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LETTER

The unfolding of plant growth form-defence syndromes along elevation gradients

Emmanuel Defossez,[1]
Loïc Pellissier[2,3] and
Sergio Rasmann[1]*

Abstract

Understanding the functional economics that drives plant investment of resources requires investigating the interface between plant phenotypes and the variation in ecological conditions. While allocation to defence represents a large portion of the carbon budget, this axis is usually neglected in the study of plant economic spectrum. Using a novel geometrical approach, we analysed the co-variation in a comprehensive set of functional traits related to plant growth strategies, as well as chemical defences against herbivores on all 15 *Cardamine* species present in the Swiss Alps. By extracting geometrical information of the functional space, we observed clustering of plants into three main syndromes. Those different strategies of growth form and defence were also distributed within distinct elevational bands demonstrating an association between the functional space and the ecological conditions. We conclude that plant strategies converge into clear syndromes that trade off abiotic tolerance, growth and defence within each elevation zone.

Keywords

Glucosinolates, herbivore pressure, leaf economic spectrum, plant defence syndromes, plant–herbivore interaction, resource availability hypothesis, volatile organic compounds.

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INTRODUCTION

Two general frameworks have been proposed to explain the variation in plant forms and functions: the plant economic spectrum and the plant defence theory. The plant economic spectrum (e.g. Wright *et al.* 2004; Díaz *et al.* 2016) generalises how functional traits and plant growth strategies relate to plant carbon and nutrients investment across variation in the abiotic environment. In contrast, the plant defence theories (Stamp 2003) formalise the different plant defence phenotypes in responses to variation in herbivore pressure (Coley *et al.* 1985) and co-evolutionary dynamics (Ehrlich & Raven 1964). Moreover, plant defence theories have been extended to include complex multi-trait approaches (Agrawal & Fishbein 2006), which now overlap with the plant economic spectrum. Nevertheless, we are still missing an integrative framework that links those theories for comprehensively understanding the multivariate plant trait co-expression along large-scale environmental clines (e.g. Moles *et al.* 2013; Turley *et al.* 2013; Reese *et al.* 2016). Most of the existing studies on plant defence strategies have usually focused on single traits or pairwise trade-off approaches for understanding how plants are constrained in evolving specific trait values (Agrawal & Fishbein 2006). Therefore, a multi-dimensional framework is necessary for understanding how plant strategies of growth and defence evolve along environmental gradients (Blonder *et al.* 2014; Díaz *et al.* 2016). Finally, the linkage between the plant economic spectrum and plant defence theories will necessitate the concomitant statistical representation of the multivariate space (e.g. the functional hypervolume of Blonder *et al.* (2014)) and the trait-based correlations and syndromes (e.g. Agrawal & Fishbein 2006).

The plant economic spectrum indicates that the radiation of plant lineages into different habitats, or along ecological gradients, requires the evolution of a variety of life-history traits and forms to tolerate shifting abiotic and biotic conditions (Ackerly *et al.* 2006). In addition, because plant functional processes are inherently economic in nature (Bloom *et al.* 1985; Givnish 1986), practically all plants set themselves along a series of allometrically constrained sets of functional trait values (Wright *et al.* 2004; Kunstler *et al.* 2016). For instance, classic categories of plants are aligned along the spectrum of maintenance vs. growth, fast vs. slow growth, high vs. low carbon storage, N acquisition, soft vs. hard leaves, or resistance (Mark Mark *et al.* 2002; Díaz *et al.* 2016; Kunstler *et al.* 2016). While the plant economic spectrum provides an excellent framework for elucidating constraints on habitat adaptation using a set of readily measurable and quantifiable traits, additional important ecological axes, such as the inclusion of specific traits related to defence against herbivores, which also require investment and hence shape the economic spectrum, have so far been omitted [see discussion in Wright *et al.* (2004)].

Plant defences against herbivores include chemical and mechanical traits that act directly on the herbivores (Agrawal 2007; Carmona *et al.* 2011; Mithoefer *et al.* 2012), or indirectly, by providing shelter, reward, or information cues that foster predators recruitment and presence near the plant

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[1] *Laboratory of Functional Ecology, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland*

[2] *Landscape Ecology, Institute of Terrestrial Ecosystems, ETH Zürich, Zürich, Switzerland*

[3] *Swiss Federal Research Institute WSL, 8903 Birmensdorf, Switzerland*

**Correspondence: E-mail: sergio.rasmann@unine.ch*

(Kessler & Heil 2011). Previous studies showed how the general plant form and function could be associated with defence strategies. Coley and colleagues (Coley 1983, 1993; Coley *et al.* 1985; Fine *et al.* 2004) showed that soil resource availability could influence the plant growth-defence trade-off. For instance, tropical plant species colonising sandy soils display different suites of defence traits, compared to species colonising the more nutrient-rich clay soil (Fine *et al.* 2004). This habitat-driven variation in plant defences resulted in divergent patterns of herbivory (Fine *et al.* 2004, 2006). Kursar & Coley (2003) have also shown that tropical trees (*Inga* spp.) could be classified along an escape (few chemical defences but rapid leaf expansion)–defence (high toxin concentrations but slow growth) continuum. Agrawal & Fishbein (2006) later formalised the concept of plant defence syndromes, by categorising *Asclepias* species into either employing a tolerance/escape strategy, a high nutrient and high defence strategy, or a low nutritional quality strategy. Given the link between leaf palatability traits and plant resource use strategies (Wright *et al.* 2004), plant defence theories are inherently associated with the plant economic spectrum, and both should be integrated in a cohesive framework.

To address the challenge of integrating the plant economic spectrum and plant defence theories, here we characterised the functional space of a group of closely related plant species growing along a steep environmental gradient. We measured a wide set of traits that are related to both the plant economic spectrum as well as to plant defences against herbivores. For integrating the representation of the trait-based hypervolume (Blonder *et al.* 2014) with a description of trait's correlations (positive and negative) and syndromes (Agrawal & Fishbein 2006), we developed a novel geometrical framework for characterising the shape of the functional space of each of the 15 *Cardamine* (Brassicaceae) species that occupy the entire elevational gradients in the Alps. We used elevation gradients to study plant functional trait variation because the mountain heterogeneity provides powerful 'natural experiments' for testing ecological and evolutionary responses (Körner 2007). By limiting spatial area while maximising the range of climatic conditions, elevation gradients allow to disentangle how abiotic, biotic and phylogenetic factor concomitantly influence the shape of functional niche.

Specifically, we had the following predictions derived from the plant economic spectrum and plant defence theories: (1) Plants growing within the same elevation zone converge into the same growth form-defence syndrome, independently of their shared phylogenetic history, but moulded by changes in environmental conditions. (2) The co-variation or trade-offs among traits involved in abiotic tolerance, growth and defence shape the functional space of plants. (3) The inclusion of direct and indirect defence-related traits into the functional space of plants redefines and expands the categorization of plant functional types along ecological gradients. Through an analysis of plant traits associated with both growth and defence, our main objective is to demonstrate the existence of a generalisable, habitat-driven, growth form-defence spectrum into which plants can adapt.

MATERIAL AND METHODS

Study system

To assess the putative presence of growth form-defence syndromes along elevation gradients, we sampled 15 out of the 19 described species of *Cardamine* (Brassicaceae), currently growing in Switzerland, accounting for approximately 40-% of all European *Cardamine* described species (Aeschimann *et al.* 2004). Species choice was based on eliminating the extremely rare occurrences (e.g. *C. asarifolia*) and the sub-species taxonomic level. Collectively, species ranged from growing as low as 300 m above sea level (m a.s.l.) (e.g. *C. bulbifera*) to up to more than 3000 m a.s.l. (e.g. *C. alpina*). For each species, we quantified the realised climatic niche in Switzerland using the occurrence datapoints obtained from the National Data Center and Information on the Flora of Switzerland (www.infoflora.ch). For each accession, we extracted elevation and climatic data, including degree-days (i.e. the cumulative number of degrees in a month, or a measure of temperature), and potential evapotranspiration (i.e. humidity), monthly average precipitation and number of frost days in a year, which represent the most symptomatic niche values of elevation clines (Körner 2007). Additionally, for each species, we extracted ecological indicator values for soil fertility based on Landolt (2010). Finally, to control for phylogenetic non-independence, we pruned the *Cardamine* phylogeny described by Pellissier *et al.* (2016) to create a community phylogeny of the species included in this study.

Sampling of plant functional traits

For each *Cardamine* species, we *in situ* sampled 10 functional traits related to abiotic tolerance, growth and defence (Table S1); plant biomass, plant maximum height, SLA, chlorophyll levels, leaf toughness, carbon to nitrogen ratio (C/N), constitutive and inducible direct (glucosinolates – GSL) and indirect (volatile organic compounds – VOCs) defences.

Traits were chosen according to classical literature (Karban & Baldwin 1997; Wright *et al.* 2004; Cornelissen *et al.* 2008; Díaz *et al.* 2016), and represent the plant functional dimensions along three axes of plant growth (plant size, biomass, chlorophyll, SLA, C/N), resistance against abiotic stress (toughness, SLA), and defences against herbivores (C/N, toughness and secondary metabolites).

Upon herbivore damage, GSLs enter in contact with myrosinase enzyme and are degraded into toxic sulphur- and nitrogen-containing by-products (Hopkins *et al.* 2009), toxic to both generalist and specialist herbivores (e.g. Louda & Rodman 1983; Daxenbichler *et al.* 1991; Montaut & Bleeker 2011). In addition, similar to most other vascular plant species, *Cardamine* leaves release low molecular weight molecules (VOCs), which are generally induced after mechanical or herbivore damage, and have been shown to attract natural enemies of herbivores in a variety of systems (e.g. Dudareva *et al.* 2004; Gols & Harvey 2009; Harvey *et al.* 2011). While other classes of secondary metabolites (e.g. phenolics) might be present in these plants that would affect herbivore performance, only for GSLs and VOCs, their putative effect as defence traits has been acknowledged.

Specifically, from May until August 2012, we visited 1–3 populations per species growing in their optimal habitat (Table S2), and following the natural phenology of the plants. All species were sampled at the flowering stage in order to avoid ontogenetic effects on plant growth and chemistry. In addition, we chose to sample at the flowering stage since most species flower very rapidly, or for long period of times, sometimes throughout the whole growing season (e.g. *Cardamine hirsuta*). We collected individuals from each species at the centre of their elevational distribution to be conservative with regard to within-species variation, but we acknowledge that we are not accounting for intraspecific variation that may exist along the elevation gradient. All control plants used for trait sampling were chosen within each population to display the least amount of damage in order to reduce the confounding effect of plants being already naturally induced (Pellissier *et al.* 2016).

Within each population, we selected eight plants, half of which were induced with methyl jasmonate (JA) (CAS number 39924-52-2, Sigma, St. Louis, IL, USA) (hereafter JA induction) 24 h. prior to sampling by bagging the aboveground parts of the plants with nalophan bags (1 L). JA-treated plants were induced by applying 5 μL of the pure compound to a cotton bud, which was placed in the bags. The other half of the plants were used as control plants, which also received cotton buds, but without JA application. JA was used as a broad mimic of chewing herbivore damage, as was done in several previous experiments (e.g. Halitschke *et al.* 2008). On the following day, the bags were replaced with new bags to avoid sampling highly concentrated scent. VOCs were then sampled for 4 h by pulling air through a charcoal filter trap (Orbo-32; Supelco, Bellefonte, PA, USA) at a flow rate of 200 mL min^{-1}. VOCs were later eluted with 250 μL dicholormethane and analysed with gas chromatography-mass spectrometry (GC-MS) (Pellissier *et al.* 2016). Leaf GSL profiles of each plant were obtained for three, fully expanded, leaves per plant sampled immediately after the volatile collection, and analysed using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) as described in Pellissier *et al.* (2016). Constitutive defences were thus obtained from the averages of control plants, while inducible defences were obtained from the differences between the average JA-induced plants minus the control plants.

For each plant, we measured the height from the ground to the highest leaf. SLA was calculated as the ratio of 6 mm diameter leaf disk area to its dry mass (mm^2 mg^{-1}). The same leaf disks were dried and ground to powder for measuring C/N content using an elemental analyser. Toughness was measured as the force (in grams) needed to pierce a hole into a leaf, and was done by punching three holes in three leaves per plant with a custom-made manual force gauge (Figure S1). Chlorophyll content was measured on the same leaves, three times per leaf, using a SPAD-502 Plus chlorophyll metre (Konica Minolta, Investment Ltd, China). During each sampling, we also recorded the amount of herbivore damage on each analysed plant using a percentage scale from 0% = no visible damage to 100% = all leaves were completely eaten, and using percentage increments as follows 0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%. Finally, all plants were cut near the ground, oven-dried at 40 °C for 7 days and weighed.

Grouping of species into syndromes

To detect potential growth form-defence syndromes, we organised the data into a 10 traits × 15 species matrix. We submitted the matrix to the unsupervised k-means clustering algorithm using the function *kmeans* in R (R Development Core Team 2016). The algorithm randomly assigns each observation to a cluster, and finds the centroid of each cluster until the within-cluster variation cannot be reduced any further. Because, k-means needs to specify the number of clusters, we opted to assign a minimum of three clusters based on natural history observations of plants growing in low-elevation warm sites, mid-elevation forested sites, or high-elevation alpine sites (see Figure S2 for details of the optimal distribution range and habitat of each species). Following the k-means grouping, we performed a redundancy analysis based on the correlation matrix of variables, in which data are centred and standardized by standard deviation (*rda* in the package *vegan* in R [Oksanen *et al.* 2013)]. We then projected all measures unto the first two components, PC1 and PC2, to create a two-dimensional space of measures.

Because species were clustered according to the k-means grouping (see Results), we were able to describe plant strategies along the growth form-defence syndrome based on the projection of principal component analysis (PCA) variables on the two-dimensional space. Next, in order to statistically assess variation in the plant form-defence syndromes observed in the PCA and explore their variations across both biotic and abiotic factors, we visualised the multi-dimensional functional space using radial plots. This allowed generating a unique geometrical shape of the functional space for each species. Radial plots were built by plotting the numeric values of each trait as the distances from the centre of a circular field along 10 directions (one per trait), and the direction of each axis was defined by the PCA. The position of axes corresponds to the order of the PCA to reflect the variation between strategies driven by the naturally observed trade-offs and positive correlations between traits. Overall traits data were centred, standardized by standard deviation and then rescaled between -1 and 1 across all species (See Figure 2, and additional dynamic plot in Appendix 1). From the radial plots, we were able to extract geometrical information, including the area and the centroid coordinates of the polygon for each species using the package *pracma* in R (Borchers 2015).

Based on the overall trait correlation matrix (as represented by the PCA), and based on the geometrical form of the radial plots, we had the following expectations: the position of the centroid would inform on the preponderance of a particular growth-defence strategy, while the area of the shape would indicate higher resource use. Traits were treated such that greater values of each trait correspond to greater resource allocation to the trait. For instance, higher levels of plant biomass, plant maximum height, chlorophyll, leaf toughness, constitutive and inducible GSL or VOCs are all related to higher energy expenditure. Higher SLA levels are positively correlated with potential relative growth rate across species, photosynthetic rate, or leaf nitrogen (N). In general, species in resource-rich environments tend, on average, to have a higher SLA than do those in resource-poor environments (Garnier & Laurent 1994; Poorter & Garnier 2007). Finally, higher C/N values indicate higher carbon storage per nitrogen molecules.

Statistical analyses along the gradient

We built distance matrices using: (1) the phylogenetic relationships between *Cardamine* species (function *cophenetic* in R); (2) the interspecific variability in the 10 selected traits; (3) the climatic variables; (4) the average herbivore damage; (5) the area of the radial plots and (6) the positions of the centroids [package *spatial.tools* in R (Greenberg 2014)]. Based on the distance matrices, we assessed all pairwise potential correlations among explanatory factors using classical Mantel tests ($n = 10\ 000$ permutations). Next, we performed multiple matrix regressions with Mantel permutations (10 000 iterations) using the function *multi.mantel* in *Phytools* (Revell 2012) to test for the relative importance of the phylogenetic, environmental and herbivore pressure distance matrices on the functional, the area and the centroid distance matrices. Finally, we (1) mapped the area of each species on the phylogeny of *Cardamine,* and (2) produced 3D plots between the two coordinates of the centroids and the linear representation of the environmental gradient based on the first axis of a PCA including all the climatic variables.

To evaluate how the axial organisation of the traits could potentially affect the polygon area and the shape, and consequently their relationships with biotic and abiotic factors, we estimated the stability of our results using 10 000 permutations of axes positions and analysed the variation in estimated coefficient of correlations and *P*-value. All analyses were performed using R cran (R Development Core Team 2016).

RESULTS

Syndrome detection and description

The three clusters extracted from the k-means analysis altogether explained 51% of the total variability shared among traits involved in both plant growth form and defences (Figure S3). These clusters represent three syndromes related to the growth-defence trade-offs, and within-traits correlations (Fig. 1), and which correspond to three characteristic zones along the studied elevation gradient (Figure S2). The geometrical representation of the functional dimension representing each species using the radial plot approach revealed variability in shapes across species, and again highlighted three distinct forms for each syndrome (see average syndrome shape in Fig. 2, and see dynamic plot showing the transition, and overlap among syndromes along the climatic gradient in Appendix 1).

Syndrome 1 (blue area in Fig. 1) included species with high levels of chlorophyll, tough leaves, high constitutive GLS, but low inducibility of both GLS and VOCs and generally small-size plants. This combination of traits characterises a global strategy of high constitutive defence, high abiotic resistance (low SLA values) and slow growth for species living at high elevation (alpine to mountain zones), and includes seven species (*C. alpina*, *C. resedifolia*, *C. rivularis*, *C. amara*, *C. pratensis* and *C. trifolia*).

Syndrome 2 (green area in Fig. 1) included species with high biomass production, high C/N ratio and globally low levels of

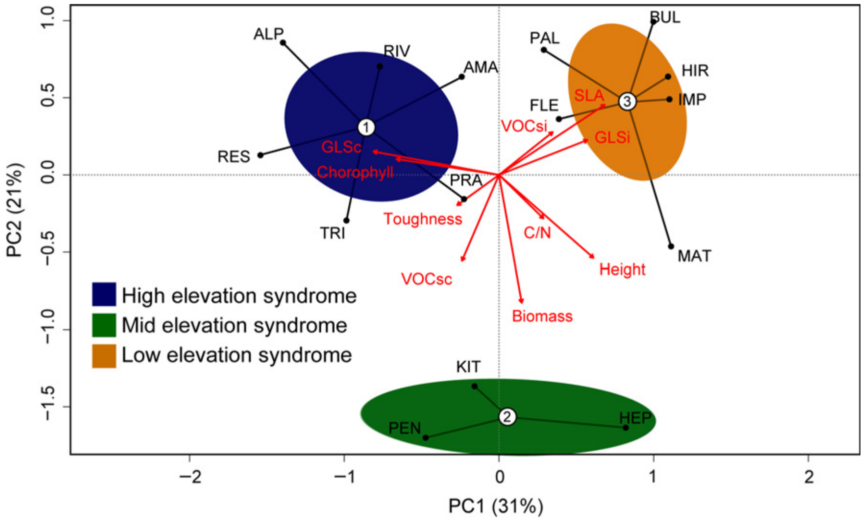

Figure 1 Clustering of the 15 *Cardamine* species into three syndromes. Scatter plot of principal component analysis of the functional trait variation with 95% confidence interval ellipses: Blue = Syndrome 1 of high-elevation alpine species, green = Syndrome 2 of forested mid-elevation species, orange = Syndrome 3 of low-elevation species. The first two vectors account for > 51% of functional variation. Species belonging to Syndrome 1 are: *C. alpina* (ALP), *C. rivularis* (RIV), *C. amara* (AMA), *C. pratensis* (PRA), *C. trifolia* (TRI), *C. resedifolia* (RES). Species belonging to Syndrome 2 are: *C. kitaibelii* (KIT), *C. heptaphylla* (HEP), *C. pentaphyllos* (PEN). Species belonging to Syndrome 3 are: *C. bulbifera* (BUL), *C. hirsuta* (HIR), *C. impatiens* (IMP), *C. matthioli* (MAT), *C. flexuosa* (FLE), *C. palustris* (PAL). Clustering of species into the three syndromes was based on k-means clustering analysis (see Figure S3).

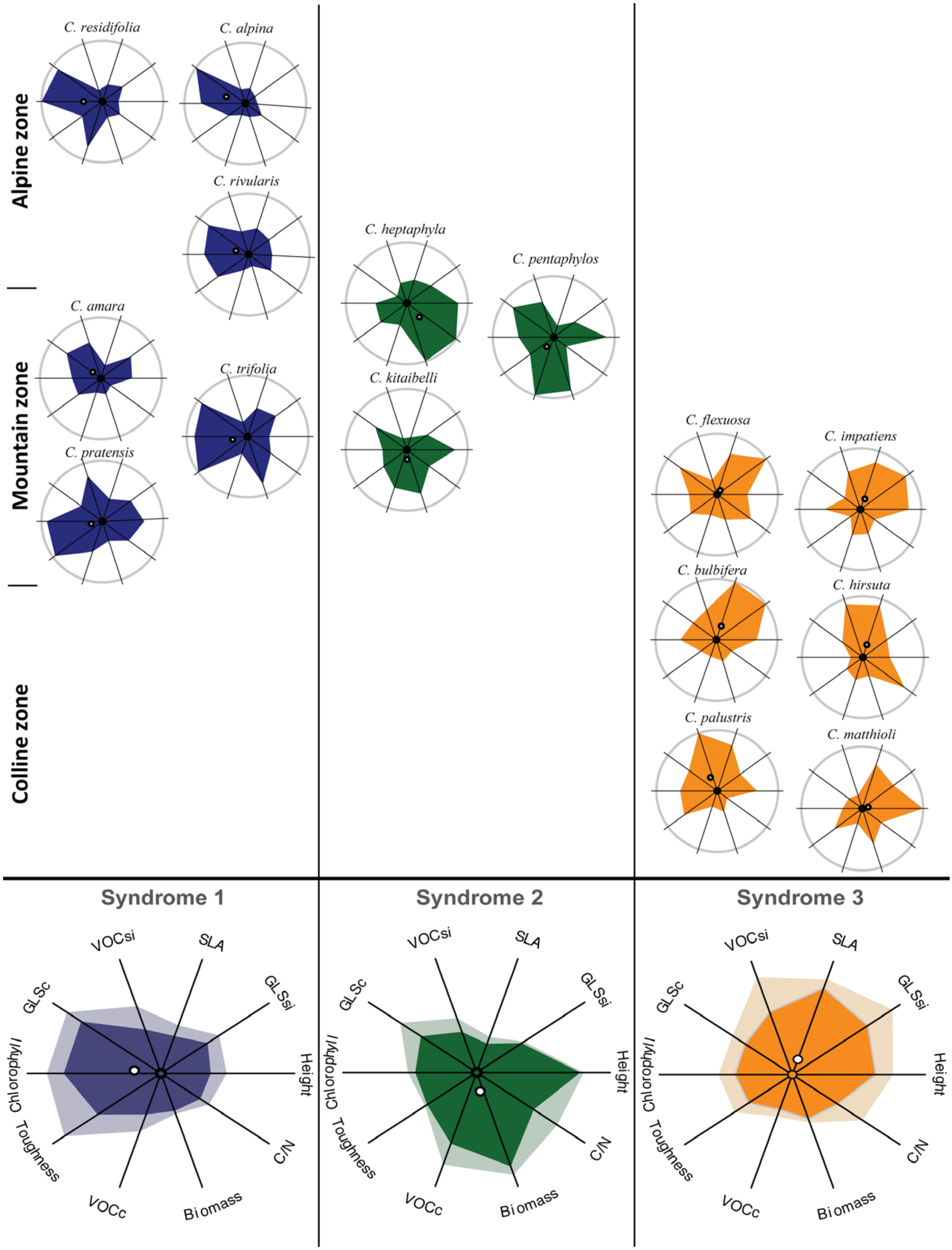

Figure 2 Polygonal representation of the functional niche. Each axis of the radial plot corresponds to one of the 10 functional traits measured (plant biomass, plant maximum height, SLA, chlorophyll levels, natural herbivory levels, leaf toughness, carbon to nitrogen ratio (C/N), constitutive and inducible glucosinolates (GLSc and GLSsi respectively) and constitutive and inducible volatile organic compounds (VOCsc and VOCsi respectively). The functional space of all species of *Cardamine* studied is placed in relation to the optimal elevation zone of each species, while at the bottom, an average functional space for each syndrome (blue for high-elevation, green for mid-elevation and orange of low-elevation syndromes) is depicted. The organisation of the radial plots corresponds to the organisation of the trait based on the PCA of Fig. 1. The white dots within the radial plot show the centroid of each polygon.

both mechanical and chemical defences. This syndrome characterises a potential strategy of high tolerance against herbivore pressure and encompasses a group of four species associated with mid-elevation conditions of forest habitats (*C. pentaphyllos*, *C. kitaibelii*, *C. heptaphylla*, *C. matthioli*).

Syndrome 3 (orange area in Fig. 1) included species with high stature (fast growth), high SLA (soft leaves), high inducibility of both GLSs and VOCs (chemical defences) and low constitutive defences. This syndrome characterises a global strategy of fast resource use for fast growth but low chemical and physical defences for five low-elevation species (*C. bulbifera*, *C. palustris*, *C. flexuosa*, *C. hirsuta*, *C. impatiens*).

Multivariate analysis of traits of forms and defences

The PCA structure revealed that the trait co-variations are driven by two interdependent trade-offs resulting in three equidistant directions. The scatter plot (Fig. 1) suggests that traits related to growth (such as plant biomass, maximum height) are negatively correlated with trait related chemical defences (particularly constitutive glucosinolate production). When only looking at chemical defences, a second clear trade-off appears between constitutive and inducible defences for both glucosinolates and volatile organic compounds. Moreover, constitutive glucosinolates were positively correlated with high leaf toughness, a trait also related to stressful abiotic conditions. At the opposite, inducible defences showed a positive correlation with SLA, which is generally linked to milder condition habitats. Across these three directional backgrounds, species are globally distributed along the first PCA axis, reflecting the elevational organisation as displayed in nature.

Relationship between environmental gradient and the functional space

We found no correlation between all of the four explanatory variables related to the abiotic (climate, soil nutrients) and the biotic (herbivory pressure, phylogeny) environment. This allowed exploring the effect of each factor in explaining the functional space independently. Using the basic distance matrix based on the functional traits, we could not detect significant correlations with the environmental variables (Table 1). In contrast, using the geometrical representation in the form of the radial plots, we found that the area of the polygon was correlated with the phylogenetic distance among species as well as to climatic conditions (Fig. 3, Table 1). Closely related species tend to have a similar area size (Fig. 3), and that species found in colder and more humid environments (i.e. high elevations) have a reduced size of the functional space (Figure S5). The place of the centroid of the radial plots was also related to climatic conditions of plant growth (Table 1, Fig. 4a), as well as by the levels of herbivory (Table 1, Fig. 4b) and marginally by nutrient availability in the soil (Table 1). In other words, the shape of the functional space represented by the radial plots was related to both abiotic and biotic factors. As a consequence, the three syndromes characterised by a comprehensive trait combination and a specific geometric signature showed a strong association with the environmental gradient, principally driven by elevation.

Table 1 Multiple matrix regression analyses table

Functional space variables	Biotic and abiotic factors	R^2	Coeff.	*t*	*p*
Functional	Phylogeny	0.01	21.89	0.94	0.49
	Climate		−0.04	−0.33	0.86
	Herbivory		−5.72	−0.79	0.54
	Nutrients		−13.74	−0.16	0.88
Area	Phylogeny	0.15	0.11	3.01	0.04*
	Climate		< 0.001	2.66	0.05°
	Herbivory		−0.01	−1.23	0.29
	Nutrients		0.03	0.19	0.86
Centroid	Phylogeny	0.29	0.01	1.02	0.37
	Climate		0.0002	4.32	0.003**
	Herbivory		0.01	2.82	0.02**
	Nutrients		0.08	2.10	0.06°

Shown is the relative effect of phylogenetic, climatic, herbivore pressure and nutrient availability pairwise distances across the 15 species of *Cardamine* studied on overall functional trait pairwise distance, the pairwise distance of the area size obtained from the radial plots, as well as their centroid position.

$°p < 0.1$, $*p < 0.05$, $**p < 0.001$.

These results are robust to axis rotation. The permutation tests showed that all significant correlations observed with the PCA-based organisation of the axes (area with phylogeny, area with environment, centroid with environment, centroid with herbivory), remain stable, independently of the axes rotation (see large proportion of significant values in Figure S6).

DISCUSSION

We here combined the traditional functional plant growth form axis with the plant defence axis into a cohesive representation of the plant functional dimensions. This approach showed that *Cardamine* species cluster into three main syndromes of plant functional traits that are aligned along the variation in the growth form-defence spectrum (see animation in Appendix S1). The multi-dimensional functional spaces are related to the phylogenetic distance among species, as well as to both biotic (herbivore pressure) and abiotic (climate) local conditions. In particular, the area delimited by the functional dimensions is associated with both a phylogenetic signal and abiotic conditions, while the centroid is mainly associated with both abiotic and biotic conditions. We propose that the phenotype of a given species is determined by local abiotic and biotic conditions that shape trait expression and trait co-variation, and thus the convergence of the forms of the functional dimension at a given site.

Trait co-variation and prevalence along elevation gradients

Across the 15 species of *Cardamine* studied, we found both positive and negative correlations among functional traits (Figure S4), which is in accordance with classical energy-allocation theory (Chapin *et al.* 1993; Wright *et al.* 2004). Particularly, we found trade-offs between growth and stress-tolerance (Herms & Mattson 1992), and between physical and

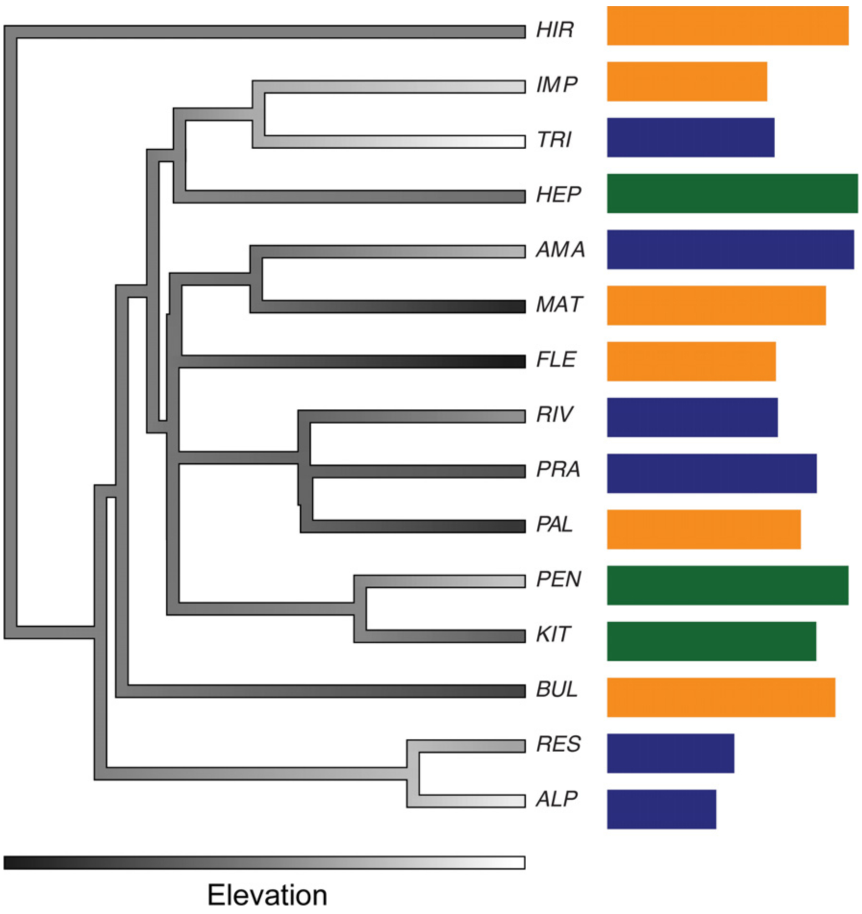

Figure 3 Area of the *Cardamine* spp. functional niche. Shown is the phylogenetic relationship among the 15 *Cardamine* spp. studied. Mapped on the side of the phylogeny is the average area value of the functional niche per species (horizontal bars on the right of the phylogenetic tree), and on the phylogeny the optimal elevation of each species is represented with different shades of grey (darker colours represent lower elevations, while lighter colours represent higher elevations).

chemical constitutive defences and inducible chemical defences. Species with high SLA do not share the same habitat with species that are small in size and have hard and tough leaves. By evolving fast-growing capabilities such as having high SLA values where resources are plentiful, a plant is able to escape competition for light (Gaudet & Keddy 1988). Yet, tall plants with soft and large leaves render a plant more sensitive to harsh climatic conditions such as desert habitats (Díaz *et al*. 2016), or alpine environments (Korner *et al*. 1989). Along these lines, we found that small plant size, tough leaves and low SLA values were associated with both of the most alpine species (*C. alpina* and *C. resedifolia*), while classical low-elevation plants such as *C. bulbifera* and *C. impatiens* clearly show the opposite strategy.

Moreover, we found trade-offs between the constitutive expression of defences and their inducibility. Within the context of plant defence theories, trade-offs between different types of defences and deployment strategies have also been postulated (Agrawal *et al*. 2010). Among those, a trade-off between constitutive defences and their inducibility is inherent to the fact they both use the same set of resources (Thaler & Karban 1997). It was postulated that the patterns of defence induction depend on the probability of attack and the value of the tissues eaten (Zangerl & Rutledge 1996). Hence, plants or organs that are regularly attacked or that are highly valuable (including cases in which they are valuable because they cannot be replaced in resource-poor environments such as Amazonian white sands Fine *et al*. 2004) should have greater constitutive defences. For instance, *Vicia sepium* plants growing in environments where herbivore pressure is strong produce constitutively a copious amount of VOCs, while in environments where herbivore is more haphazardly spread (e.g. high elevation), the inducibility of VOCs production is higher (Rasmann *et al*. 2014). In our study, we found the postulated trade-off between constitutive and inducible defences. However, and contrary to expectations, we observed that plants generally living under stronger and more regular herbivore pressure (i.e. lowlands) invest more in the induction of defences, while high-elevation plants, under generally average lower herbivore pressure (Figure S7), promotes higher levels of constitutive direct defences (GLSs). At high elevation, the cost to recover tissue lost is strongly limited by paucity of resources and cold temperatures. Therefore, for these alpine species, the fitness costs of herbivory cannot be outweighed by the energy saved in reduced levels of defences. The deployment of defence strategies is therefore more linked to the

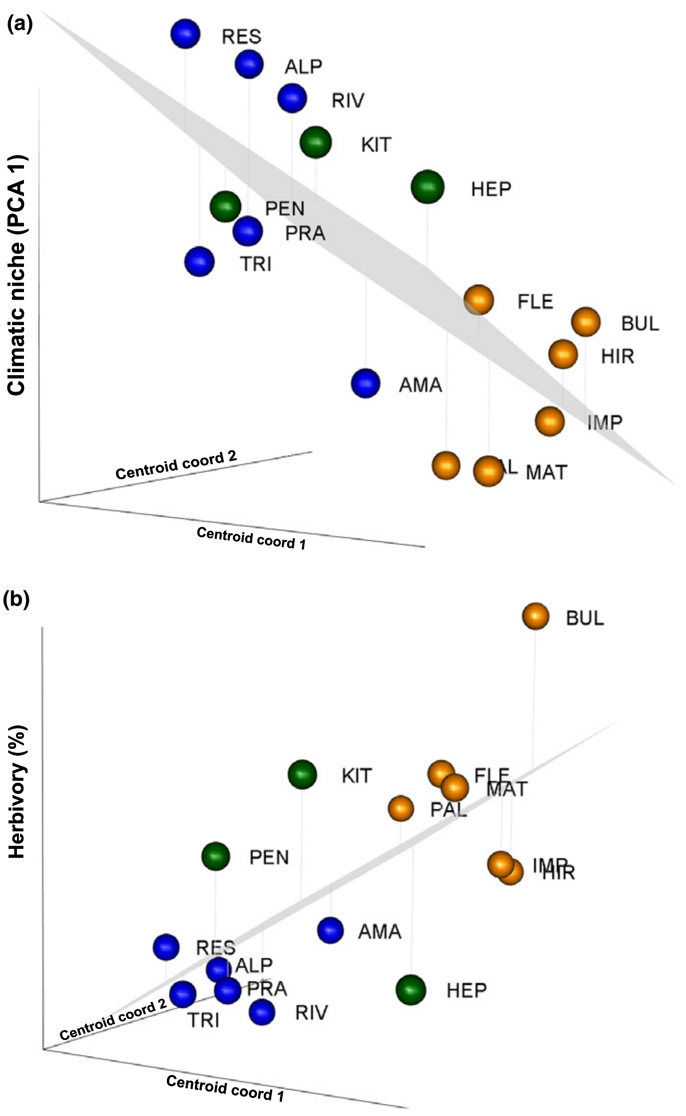

Figure 4 The shape of the *Cardamine* spp. functional niche. Shown is (a) the species level correlation between the positions of the centroid of the polygon and the climatic niche, depicted as the first axis of the climatic PCA, and (b) between centroid values and the average amount of herbivore damage measured for each species. The grey shading represents the plane derived from the two-dimensional centroid space. Dots represent species averages, and colours represent the three syndromes as detailed in Fig. 1.

impact of herbivory based on resources available, than solely on herbivore pressure.

Our approach enabled the integration of classic functional traits (SLA, chlorophyll, toughness, CN, biomass), with anti-herbivore defences into complex positive and negative relationships. For instance, we found that higher biomass and height negatively correlates with chemical defences. This is in line with postulated growth-defence trade-offs (Herms & Mattson 1992). Using radial plots, such a trade-off can be visualised even within the complex multi-trait matrix of each individual plant.

Finally, while the existence of plant functional groups that separate themselves along a continuum of tolerance to abiotic stress and resource-rich environment has been predicted by ecologists since more than 40 years now (Grime 1977; Tilman 1980), the link between plant growth form and defence was so far still missing. Therefore, while our findings are in line with previous observations of within-species trait correlations (e.g. Ackerly 2004; Díaz *et al.* 2016), we here argue that the incorporation of defence traits into plant functional space description enhances the overall resolution and habitat-based prediction abilities of how the environment shapes plant functionality.

Variation in the geometrical shape of the growth form-defence syndrome along elevation gradients

We used a radial representation of the functional space for the identification of growth form-defence syndromes along elevation gradients. From the different polygons generated for each species, we found that specific characteristics of the geometrical representation, such as the area or the centroid position, are correlated with variation in both abiotic (climate, nutrients) and biotic (herbivory) factors. This geometrical approach allowed detecting how biotic and abiotic factors drive the geometrical structure of the functional dimensions of each species.

Specifically, we found that the area of the polygon is related to both the phylogenetic structure and climate. First, this indicates that the size of the functional trait space (area) potentially carries a certain amount of phylogenetic signal, in which more closely related species tend to have more similar area values. In addition, we found that plants radiating into colder environments have a generally smaller functional area. While this is in line with our expectations that harsher (alpine) environment inhibits plant functional resource use, a higher diversity of functional traits should be included in future studies to confirm our trend and explore specifically the question of the functional area range. In addition, because we found no correlation between phylogenetic distance and the climatic distance, both phylogenetic structure and environmental conditions seem to independently drive the trait value. However, for now, our reduced number of species cannot fully tease this apart (Pearman *et al.* 2014).

The radial plot approach also allowed uncovering the relationships between the shape of the polygons (measured as the position of the centroids in the radial plots) and both the environmental conditions and herbivory. The structure of the trait space, which is itself influenced by pairwise positive- and negative trait correlations, is driven by both biotic and abiotic conditions of the local habitat. This convergence of forms indicates that there is a limited number of potential strategies a plant can evolve to when radiating into a specific environment (Grime 1977). While this is in opposition with the limiting similarity theory when plants are under competition (Macarthur & Levins 1967), our model system does not imply direct competition within the *Cardamine* species growing along the elevation gradient. While a comparative phylogenetic approach as this one allows highlighting that during the radiation of a group, habitat filtering and plant–herbivore interaction shape the structure of the functional space into syndromes (as described in Fig. 5), it does not address how plant–plant interaction drives the evolution of the functional space as might be described for community assembly theory (Kraft *et al.* 2015). But how plant–plant competition affect trait space could also be modelled in future community

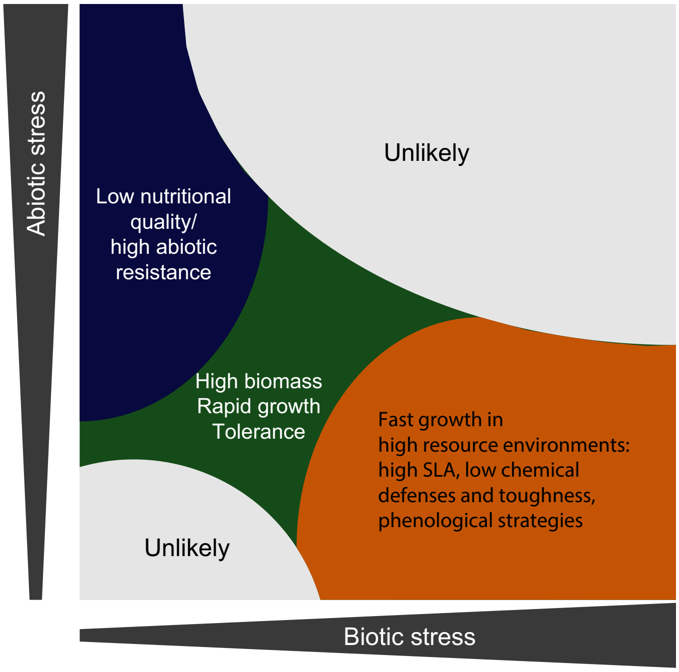

Figure 5 The unfolding of the plant growth-defence syndrome along ecological gradients. The low-elevation high resources/high biotic interaction syndrome (orange) is arranged around fast growth, and high inducibility of defences. The mid-elevation forest species syndrome (green) is characterised by high biomass, and low levels of defences typical of resource-rich soil environments and moderate levels of herbivory. The high-elevation high abiotic stress syndrome (blue) favours slow growth, high physical resistance and high levels of direct defence. Grey areas represent zones of empty functional space in our study, thus considered unlikely to occur.

assembly-based experiments using the radial plot approach described here.

Finally, it is worth noting that the shape of the functional space as described with the radial plots is inherently linked to the choice of the traits measured (e.g.: SLA vs. 1/SLA = leaf mass per area (LMA); N vs. C/N; induced defences (I) vs. inducibility of defences (I-C)). Therefore, while an across-species comparative analyses, as we preformed, here remain always valid, we advocate caution in interpreting absolute area sizes and centroid position values if not placed into context.

CONCLUSION

We here present the plant spectrum of form and defences that integrates both classic plant defence theories (e.g. Ehrlich & Raven 1964; Feeny 1976; Bryant *et al.* 1983; Coley *et al.* 1985), plant growth form and the leaf economic spectrum (e.g. Grime 1977; Chapin *et al.* 1993; Wright *et al.* 2004; Díaz *et al.* 2016). Our geometrical approach allows the simultaneous exploration of the effect of the environment on plant strategies, as well as the within-species trait co-variation driven by both biotic and abiotic conditions. We advocate the future consideration of classical plant functional traits together with plant defences strategies to fully understand the simultaneous and coordinated evolution of plant traits within communities and along environmental gradients.

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AUTHOR'S CONTRIBUTION

SR initiated the project and collected the data;, EM, SR and LP analysed the data; all authors wrote the manuscript.

DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.6pq3250, and used under the Creative Commons Attribution licence.

REFERENCES

Ackerly, D.D. (2004). Adaptation, niche conservatism, and convergence: comparative studies of leaf evolution in the California chaparral. *Am. Nat.*, 163, 654–671.

Ackerly, D.D., Schwilk, D.W. & Webb, C.O. (2006). Niche evolution and adaptive radiation: testing the order of trait divergence. *Ecology*, 87, S50–S61.

Aeschimann, D., Lauber, K., Moser, D.M. & Theurillat, J.-P. (2004). Flora Alpina. Haupt Berne, Switzerland.

Agrawal, A.A. (2007). Macroevolution of plant defense strategies. *Trends Ecol. Evol.*, 22, 103–109.

Agrawal, A.A. & Fishbein, M. (2006). Plant defense syndromes. *Ecology*, 87, S132–S149.

Agrawal, A.A., Conner, J.K. & Rasmann, S. (2010). Tradeoffs and adaptive negative correlations in evolutionary ecology. In: *Evolution After Darwin: The First 150 Years* (eds Bell, M.A., Futuyma, D.J., Eanes, W.F. & Levinton, J.S.). Sinauer Associates, Inc., Sunderland, MA, USA, pp. 243–268.

Blonder, B., Lamanna, C., Violle, C. & Enquist, B.J. (2014). The n-dimensional hypervolume. *Global Ecol. Biogeogr.*, 23, 595–609.

Bloom, A.J., Chapin, F.S. & Mooney, H.A. (1985). Resource limitation in plants - an economic analogy. *Annu. Rev. Ecol. Syst.*, 16, 363–392.

Borchers, H.W. (2015). Pracma: practical numerical math functions. R package version 1.8.3. http://CRAN.R-project.org/package=pracma. Last accessed 30 January 2018.

Bryant, J.P., Chapin, F.S. & Klein, D.R. (1983). Carbon nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, 40, 357–368.

Carmona, D., Lajeunesse, M.J. & Johnson, M.T.J. (2011). Plant traits that predict resistance to herbivores. *Funct. Ecol.*, 25, 358–367.

Chapin, F.S., Autumn, K. & Pugnaire, F. (1993). Evolution of suites of traits in response to environmental-stress. *Am. Nat.*, 142, S78–S92.

Coley, P.D. (1983). Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecol. Monogr.*, 53, 209–233.

Coley, P.D. (1993). Gap size and plant defenses. *Trends Ecol. Evol.*, 8, 1–2.

Coley, P.D., Bryant, J.P. & Chapin, F.S. (1985). Resource availability and plant antiherbivore defense. *Science*, 230, 895–899.

Cornelissen, T., Fernandes, G.W. & & Vasconcellos-Neto, J. (2008). Size does matter: variation in herbivory between and within plants and the plant vigor hypothesis. *Oikos*, 117, 1121–1130.

Daxenbichler, M.E., Spencer, G.F., Carlson, D.G., Rose, G.B., Brinker, A.M. & Powell, R.G. (1991). Glucosinolate composition of seeds from 297 species of wild plants. *Phytochemistry*, 30, 2623–2638.

Díaz, S., Kattge, J., Cornelissen, J.H.C., Wright, I.J., Lavorel, S., Dray, S. *et al*. (2016). The global spectrum of plant form and function. *Nature*, 529, 167–171.

Dudareva, N., Pichersky, E. & Gershenzon, J. (2004). Biochemistry of plant volatiles. *Plant Physiol*., 135, 1893–1902.

Ehrlich, P.R. & Raven, P.H. (1964). Butterflies and plants - a study in coevolution. *Evolution*, 18, 586–608.

Feeny, P. (1976). Plant apparency and chemical defense. In: *Biochemical Interaction Between Plants and Insects: Proceedings of the Fifteenth Annual Meeting of the Phytochemical Society of North America* (eds Wallace, J. & Mansell, R.). Plenum Press, New York, pp. 1–40.

Fine, P.V.A., Mesones, I. & Coley, P.D. (2004). Herbivores promote habitat specialization by trees in amazonian forests. *Science*, 305, 663–665.

Fine, P.V.A., Miller, Z.J., Mesones, I., Irazuzta, S., Appel, H.M., Stevens, M.H.H. *et al*. (2006). The growth-defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology*, 87, S150–S162.

Garnier, E. & Laurent, G. (1994). Leaf anatomy, specific mass and water content in congeneric annual and perennial grass species. *New Phytol*., 128, 725–736.

Gaudet, C.L. & Keddy, P.A. (1988). A comparative approach to predicting competitive ability from plant traits. *Nature*, 334, 242–243.

Givnish, T.J. (1986). *On the Economy of Plant Form and Function*. Cambridge Univ. Press Cambridge/New York City, New York.

Gols, R. & Harvey, J.A. (2009). Plant-mediated effects in the Brassicaceae on the performance and behaviour of parasitoids. *Phytochem. Rev*., 8, 187–206.

Greenberg, J. (2014). spatial. tools: R functions for working with spatial data. R package version, 1.

Grime, J.P. (1977). Evidence for existence of 3 primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat*., 111, 1169–1194.

Halitschke, R., Stenberg, J.A., Kessler, D., Kessler, A. & Baldwin, I.T. (2008). Shared signals –'alarm calls' from plants increase apparency to herbivores and their enemies in nature. *Ecol. Lett*., 11, 24–34.

Harvey, J.A., van Dam, N.M., Raaijmakers, C.E., Bullock, J.M. & Gols, R. (2011). Tri-trophic effects of inter- and intra-population variation in defence chemistry of wild cabbage (Brassica oleracea). *Oecologia*, 166, 421–431.

Herms, D.A. & Mattson, W.J. (1992). The dilemma of plants - to grow or defend. *Q. Rev. Biol*., 67, 283–335.

Hopkins, R.J., van Dam, N.M. & van Loon, J.J.A. (2009). Role of Glucosinolates in Insect-Plant Relationships and Multitrophic Interactions. *Annu. Rev. Entomol*., 54, 57–83.

Karban, R. & Baldwin, I.T. (1997). *Induced Responses to Herbivory*. The University of Chicago Press, Chicago.

Kessler, A. & Heil, M. (2011). The multiple faces of indirect defences and their agents of natural selection. *Funct. Ecol*., 25, 348–357.

Körner, C. (2007). The use of 'altitude' in ecological research. *Trends Ecol. Evol*., 22, 569–574.

Korner, C., Neumayer, M., Menendezriedl, S.P. & Smeetsscheel, A. (1989). Functional-morphology of mountain plants. *Flora*, 182, 353–383.

Kraft, N.J.B., Adler, P.B., Godoy, O., James, E.C., Fuller, S. & Levine, J.M. (2015). Community assembly, coexistence and the environmental filtering metaphor. *Funct. Ecol*., 29, 592–599.

Kunstler, G., Falster, D., Coomes, D.A., Hui, F., Kooyman, R.M., Laughlin, D.C. *et al*. (2016). Plant functional traits have globally consistent effects on competition. *Nature*, 529, 204–207.

Kursar, T.A. & Coley, P.D. (2003). Convergence in defense syndromes of young leaves in tropical rainforests. *Biochem. Syst. Ecol*., 31, 929–949.

Landolt, E. (2010). *Flora Indicativa*, 1st edn.. Haupt Verlag, Bern.

Louda, S.M. & Rodman, J.E. (1983). Concentration of glucosinolates in relation to habitat and insect herbivory for the native crucifer *Cardamine cordifolia*. *Biochem. Syst. Ecol*., 11, 199–207.

Macarthur, R. & Levins, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. *Am. Nat*., 101, 377–385.

Mark, W., Daniel, S.F., Angela, T.M., Peter, A.V. & Wright, I.J. (2002). Plant ecological strategies: some leading dimensions of variation between species. *Annu. Rev. Ecol. Syst*., 33, 125–159.

Mithoefer, A. & Boland, W. (2012). Plant defense against herbivores: chemical aspects. In: *Annual Review of Plant Biology*, Vol. 63 (ed. Merchant, S.S.), pp. 431–450.

Moles, A.T., Peco, B., Wallis, I.R., Foley, W.J., Poore, A.G.B., Seabloom, E.W. *et al*. (2013). Correlations between physical and chemical defences in plants: tradeoffs, syndromes, or just many different ways to skin a herbivorous cat? *New Phytol*., 198, 252–263.

Montaut, S. & Bleeker, R.S. (2011). *Cardamine* sp. - A review on its chemical and biological profiles. *Chem. Biodivers*., 8, 955–975.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B. *et al*. (2013). vegan: Community Ecology Package. http://vegan.r-forge.r-project.org/. Last accessed 24 January 2018.

Pearman, P.B., Lavergne, S., Roquet, C., Wüest, R., Zimmermann, N.E. & Thuiller, W. (2014). Phylogenetic patterns of climatic, habitat and trophic niches in a European avian assemblage. *Global Ecol. Biogeogr*., 23, 414–424.

Pellissier, L., Moreira, X., Danner, H., Serrano, M., Salamin, N., van Dam, N.M. *et al*. (2016). The simultaneous inducibility of phytochemicals related to plant direct and indirect defences against herbivores is stronger at low elevation. *J. Ecol*., 104, 1116–1125.

Poorter, H. & Garnier, E. (2007). Ecological significance of inherent variation in relative growth rate and its components. In *Functional Plant Ecology* (eds Pugnaire, F.I., Valladares, F.). CRC Press, Boca Raton, pp. 67–100.

R Development Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria.

Rasmann, S., Buri, A., Gallot-Lavallée, M., Joaquim, J., Purcell, J. & Pellissier, L. (2014). Differential allocation and deployment of direct and indirect defences by Vicia sepium along elevation gradients. *J. Ecol*., 102, 930–938.

Reese, A.T., Ames, G.M. & Wright, J.P. (2016). Variation in plant response to herbivory underscored by functional traits. *PLoS ONE*, 11, e0166714.

Revell, L.J. (2012). phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol*., 3, 217–223.

Stamp, N. (2003). Out of the quagmire of plant defense hypotheses. *Q. Rev. Biol*., 78, 23–55.

Thaler, J.S. & Karban, R. (1997). A phylogenetic reconstruction of constitutive and induced resistance in *Gossypium*. *Am. Nat*., 149, 1139–1146.

Tilman, D. (1980). Resources - a graphical-mechanistic approach to competition and predation. *Am. Nat*., 116, 362–393.

Turley, N.E., Godfrey, R.M. & Johnson, M.T.J. (2013). Evolution of mixed strategies of plant defense against herbivores. *New Phytol*., 197, 359–361.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F. *et al*. (2004). The worldwide leaf economics spectrum. *Nature*, 428, 821–827.

Zangerl, A.R. & Rutledge, C.E. (1996). The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. *Am. Nat*., 147, 599–608.

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