004). Specifically, our model states that variation of biotic and biotic pressure along elevation gradients imposes variation in the degree of which functional traits converge or diverge within a given community, by modifying individual species, and all communities average trait values.

Modelling plant defence trait variation along elevation gradients

Below, we outline three potential outcomes of trait average production and variation, respectively, at the community

level (Fig. 1). The underlying assumptions of the models are that along elevation, the magnitude of biotic interactions rapidly declines when moving from low to high elevation (Pellissier et al. 2012). At the same time, high-elevation plants are exposed to lower levels of resources, cold temperatures, high-climatic variability, and short growing seasons (in short; harsher climatic condition) as compared to low elevation plants (Körner 2003) (Fig. 1).

Community-level trait convergence/divergence patterns

For case i in Fig. 1, we expect traits related to anti-herbivore defence to diverge more at low elevation, where ecological conditions are mainly characterized by strong biotic interactions (Coley and Aide 1991; Rasmann and Agrawal 2011; Rasmann et al. 2014). That herbivore pressure forces defence trait divergence has been corroborated in the tropics, on several systems (Kursar et al. 2009; Lokvam and Kursar 2005; Salazar et al. 2016).

For case ii in Fig. 1, we expect that if a trait is principally affecting plant tolerance against abiotic stresses, we expect this trait to diverge more at high elevation. For instance, a reduction in nutritive resources at high-elevation is expected to promote the co-existence of different resource-use strategies. Indeed, studies along nutrient gradients have shown higher functional diversity in nutrient poor habitats, either

using multi-trait approaches (Helsen et al. 2014) or single-trait approaches (Bernard-Verdier et al. 2012).

For case iii in Fig. 1, we expect that if a trait confers resistance to both biotic and abiotic stresses it should diverge more at mid-elevation where the array of environmental factors for which the trait functions are maximal (i.e., both biotic and abiotic forces are maximal at mid-elevation and should promote trait divergence).

Community-weighted-means

For case iv in Fig. 1, we expect anti-herbivore traits to be highly expressed at low elevation, as the strength of selection from herbivory increases. These predictions are in line with studies highlighting positive correlations between herbivore pressure and the expression of traits conferring anti-herbivore defence (e.g., Ibanez et al. 2013).

For case v in Fig. 1, if a trait is favoured in a habitat where abiotic conditions are very stressful we should expect this trait to be maximal at high elevation. For instance, small plant and leaf size should be maximal in high-elevation plant communities. Indeed, plant height is negatively correlated to injury temperatures (Squeo et al. 1991) and, as a consequence, generally decreases with elevation (Körner 2003).

For case vi in Fig. 1, at mid-elevation, trait that confers resistance to both biotic and abiotic stresses should be more strongly expressed in response to concomitant selective pressures from biotic and abiotic agents.

Functional variation along elevation gradients: a case study in the Swiss Alps

To test the predictions shown in model of Fig. 1, we sampled plant communities growing on the mountain slopes of the Swiss Alps. For each species, we sampled functional traits relating to both biotic and abiotic resistance, and measured average community trait values and the degree of trait divergence.

Survey of plant communities

Surveys of plant communities were conducted in grasslands across five transects separated by a 15 km minimum distance (Fig. S1 in Electronic Supplementary Material, ESM). Sampling sites ranged between 1040 and 3100 m to avoid low-land forests. Along each transect, sampling sites were chosen based on similar slopes and were evenly distributed between the minimum and the maximum altitudes. In total, plant species were inventoried in 32 sites (i.e., 32 plant communities) of 40 m² each (Vittoz and Guisan 2007). The specific covers were estimated according to a 10-class Braun-Blanquet scale. Among the 155 different plant species identified, we only retained species that covered more than 5% of at least

one of the sampling sites. According to this selection, statistical analyses of plant communities were performed on the 66 plant species that were the most common to alpine grasslands (Table S1 in ESM).

Plant functional traits

We analysed functional diversity of plant communities based on seven plant traits including maximum height (cm), specific leaf area (SLA; the ratio of leaf area to dry mass; mm²/mg), leaf-dry-matter-content (LDMC; the ratio of the leaf dry mass to fresh mass; mg/g), carbon-to-nitrogen ratio (CN), silicium content in leaves (Si; % of dry mass), the richness of representative primary and secondary metabolites (i.e., the number of these metabolites found per species), and the presence of trichomes (see Table S2 in ESM for species-level trait values).

We used a Swiss Flora (Hess et al. 1984) to retrieve the presence of simple, glandular or star-shaped trichomes on leaves, stems or flowers on mature plants and organs. For each plant species, we assigned a score of one when any of these cases occurred (i.e., the specific scores ranged from zero to nine). Silicium content in leaves was estimated with the molybdenum blue colorimetry spectrophotometric procedure described by Hallmark et al. (1982). The silicium content per species was obtained by averaging 1–18 individuals according to natural occurrences at three different altitudes (1800, 2050 and 2300 m). Plant height, SLA, LDMC and CN were retrieved from previous research projects (Callis-Duehl et al. 2017; Dubuis et al. 2013).

Finally, plant secondary metabolites were retrieved from untargeted metabolomics analyses performed with ultra-high pressure liquid chromatography -quadrupole-time-of-flight mass spectrometry (UHPLC-QTOFMS) (following Gaillard et al. 2018) with minor modifications. In brief, on an Acquity UPLC BEH C18 column (50×2.1 mm, 1.7 µm; Waters), a linear reversed-phase UHPLC gradient (2–100, 0.05% formic acid in acetonitrile and water as mobile phase in 6 min) was applied and detection was performed over a mass range of 85–1200 Da in electrospray positive ionization. Specific metabolite richness was estimated by averaging the number of different compounds detected in the youngest fully expanded leaves of 1–3 individuals per species at the same sites used to estimate silicium content. All chromatograms obtained in UHPLC-QTOFMS were processed with R 3.4.3 (R Development Core Team 2017) and the package *xcms* (Smith et al. 2006), [see Glauser et al. (2013) for parameters used], combined with *CAMERA* (Kuhl et al. 2012), *perfwHM* = 1/3. In short, after excluding adducts and isotopes, the individual molecule detection was based on fragment correlation probabilities and co-elution. In other words, the software estimated the number of individual compounds based on how often a specific fragment (out of the > 10,600

detected) is present with another one at a specific elution time. While this detection method implies an underestimation of the actual number of compounds present, it is conservative in that it reduces the number of single fragments to be identified as complete molecules. Because this potential underestimation is the same across species, species remain comparable. The total metabolite richness estimated from our analytical method for the 31 species collected ranges from 83 to 104 different metabolites (Table S2 in ESM).

Statistical analyses of functional diversity

We studied the range and the distribution of individual functional trait values at the community level. Among the different indices that have been developed to quantify different functional facets of biodiversity, we retained the Rao's quadratic entropy (*RaoQ*) and the community-weighted-mean (*CWM*) indices (Mouchet et al. 2010; Ricotta and Moretti 2011). The *RaoQ* is the sum of pairwise distances between species weighted by their respective covers:

$$RaoQ_i = \sum_{j,k}^S d_{jk} \times p_j \times p_k$$

in which S is the specific richness of plant community i , d_{jk} is the functional distance between species j and k , p_j is the relative cover of species j and p_k is the relative cover of species k . This index reflects the expected dissimilarity between two species of a given plant community. The *RaoQ* takes into consideration both functional richness and functional dispersion and has been shown to perform better in detecting trait convergence/divergence as compared to other functional indices (Botta-Dukat and Czucz 2016). The *CWM* is the average of specific trait values weighted by the relative cover of each species composing the community:

$$CWM_i = \sum_j^S p_j \times x_j$$

where S is the specific richness of plant community i , p_j is the relative cover of species j and x_j is the average trait value for species j .

For each trait, the *RaoQ* and the *CWM* for the 32 plant communities were measured based on the cover of plant species for which the specific mean value of trait was available. Both indices were calculated with the function *dbFD* in the package *FD* (Laliberté et al. 2014). We applied a square root correction method to the species distance matrix when this latter was not Euclidean (argument *corr* of the *dbFD* function). The observed *RaoQ* and the *CWM* were then compared to indices computed with null models (i.e., artificial plant community randomly assembled). We simulated 999 artificial plant communities where each species cover was randomly sampled

among the 32 cover values observed in the surveyed plant communities. For the *RaoQ*, we then measured the standard effect size (SES_Q) as following:

$$SES_{Qi} = \frac{Q_i - \widehat{Q}_{null}}{\sigma_{Q_{null}}}$$

in which SES_{Qi} is the standard effect size of plant community i , Q_i is the *RaoQ* index of plant community i , \widehat{Q}_{null} and $\sigma_{Q_{null}}$ are the mean and the standard deviation of *RaoQ* indices assessed for the randomly assembled plant communities. A positive SES_Q indicates that functional dispersion is higher than expected if individuals were randomly distributed along the elevation gradient, while a negative SES_Q indicates functional convergence.

We visualized the average phenotype of a given community using principal component analyses (PCA); function *dudi.pca*; package *ade4* (Dray and Dufour 2007) with matrices including either SES_Q or *CWM* estimated for the single-traits studied. Data were centred and scaled before analyses. The spatial positions of communities derived from PCA performed on SES_Q highlight the main traits driving functional diversity of the average phenotype. PCA performed on *CWM* aimed at describing the functional identity of a given community. In addition, we also measured the Pearson correlation coefficients (function *cor*) between the seven traits, both for their respective diversity (SES_Q) and their expression (*CWM*), to better understand how they co-vary.

Second, we analysed the distribution of SES_Q and *CWM* for individual traits along elevation through regression models. For both *RaoQ* and *CWM* of each trait, we performed an exact binomial test (function *binom.test*) to identify the proportion of null model indices that deviated from the observed indices ($P \leq 0.05$, $\alpha = 0.95$). We indicated significant deviations when 90% of null model indices were higher or lower to observed indices. We performed single trait analyses because we were interested in how individual trait expression at the community level changes along elevation gradients. While we acknowledge potential trait co-variation (see Fig. S2 in ESM), this approach should not affect Type-I errors, as the measure of community-level changes on one traits does not affect the probability of finding a similar response on another trait (Garnier et al. 2004; Ricotta et al. 2011). All the statistical analyses were performed with R, version 3.4.3 (R Development Core Team 2017).

Results

Average phenotype of plant defence at the community level

The first two principal components of PCA, performed with SES_Q (Fig. 2a) or *CWM* (Fig. 2b), accounted for 52

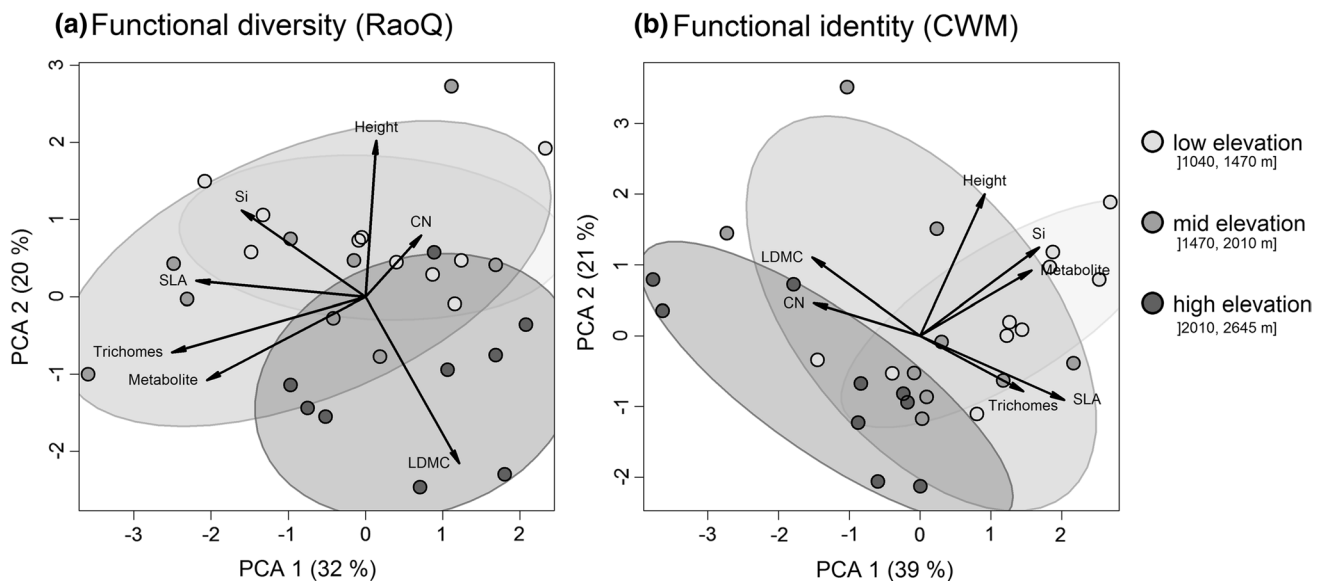


Fig. 2 Principal component analyses showing **a** multi-trait functional diversity based on SES_Q and **b** multi-trait functional identity based on CWM along an elevation gradient. Scores for each plant community were obtained from relative importance of functional indices (SES_Q or CWM) assessed for seven plant traits: plant height, specific

leaf area (SLA), leaf-dry-matter-content (LDMC), carbon-to-nitrogen ratio (CN), silicium content in leaves (Si), the presence of trichomes and the richness in total secondary metabolites. The proportion of variance explained by each axis appears in parentheses. Ellipses contain 75% of samples within each elevation class

and 60% of the total variance, respectively. For both PCA, high- and low elevation plant communities were clearly discriminated along the first two principal components, while mid-elevation plant communities partially overlapped low- and high-elevation plant communities. Concerning the functional diversity, plant communities occurring at low elevation displayed the highest SES_Q related to plant height and silicium content (Fig. 2a). On the contrary, high-elevation plant communities were characterized by low SES_Q related to plant height and silicium content. However, at high elevation, plant communities showed high inter-specific diversity in LDMC (Fig. 2a). Concerning the functional identity, the average phenotype of plant defence expressed by low-elevation plant communities displayed high plant height, silicium content and metabolite richness (Fig. 2b). High-elevation plant communities were mainly grouped based on a negative correlation with the expression of those three traits. In addition, plant communities found at high elevations were segregated along an axis including LDMC and SLA expression (Fig. 2b).

Overall, these observed changes in plant defence syndromes are only marginally affected by trait co-variation, since very few correlations between the indices assessed for the seven traits were significant. Specifically, for the SES_Q , we observed only three significant correlations between traits (Fig. S2a in ESM), and six correlations between the CWM of single traits were significant (Fig. S2b in ESM).

Community-level trait variation

The functional diversity computed for six plant traits significantly varied with elevation (Fig. 3). Along most of the elevation gradient, significant convergences were recorded for plant height and SLA, although both traits followed different spatial pattern regarding the magnitude of convergence (Fig. 3a, b). The functional diversity of plant height linearly decreased with elevation (Fig. 3a: coefficient = 5.5×10^{-4} , $t = -2.77$, $P < 0.01$). The distribution of SLA displayed a concave relationship with elevation (Fig. 3b: coefficient 2 = -1.57 , $t = -2.42$, $P = 0.022$). The LDMC followed a completely different linear pattern along mountain slopes (Fig. 3c: coefficient = 9×10^{-4} , $t = 2.08$, $P = 0.047$). While low-elevation plant communities were characterized by a convergence in LDMC values, the same trait diverged as elevation increased. Community assembly pattern did not deviate from random expectations concerning the functional diversity of CN (Fig. 3d). From low to high elevation, we observed a sharp decrease in the functional variation of leaf silicium content (Fig. 3e; coefficient = -1.1×10^{-3} , $t = -4.10$, $P < 0.001$). The functional variation for metabolites and trichomes, two traits likely involved in both biotic and abiotic stresses, showed similar concave relationships with elevation (Fig. 3f: coefficient 2 = -2.16 , $t = -2.53$, $P = 0.02$, and Fig. 3g: coefficient 2 = -1.91 , $t = -2.01$, $P = 0.05$, respectively).

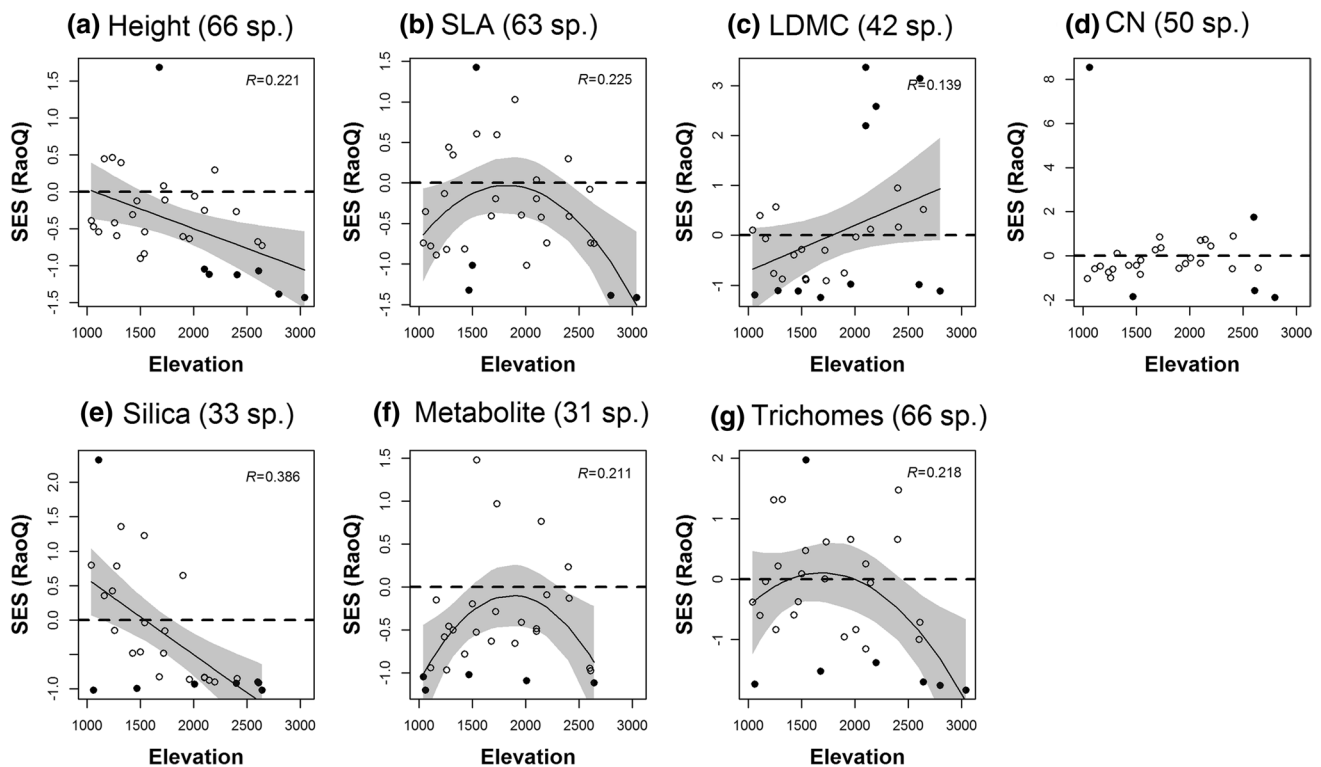


Fig. 3 Standard effect sizes (SES) of community functional diversity based on RaoQ index for **a** the plant height, **b** the leaf surface area, **c** the leaf-dry-matter-content, **d** the carbon-to-nitrogen ratio, **e** the leaf silicium content, **f** the richness of total metabolite and **g** the presence of trichomes. Positive and negative SES value indicates that functional diversity is higher and lower, respectively, than expected

for randomly assembled plant community. Filled circles represent significantly high or low functional diversity (exact binomial test, $P \leq 0.05$). Curves and grey areas depict the significant coefficient estimates ($P \leq 0.05$) and the confident intervals ($\alpha = 0.95$) retrieved from linear or 2nd degree polynomial models. R indicates the coefficient of determination for each model

Community-level trait average

The CWM of several measured plant functional traits varied along elevation (Fig. 4). Plant height displayed a sharp linear decrease with elevation (Fig. 4a; coefficient = -0.025 , $t = -4.43$, $P < 0.001$). SLA showed a convex relationship with elevation (Fig. 4b; coefficient 2 = 9.04 , $t = 2.87$, $P = 0.008$). Regarding LDMC, despite a significant pattern for functional diversity, trait expression was highly variable along mountain slopes (Fig. 4c). Similar to functional diversity, the CWM of CN did not depend on elevation (Fig. 4d). On the other hand we found that silicium content and metabolite richness sharply declined with elevation (Fig. 4e; coefficient = -0.0002 , $t = -2.93$, $P = 0.007$, and Fig. 4f; coefficient = -0.005 , $t = -317$, $P = 0.004$, respectively). Finally, we found that mid-elevation plant communities displayed the lowest amounts of trichomes (Fig. 4g; coefficient 2 = 1.37 , $t = 3.06$, $P = 0.005$).

Discussion

We here provide a theoretical framework for addressing the causes of plant defence trait variation at the community level in nature. In particular, we advocate for considering the relative role of biotic and abiotic forces for driving variation of individual traits, depending on the relative functional role of each individual plant trait. Indeed, we found strong variability in community-level divergence patterns of traits, as well as community-weighted mean values, along elevation gradients depending on the trait measured. We discuss these results in light of the putative functional roles of each trait for plant species.

Elevation patterns of community-level trait variation

Among the seven traits studied, we found that plant communities were more divergent than expected in

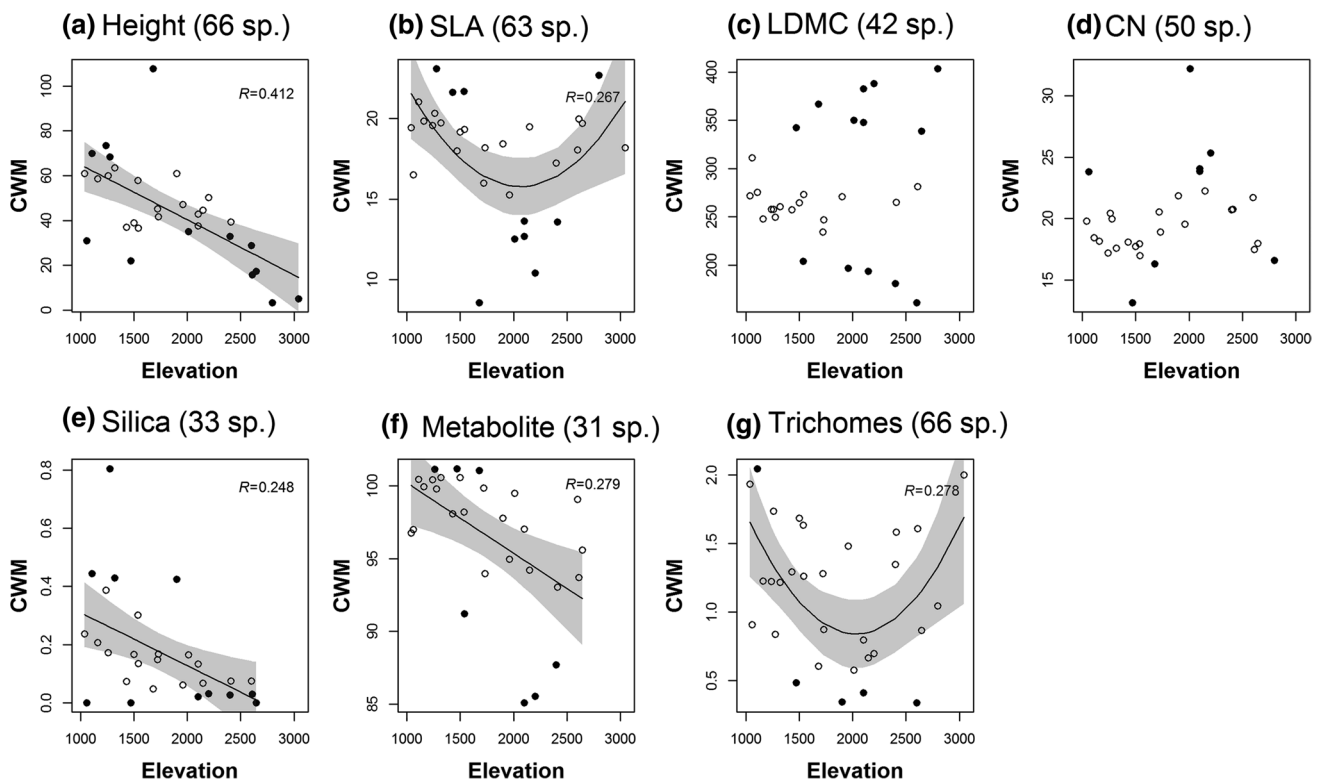


Fig. 4 Range of plant trait expression at the community level based on the community-weighted mean index (CWM) for **a** the plant height, **b** the leaf surface area, **c** the leaf-dry-matter-content, **d** the carbon-to-nitrogen ratio, **e** the leaf silicium content, **f** the richness of total metabolite and **g** the presence of trichomes. Filled circles represent significantly higher or lower trait expression at the community

level as compared to randomly assembled plant communities (exact binomial test, $P \leq 0.05$). Curves and grey areas depict the significant coefficient estimates ($P \leq 0.05$) and the confident intervals ($\alpha = 0.95$) retrieved from linear or 2nd degree polynomial models. R indicates the coefficient of determination for each model

randomly-assembled communities only for the trait LDMC (Fig. 3c). This indicates that harsh alpine climates select for niche partitioning along the axis of accumulation of leaf-dry-matter content. Since this trait is related to resource use and nutrient uptake strategies (Pérez-Harguindeguy et al. 2013; Zheng et al. 2017), the segregation of ecological niches based on LDMC may greatly contribute to species co-existence within plant communities exposed to abiotic stresses.

Contrary to similar expectations, we found that other traits related to carbon storage (plant height and SLA) tended to converge at high elevation (Fig. 3a, b, respectively). Indeed, several studies have shown that stressful conditions related to abiotic factors result in convergent trait syndromes (i.e., filtering) (Kraft et al. 2015). In addition, Figs. 3a and 4a show that plant height converges with elevation towards small sizes, likely in response to injury temperatures (Squeo et al. 1991). Since SLA is negatively correlated to plant growth rate, it is perhaps not surprising that we observe convergence of this trait around high values above 2000 m, likely reflecting pulse-resource availability and/or short growth seasons and, thus, fast development of plants

(Díaz and Cabido 1997; Pérez-Harguindeguy et al. 2013). Those increases in trait expressions towards optimal values (i.e., small plant size, high SLA) fit with the case v in the Fig. 1. Taken together, these results suggest that mechanisms leading to similar plant structures regarding plant height and SLA are related to habitat filtering, while divergent assembly rules govern the distribution of LDMC values for facilitating niche divergence and plant co-existence. For instance, when moving from high to low elevations, SLA may gradually escape abiotic pressures and convergent assembly rules, whereas an increase in biotic interactions and plant–plant competition may drive the convergence of SLA around high values that confer competitive dominance through, for instance, faster plant growth rate or higher photosynthetic activity (Pérez-Harguindeguy et al. 2013).

As predicted, we observed pronounced community-level divergence for traits conferring resistance against biotic stresses at low elevation (silicium, trichomes, and only partially for plant secondary metabolites). However, while low elevation plant communities converge to the maximal values for silica and secondary metabolite richness, trichomes are mostly featured at mid-elevation.

While for silicium high divergence and high mean values at low elevation highlight niche partitioning and silicium importance driven by habitat specific characteristics (e.g., high herbivory and high plant–plant competition), these results emphasize the multiple functionality of several traits (Lavorel et al. 2011). For instance, the convergence of trichomes values toward their maxima at the edges of the elevation gradient may result from fitness advantage in relation to high herbivore pressures at low elevation (Bjorkman et al. 2008), and high levels of UV radiation at high elevation (Yan et al. 2012). At mid-elevation, a relaxation in both selective pressures likely explains the relative divergent assembly patterns we observed.

Our estimation of the numbers of metabolites present in the plants is relatively low compared to other systems (Coley et al. 2018), therefore, only giving a partial picture of actual metabolite richness found in plants. At the community level, species showed maximum divergence at mid-elevation, suggesting that multiple selective pressures are maximized at mid-elevation (Callis-Duehl et al. 2017; Holt and Lawton 1993; Kursar et al. 2009; Salazar et al. 2016). While our model assumes maximal levels of herbivory at the lowest end of the gradient, recent analyses indicated that herbivore abundance seems to be highest around 1500–2000 m of elevation in the Alps, probably due to a mid-domain effect of species richness, or high anthropogenic impact at low elevation (Pellissier et al. 2012). Independently of the precise causes, such a diversity pattern would indeed favour species phytochemical divergence at mid-elevation. However, low elevation plants tend to produce higher numbers of metabolites, suggesting that average production is correlated with diversity of phytochemicals in plants and indirectly with overall within-community plant diversity (Rasmann and Agrawal 2011; Richards et al. 2015).

Conclusions

Within communities, trait expression and variation is the result of multiple biotic and abiotic forces. While plant functional traits relating to the leaf economic spectrum have been historically used for deciphering community assembly patterns (Ackerly and Cornwell 2007; Díaz et al. 2016; Spasojevic and Suding 2012), plant traits related to anti-herbivore resistance are also increasingly acknowledged for contributing to community assembly (Kursar et al. 2009; Salazar et al. 2016). Analyses along steep environmental gradients suggest that each trait, independently, contributes differentially to the community assembly process (Read et al. 2014), in which, the magnitude of expression of each trait is governed by its contribution to increased plant fitness in a given environment. Therefore, studying species-specific fitness consequences of individual traits in the context of

different biotic and abiotic pressures will be required to better predicting community formation and structure along ecological gradients.

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Author contribution statement SM originally formulated the ideas. SM and AK developed methodology. AK, LP, PD collected the data. GG, AK analyzed the data. SM and AK wrote the manuscript with the help of all co-authors

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