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Intraspecific functional variability: extent, structure and sources of variation

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Summary

1. Functional traits are increasingly used to investigate community structure, ecosystem functioning or to classify species into functional groups. These functional traits are expected to be variable between and within species. Intraspecific functional variability is supposed to influence and modulate species responses to environmental changes and their effects on their environment. However, this hypothesis remains poorly tested and species are mostly described by mean trait values without any consideration of variability in individual trait values.

2. In this study, we quantify the extent of intraspecific plant functional trait variability, its spatial structure and its response to environmental factors. Using a sampling design structured along two direct and orthogonal climatic gradients in an alpine valley, we quantified and analysed the intraspecific variability for three functional traits (height, leaf dry matter content and leaf nitrogen content) measured on sixteen plant species with contrasting life histories.

3. Results showed a large variability of traits within species with large discrepancies between functional traits and species. This variability did not appear to be structured within populations. Between populations, the overall variability was partly explained by the selected gradients. Despite the strong effects of temperature and radiation on trait intraspecific variability, the response curves of traits along gradients were partly idiosyncratic.

4. *Synthesis*. Giving a comprehensive quantification of intraspecific functional variability through the analysis of an original data set, we report new evidence that using a single trait value to describe a given species can hide large functional variation for this species along environmental gradients. These findings suggest that intraspecific functional variability should be a concern for ecologists and its recognition opens new opportunities to better understand and predict ecological patterns in a changing environment. Further analyses are, however, required to compare inter- and intraspecific variability.

Key-words: alpine ecosystems, environmental gradients, intraspecific variability, leaf traits, life forms, linear mixed models, plant functional traits, radiation, response surface methodology, temperature

Introduction

Plant functional traits – any measurable features at the individual level that directly or indirectly affect overall plant fitness (Lavorel *et al.* 1997; Violle *et al.* 2007) – are increasingly used, for instance, to establish functional classifications of organisms (Lavorel *et al.* 1997), to quantify the functional diversity of communities (de Bello *et al.* 2009) or to parameterize plant functional types in vegetation models (Woodward & Diament 1991). These functional traits are variable between and within species. Contrasts between species' trait values have been largely investigated in terms of response of functional traits to gradients or trait trade-offs (Reich *et al.* 2003; Diaz *et al.* 2004; Thuiller *et al.* 2004; Wright *et al.* 2005). However, species are commonly described by functional trait measurements collected from a few individuals from one or few populations and averaged at the population or species level, disregarding the intraspecific functional variability (FV). A large number of

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studies have been published investigating, on a regional to global scale, the response of functional, ecophysiological or demographic traits to environmental gradients (Cordell *et al.* 1998; Meziane & Shipley 1999; Reich *et al.* 1999; Ryser & Aeschlimann 1999; Dyer *et al.* 2001; Garnier *et al.* 2001). However, the available literature gives an incomplete description of intraspecific FV because studies have not been carried out with this objective in mind; it thus reveals a lack of knowledge about the general spatial structuring of intraspecific FV and its response to environmental gradients (McGill *et al.* 2006; Tables S1 and S2 in Supporting Information). Moreover, most studies dealing with intraspecific FV have focused on indirect gradients, namely altitude, latitude or longitude (Cordell *et al.* 1998; Ryser & Aeschlimann 1999). These indirect gradients, *sensu* Austin, Cunningham & Fleming (1984), are unknown combinations of multiple direct environmental gradients that impact plant physiology directly (e.g. temperature, nutrient availability). Indirect gradients should not be used to describe ecological patterns as they are not comparable over time and space and do not have any ecological meaning (Körner 2007).

Several recent studies, however, have shown that intraspecific FV can have significant effects on ecosystem functioning (nutrient and carbon cycles:Lecerf & Chauvet 2008; response to herbivory: Boege & Dirzo 2004). These studies called for a better understanding of intraspecific FV, its extent and structure and the way traits vary along environmental gradients. Intraspecific FV could then be fully integrated into ecological theories and studies and help to further improve our understanding of biodiversity and ecosystems functioning and to predict their response to global changes (McGill *et al.* 2006). Observed intraspecific FV expresses the range of possible trait values as well as the variability of what is functionally experienced by individuals of a given species living in different environmental conditions. It results from genetic diversity and phenotypic plasticity of the different genotypes, both of which are potentially complementary and not exclusive (Joshi *et al.* 2001; Byars, Papst & Hoffmann 2007). Intraspecific variability enables plant species to survive, grow and reproduce under new environmental conditions (Joshi *et al.* 2001; Byars, Papst & Hoffmann 2007). It will thus influence the response of populations and species to environmental changes and the resulting changes in communities and ecosystems. Intraspecific FV can occur at different levels: (i) as differences between mean traits of populations living in contrasted environmental conditions, (ii) as differences between individuals of a population or (iii) as differences between leaves of an individual (Shipley 1995).

In this study, we investigated the extent of intraspecific FV, without distinguishing genetic and plastic effects, because: (i) phenotypic variability gives a general idea of the species' ability to cope with new environmental conditions (Milla, Escudero & Iriondo 2009); (ii) from a functional point of view, individual phenotypic expression and its consequences for higher levels of organization (populations, communities, ecosystems) make the identification of the underlying processes that are responsible for such variability a secondary priority, albeit relevant for other questions such as evolution of species niches. We examined the magnitude and structure of intraspecific FV through the analysis of an original data set collected from 16 contrasting species following a unique sampling design and protocol for this purpose. The study focussed on alpine ecosystems that provide, over small spatial scales, steep climatic gradients that constrain the functional make-up of species (Körner 1999). Such combinations of contrasting conditions and species allowed us to address the following questions: (i) Magnitude: how variable are functional traits within species? (ii) Structure: how is intraspecific FV structured spatially (between individuals, subpopulations, populations)? As neighbouring individuals are expected to be genetically related, the variability is expected to be higher between than within populations. (iii) Response to gradients: how do traits respond to climatic gradients (shape and strength)? (iv) Determinants: could the FV within each species (magnitude, structure and response to gradients) be related to species' characteristics such as growth form or niche breadth?

Material and methods

STUDY SITE

The study was conducted in the 25 km long Guisane valley (*c.* 260 km^2; 44.9° N, 6.6° E) in the central French Alps (between Briançon at 1200 m and Combe Roche Noire at 3000 m a.s.l.). The valley is characterized by strongly contrasted climatic conditions, with monthly mean temperatures ranging from −6.3 °C to −1 °C for the coldest and from 10 °C to 17 °C for the warmest months. As in other valleys of the central Alps, the landscape is a mosaic of coniferous and deciduous forests, shrub heaths, subalpine grasslands and alpine meadows, which were all included in the sampling strategy.

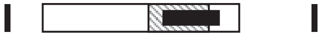
SPECIES SELECTION

We sampled, during summer 2007 and 2008, 16 species (see Table 1) (i) from different life forms (graminoïd, forbs, legume, shrub, tree) to obtain the widest possible scope for the robustness and generality of detected patterns; (ii) that were sufficiently common, i.e. widely distributed, to be found in contrasting conditions; (iii) with contrasting climatic preferences, i.e. species for which optimal conditions are lowland, subalpine or alpine, to be able to detect potential functional changes between marginal and optimal populations (Hampe & Petit 2005). We used two sets of congeners (*Juniperus: sibirica* and *communis* and *Pinus: sylvestris* and *uncinata*) because of their apparent hybridization in the study area (P. Salomez, personal communication). We checked *post hoc* that these congeners were relevant functional continua, i.e. had unimodal trait distributions. We calculated for each species the theta index to estimate its degree of specialization (Fridley *et al.* 2007). This species co-occurrence index assumes that generalist species co-occur with a larger number of species than specialists (see Appendix S1).

SAMPLING STRATEGY

Based on the results of a principal component analysis (data not shown) on a set of interpolated topoclimatic variables from the meteorological model Aurelhy at a 50-m resolution (Benichou & Le Breton 1987), we selected two uncorrelated direct gradients: mean minimal temperature in winter (temperature) and solar radiation in August (radiation). We hypothesized that functional traits would

Table 1. Characteristics of the sixteen species sampled. For all sampled species are given: name (species were sampled in 2007, except species with an asterisk, which were sampled in 2008), label, family, life form (G: grass, H: herb, S: shrub, T: tree), the number of populations where they were sampled, the number of measured individuals, the number of measured leaves per individual plant, the way the Leaf Nitrogen Content (LNC) was sampled (SP: samples pooled by subpopulations, I: measured individually). 'Niche1′ represents a species' altitudinal range [considered as the 10–90% inter-quantile length from the presence points observed by the Conservatoire National Botanique des Alpes (CBNA)], 'Niche2′ represents the optimal altitude of the species (mode of the distribution curve of presence along altitude, data from CBNA), 'Sampling' represents the altitudinal range sampled in the study and 'Spe' represents species' Fridley's specialization index (see Appendix S1). 'Environmental requirements and sampling' describes the altitudinal range of the species between 0 and 4000 m a.s.l.: (i) white rectangles give the full altitudinal range from the presence points observed by the CBNA; (ii) hatched rectangles give the inter-quantiles (10–90%) of the altitudinal range and (3) dark rectangles give the altitudinal range we sampled

Species	Label	Family	Life Form	Populations	Individuals	Leaves	LNC	Niche 1	Niche 2	Sampling	Spe	Environmental requirements and Sampling
Carex sempervirens Vill.	CS	Cyperaceae	G	12	108	1	SP	897	1990	899	0.54	
Dactylis glomerata L.	DG	Poaceae	G	10	90	1	I	1448	1073	624	0.62	
Dryas octopetala L.	DO	Rosaceae	S	15	135	10	I	771	2040	878	0.52	
Festuca paniculata (L.) Schinz & Thell.	FP	Poaceae	G	16	144	1	I	605	2040	831	0.48	
Geum montanum L.	GM	Rosaceae	H	17	153	1	I	781	2308	760	0.46	
Juniperus communis L./ *sibirica* Lodd. ex. Burgsd.	J	Cupressaceae	S	14	126	10	SP	1597	1504	1156	0.60	
Larix decidua Mill.	LD	Pineaceae	T	18	162	10	I	870	1883	747	0.62	
Leucanthemum vulgare Lam.*	LV	Asteraceae	H	12	108	1	SP	1385	1278	920	0.53	
Pinus sylvestris L./ *uncinata* Ramond ex. DC	P	Pineaceae	T	9	81	10	SP	1329	1221	720	0.59	
Polygonum viviparum L.*	PV	Polygonaceae	H	12	108	1	SP	770	2247	525	0.57	
Rhododendron ferrugineum L.	RF	Ericaceae	S	7	63	10	I	737	1981	626	0.55	
Salix herbacea L	SH	Salicaceae	S	7	63	10	SP	492	2471	329	0.45	
Sesleria caerulea (L.) Ard.	SC	Poaceae	G	11	99	1	SP	1456	1748	916	0.61	
Silene nutans L.*	SN	Caryophyllaceae	H	12	108	1	SP	1386	1621	895	0.58	
Trifolium alpinum L.	TA	Fabaceae	H	13	117	1	SP	585	2279	871	0.43	
Vaccinium myrtillus L.	VM	Ericaceae	S	14	126	10	I	1004	1683	719	0.60	
Total	16	11	4	199	1791	8595	1179	–	–	–	–	0 m a.s.l. 2000 4000

respond to these orthogonal gradients either linearly (in case of a strong limiting gradient) or following a bell-shaped curve (with an optimum trait expression along the gradient). These traits could then be expressed using the following equation with 'Temp' for the Temperature and 'Rad' for the Radiation:

$$\text{Trait} \approx a1 \times \text{Temp} + a2 \times \text{Temp}^2 + b1 \times \text{Rad} + b2 \times \text{Rad}^2 + ab \times \text{Temp} \times \text{Rad} + c$$

Then, following a hierarchical stratified sampling design based on a surface response methodology (Box & Draper 1959), at least nine samples were required to parameterize the model and account for environmental heterogeneity. To select populations for each species throughout the whole valley, we applied the environmental stratification presented above to the known occurrences of this species in the valley (data set from the French National Alpine Botanical Conservatory).

To determine the spatial structure of the intraspecific FV, we further stratified the sampling design. For each species, we selected three subpopulations within each population (50 cm× 50 cm or 1 m× 1 m for herbaceous species, and 10 m× 10 m for shrubs and trees), and in each subpopulation three random individuals (tussocks or ramets) were measured (Table 1). Subpopulations were scaled to the individual size in order to make them (i) contain at least three distinct individuals and (ii) be sufficiently homogeneous, so that differences between subpopulations integrate environmental micro-heterogeneity. For leaf trait measurement, we collected one leaf per individual for herbaceous species and 10 for shrubs and trees (see Table 1); trait values were averaged at the individual level for the analyses. Only sexually mature plants and unshaded individuals were sampled (except in woodlands). We collected non-senescent, non-grazed, non-frozen, well-developed and sun-exposed leaves. We tracked the growing season according to altitude, aspect and field observations to sample all individuals of a species at a similar phenological stage.

TRAIT SELECTION AND MEASUREMENT

We measured three functional traits to characterize the ecological strategies of the studied species. (i) Maximum vegetative height ($H_{\max}$, not for trees), i.e. the distance between the top of the photosynthetic tissue and the ground, is associated with plant competition vigour and tends to be allometrically correlated with above-ground biomass (Cornelissen *et al.* 2003). (ii) Leaf dry matter content (LDMC), the dry mass of a leaf divided by its water-saturated fresh mass (Cornelissen *et al.* 2003) expressed in mg g^{-1}, reflects plant growth rate and carbon assimilation. Leaf dry matter content is considered to be a robust trait (Roche, Diaz-Burlinson & Gachet 2004) and is usually negatively correlated with relative growth rate (Weiher *et al.* 1999). Leaf dry matter content was measured with the partial-rehydration method, which has been demonstrated to not produce significant biases as compared to the more intensive full-rehydration method (Vaieretti *et al.* 2007). (iii) Leaf nitrogen content (LNC), the total nitrogen per unit of dry leaf mass (in mg g^{-1}), is closely linked to the mass-based maximum photosynthetic rate (Cornelissen *et al.* 2003). Dried and marble-ground leaf samples of 3–5 mg were analysed with FlashEA 1112 elemental analyzer (Thermo Fisher Scientific Inc., Milan, Italy) at the individual or at the subpopulation-level for species with small leaves (Table 1).

To validate our data and compare trait values with existing literature, we extracted $H_{\max}$, LDMC and LNC from several available data bases (Fig. 1: own data and see Table S1 in Appendix S1).

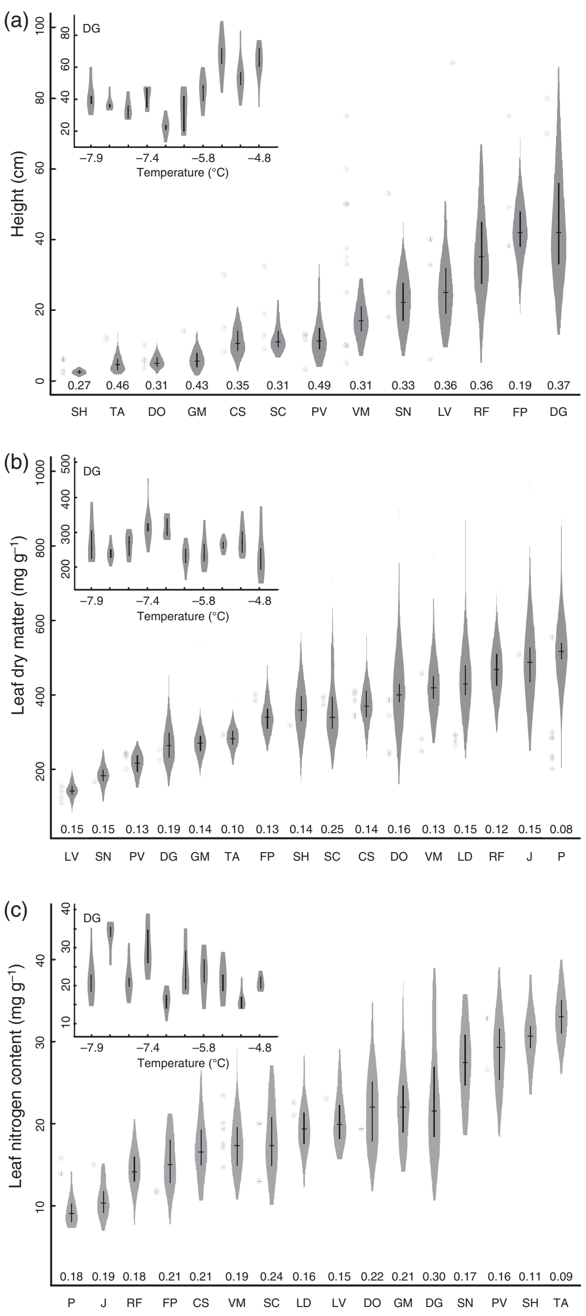


Fig. 1. Extent of the intraspecific functional variability. A violin plot is a combination of a boxplot and a kernel density plot (a non-parametric way of estimating the probability density function of a random variable). It presents (i) the density of the data estimated by kernel method (in grey) (ii) the median value (black dash) (iii) the inter-quartile range: between the first and the third ones (black segment). These figures give the extent of the intraspecific functional variability for (a) the maximum vegetative height ($H_{\max}$), (b) the leaf dry matter content (LDMC) and (c) the leaf nitrogen content (LNC), at the level of the subpopulation or the individual plant. For each trait, results are presented by species ordered by their mean values (species codes come from the Table 1) and coefficients of variation are given for each species under the violin (e.g.: 0.15 for the LDMC of *Juniperus* sp.). The grey asterisks represent data from the literature and from data bases (see Appendix S1) for comparison with our results. The inset within each graph presents data for *Dactylis glomerata* (DG); trait values are given per population sorted by increasing temperature (minimal temperature in winter).

STATISTICAL ANALYSIS

We used linear mixed models to represent the hierarchical sampling design (Pinheiro & Bates 2000) and decompose the trait variation of each species. We started with intercept random models (m_0) with only random effects (population, subpopulation nested into population) to decompose the variability of trait values ($H_{\max}$, LNC and LDMC) at the different levels of sampling. We then built more complex mixed models with linear and quadratic fixed effects of the gradients (m_1). We checked the normality of the predicted random effects and residuals, as well as for stability of the estimates obtained from different models. To determine what part of the variability at the population-level was due to the fixed effects, we calculated a measure of explained variation based on the variances at the population-level (σ^2) for the different models (Xu 2003):

$$R^2 = 1 - \frac{(\sigma_{m1})^2}{(\sigma_{m0})^2},$$

where σ_{m0} (resp. σ_{m1}) is the estimated error standard deviation at population-level estimated under model m_0 (resp. m_1). Variances were estimated by maximizing the restricted log-likelihood (REML).

We further tested if some emerging patterns in intraspecific FV could be related to species' characteristics such as growth form ('Life Form'), niche breadth ('Niche1′), optimal altitude ('Niche2′), sampling range ('Sampling') or specialisation ('Spe') (Table 1). We tested the effects of these characteristics on response variables (Y) with linear models built as follows: $Y\sim$ 'Characteristics'. To test the existence between species of general patterns in: (i) trait variability, we used for Y the coefficient of variation (CV) of the trait for each species (Lande 1977); (ii) decomposition of trait variance between the different sampling levels, we used for Y the percentage of variance at the population-level; (iii) the percentage of trait variation explained by gradients, we used for Y the maximal value of R^2 obtained for the species.

Statistical analyses were carried out with R 2.7.0 (R Development Core Team 2008) using the packages *nlme, stats, ade4* and *vioplot*.

Results

QUANTIFICATION OF INTRASPECIFIC FUNCTIONAL VARIABILITY

A comparison of the altitudinal range observed for the selected species in the French Alps and our sampling showed that the sampling strategy was rather efficient and that a large part, or at least the upper part, of the species' ranges was sampled (Table 1). We found large interspecific FV across the measured traits (Fig. 1). Species mean values ranged from 2.4 to 44 cm for $H_{\max}$ (Fig. 1a, without *Juniperus*), from 142 to 518 mg g^{-1} for LDMC (Fig. 1b) and from 9.3 to 33.1 mg g^{-1} for LNC (Fig. 1c). Each species showed large intraspecific FV. For example, the mean LDMC of *Sesleria caerulea* was 363 mg g^{-1} but it ranged between 200 and 720 mg g^{-1}. $H_{\max}$ was particularly variable with coefficients of variation (CV) for each species running from 0.19 to 0.49 while LDMC and LNC tended to be less variable (CV in 0.08–0.25 and 0.09–0.29, respectively, Fig. 1). This large variability within each species resulted from both between- and within-population-levels. The case of *Dactylis glomerata* is given as an example in the left corner of each sub-figure in Fig. 1. Study species did not have similar magnitudes of variability and no clear trend emerged across species types (Life Forms, Niche1, Niche2, Sampling, Specialization index, see Table 1). Moreover, a given species could be highly variable for one trait and not for

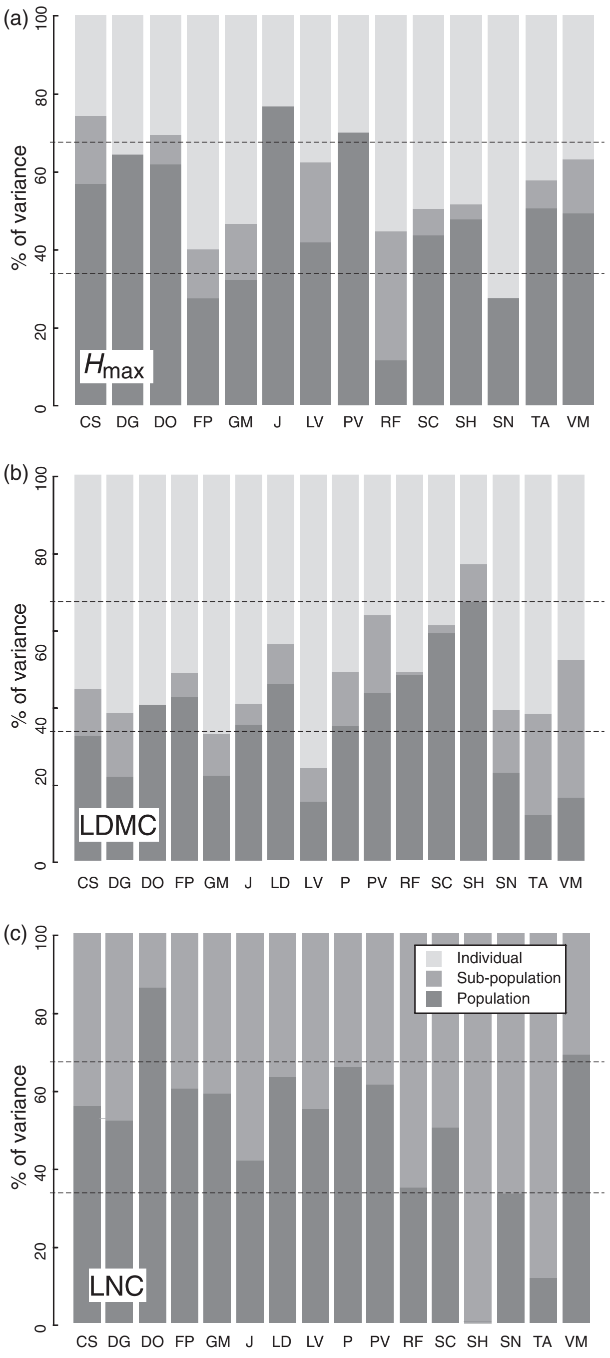


Fig. 2. Sources of variation within species. Relative variance decomposition at the individual, subpopulation and population-levels for (a) the maximal vegetative height ($H_{\max}$), (b) the leaf dry matter content (LDMC) and (c) the leaf nitrogen content (LNC, data at the subpopulation-level). Species are sorted by their names (species codes come from the Table 1) and the 33 and 66% thresholds are given in each case by a dashed line.

another (*Trifolium alpinum* had a CV of 0.46 for $H_{\max}$ and of 0.09 for LNC). Comparison of the measured values with the literature and existing data bases (Fig. 1 and Table S1 in Appendix S1) showed that they are consistent with former measurements.

ATTRIBUTION OF THE SOURCES OF VARIATION

Variance decomposition

The sources of variation varied notably across traits and species (Fig. 2). For most species, these sources varied between the three traits; for example 50% of the variance in $H_{\max}$ of *T. alpinum* was due to differences between populations, but only 12% for LNC and LDMC. On average, variability between populations represented a large part of the variance (with individual data, on average 47% for $H_{\max}$ and 35% for LDMC). There was also high individual variability, reaching as much as 60–80% (LDMC of *Geum montanum*, *Leucanthemum vulgare*, *T. alpinum*), and 53% on average for LDMC with individual data. In contrast, only a small part of the variance was due to subpopulations (with individual data, on average 10% for $H_{\max}$ and 12% for LDMC).

Analysing the structure of variability within data from literature and data bases led to results comparable to ours; however, existing data were not precise enough to achieve the same complete quantification. In particular, very few data were available for LNC and at the within-population-level (Tables S1 and S2 in Appendix S1).

Effects of environmental variables

All the calibrated models converged and most of them showed significant effects of the fixed factors. Most trait response shapes were quadratic with strong interactions between both temperature and radiation gradients. The models' results remain, however, a complex patchwork with response curves and variation explained by models differing between species and between traits (Fig. 3 & Table S3 in Appendix S1). Overall, variations in $H_{\max}$ were better explained by gradients (on average 30% of the variance at the population-level explained) than variations in LNC (on average 21%), with LDMC (26%) being intermediate. Again, there were however strong differences between traits and between species (Table S3 in Appendix S1).

Discussion

A LARGE VARIABILITY AND A COMPLEX HIERARCHICAL STRUCTURE BUT NO GENERAL PATTERN

The highly structured sampling design allowed us to answer our four key questions about the structure of intraspecific FV. (i) Trait values were highly variable within species, but this variability depended on both traits and species, suggesting that the behaviour (response to and effect on the environment) of individuals or populations of a species is not fixed and might differ depending on the conditions. (ii) A large part of the variability came from differences between populations. Moreover, the variability observed within populations was not spatially structured. In selecting random individuals in small (scaled to the individual size), homogeneous subpopulations, we assumed first, that the differences between subpopulations within a given population would integrate environmental micro-heterogeneity, and second, that individuals within a subpopulation may be genetically related, although it was beyond the scope of this study to check this assumption. We thus expected large differences between subpopulations and smaller differences within them, but we actually found the opposite with a strong individual variability within subpopulations. This indicates that the subpopulations probably did not integrate either environmental heterogeneity or genetic structure, leaving a large part of the variability unexplained. As our field measurements were not designed to tease apart local adaptation (the observed local variability would be due to the presence of several genotypes within populations) and phenotypic plasticity (the observed local variability would be due to the plasticity of given genotypes), which are both integrated by measures of phenotypic variability (Byars, Papst & Hoffmann 2007), more studies are needed to research causes for the idiosyncratic variability that we highlighted. Similar results have been obtained on other measured traits (e.g. specific leaf area, leaf carbon content, number of inflorescences, green biomass);

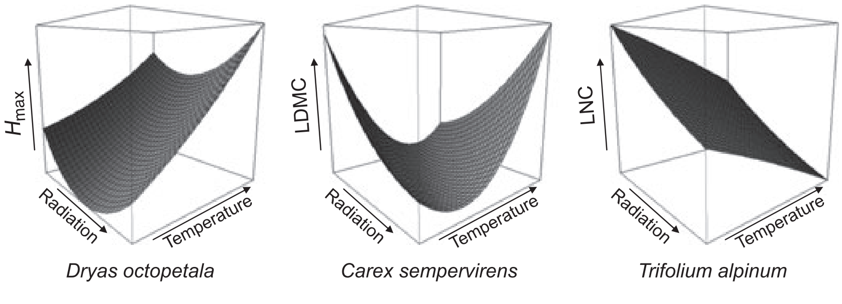


Fig. 3. Trait response shapes along gradients. These examples show the different shapes (significant relationships, see Table S3) resulting from models with the trait as the response and linear, quadratic and interaction between the gradients as the explanatory variables. *Trifolium alpinum* has been measured in the middle of its range and *Dryas octopetala* and *Carex sempervirens* have been measured at the upper end of their ranges.

however, as these traits were not available for all investigated species, we focused this paper on three important and available traits. (iii) We showed that the shapes and strengths of trait responses to environmental gradients are idiosyncratic. Depending on species and traits, climatic gradients explained more or less of the variability between populations. Moreover, including more local factors (at population-level) like soil (pH, organic matter content, texture), disturbance (grazing and mowing) or competition (vegetation cover) significantly increased the variability explained in models (the explained part of variance increased on average from 31% to 59% for $H_{\max}$, from 22% to 51% for LDMC and from 17% to 33% for LNC), but did not increase generality in patterns. (iv) Although we conducted this study in a very environmentally heterogeneous area and with contrasting species (different life forms, environmental requirements and degrees of specialization), we did not detect any pattern in terms of variability, sources of variation or response to gradients. This absence of patterns thus suggests that species are affected by the environment in idiosyncratic ways (Hultine & Marshall 2000) and corroborates the results of Gerdol (2005), who showed that two related species from the same life form (deciduous dwarf shrubs, *Vaccinium myrtillus* and *V. uliginosum*) can have very different growth performance (net primary production) and nutrient concentrations (N and P) along environmental gradients.

TRAITS AND GRADIENTS: A LONG STORY, NO HAPPY END?

Although we specifically attempted to sample trait values to identify their response shapes along environmental gradients, we did not obtain clearer patterns than previous studies. The apparent complexity and inconsistency of the curves obtained is then not due to a problem of gradient selection and comparability, or of sampling methodology. Nevertheless, traits are expected to respond to environmental gradients (Cornelissen *et al.* 2003) and patterns have been found at the interspecific level at broad spatial scales along resource, disturbance or environmental gradients (Reich *et al.* 1999; Wright *et al.* 2004).

We propose a conceptual model (Figs 4 and 5) to disentangle the apparent complexity of our results and of data sourced from the literature and data bases. We assume that within species, traits could respond in a generic way, e.g. following a bell-shaped curve along an environmental gradient, but with parameters differing between species, e.g. maximum trait value at the species' environmental optimum on the gradient (Fig. 4). Then at the intraspecific level, when studying the lower end of the relevant gradient, one should find a positive relationship between the trait value and the gradient, while at the upper end a negative relationship is expected, and at an intermediate gradient position either a bell-shaped or a flat relationship is expected (Fig. 4a). Therefore, depending on the part of the gradient sampled and the environmental requirements (range and optimum) of the different studied species, the observed relationships can be very different and inconsistent along the gradient, appearing as idiosyncratic (Fig. 4b). However, when the observed curves are combined in terms of species range (in the virtual example: lower part for species 3, higher part for species 1 and intermediate part for species 2), they lead to a bell-shaped curve, i.e. a generic intraspecific response shape along the gradient (as in Fig. 4a). Whereas, when the observed curves are combined along the whole environmental gradient, they lead to an interspecific pattern that can be very different from the generic intraspecific one (as in Fig. 4b).

Following this conceptual model, we can further interpret our results. We obtained a unimodal response for $H_{\max}$ along

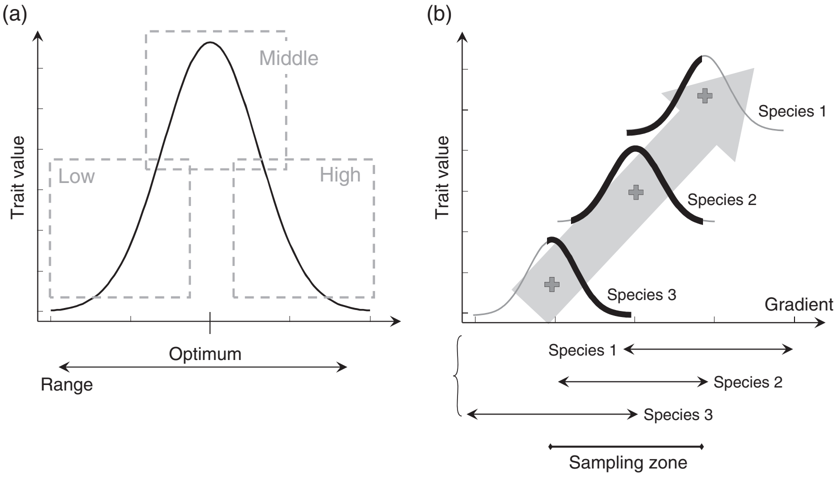


Fig. 4. Trait response along the gradients at the inter- and intraspecific levels: the conceptual model. (a) Assumed generic intraspecific trait response shape along species range: the trait is following a bell-shaped curve, reaching the maximum trait value at the species' environmental optimum on the gradient and decreasing towards the ends of the species' range. The trait can be measured in the field at the low, middle or upper part of the species range, leading to different observed relationships between trait and gradient. (b) Theoretical multi-species analysis. Three virtual species are sampled following the gradient. One species is sampled at the lower part of its range, one species at the middle and one species at the upper part: the observed relationships then appear as idiosyncratic. The interspecific pattern is very different from the generic intraspecific one.

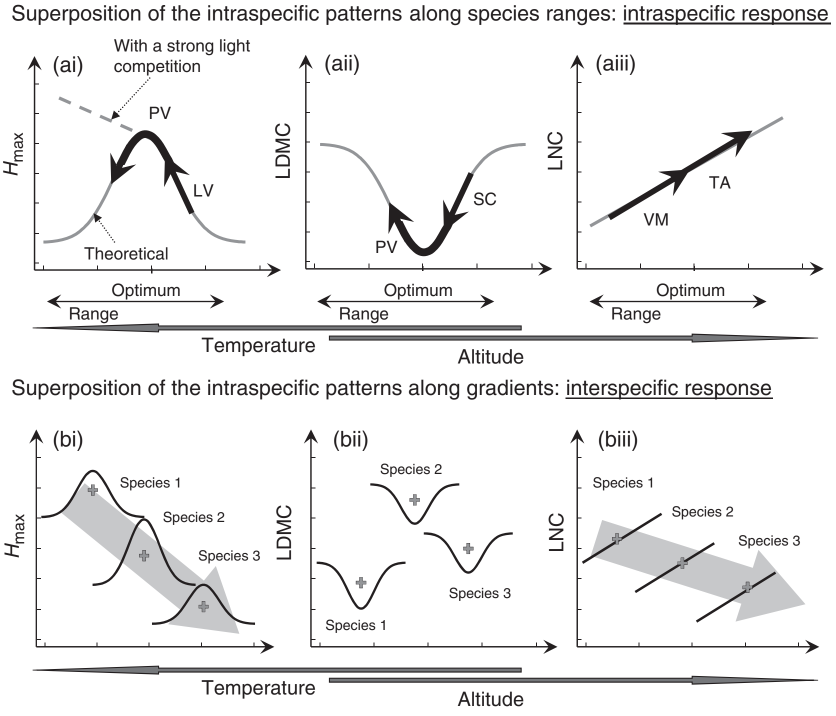


Fig. 5. Trait response along the gradients at the inter- and intraspecific levels: application of the model to the three measured traits. (a) Intraspecific variation of leaf nitrogen content (LNC), vegetative height (H_{max}) and leaf dry matter content (LDMC) (grey line) with examples for two measured species (dark arrows) along their ranges (with species range limits at low and high temperature and optimum at intermediate temperature). (b) Interspecific variation of LNC, H_{max} and LDMC (grey arrow) expressed as the overlay of intraspecific responses for three hypothetical species (dark arrows) along a gradient (decreasing temperature). Pooling intraspecific patterns along the gradient may lead to interspecific patterns that are different from intraspecific ones.

the temperature gradient, reaching a maximum for intermediate temperature values corresponding to more suitable conditions for the species (Figs 3 and 5ai). For some species (*D. glomerata*, *S. caerulea*), H_{max} might continue to increase with temperature because of strong light competition (Woodward 1986). At the interspecific level, H_{max} increased with temperature ($R^2 = 17\%$) and there was no relationship with radiation (Fig. 5bi). For LDMC, the conceptual reconstruction leads to an inverse bell-shaped response, showing lower LDMC indicating a higher growth rate for herbaceous species (Weiher *et al.* 1999) in more suitable places at the core of their distribution (Figs 3 and 5aii). We found, for example, a decreasing LDMC with increasing temperature for *S. caerulea* which was measured at the upper part of its distribution. LDMC could also increase or follow a bell-shaped response to radiation, consistent with Shipley (2003). At the interspecific level, LDMC has been shown to be either decreasing (Ryser & Aeschlimann 1999) or increasing (Roche, Diaz-Burlinson & Gachet 2004) with altitude, whereas we found no correlation between LDMC and the two studied gradients (Fig. 5bii). At the intraspecific level, LNC decreased along the temperature gradient with a clearer pattern for nitrogen content per area (mmol m^{-2}) than for nitrogen content per dry mass (mg g^{-1}, Figs 3 and 5aiii), corroborating the ecophysiological literature at the species or life form level (Woodward 1986; Körner 1989; Cordell *et al.* 1998). Nitrogen has been extensively investigated along gradients because it is a key element for a variety of metabolic functions in plants, linked to their photosynthetic capacity. At the interspecific level, LNC (mg g^{-1}) decreased ($R^2 = 10\%$) along the temperature gradient (Figs 5biii and S1 in Appendix S1), contrary to what has been found at larger scales (Craine & Lee 2003).

Thus, pooling species together masks more complex patterns that exist at the intraspecific level and can even change the direction of the relationship, as conceptualized by Shea & Chesson (2002) for the relationship between invasion success and species richness. By using species range and environmental requirements as key parameters in trait-environmental gradient relationships, our conceptual model manages to reconcile the different results from our study and the literature. As a way (i) to limit the shortcomings resulting from gradient selection and sampling range and (ii) to reconcile the general and the species-specific aspects of trait–gradient linkages, we suggest rethinking them as trait–niche relationships; with the niche defined as a synthetic index of species environmental requirements (see Fig. 4). However, methods for evaluating this niche are beyond the scope of this study (Wright *et al.* 2006; Elmendorf & Moore 2008; Thuiller *et al.* 2010).

CONSEQUENCES FOR THE FUTURE USE OF FUNCTIONAL TRAITS

This study offers a better understanding of intraspecific FV in establishing an original framework for quantifying its extent, structure and sources. Such a framework is a preliminary requisite to future analyses aiming to test whether taking this variability into account is compulsory or not and what are its implications in diverse fields of ecological research. First, we needed to advance our knowledge on the extent of intraspecific FV. Showing a large variability of functional traits within species, this study challenges the use of mean values of functional traits to describe species in single species studies, like the response of a species to environmental changes or to herbivory, or the effect of a species on community dynamics or on nutrient cycling. The behaviour of individuals and populations will likely depend on the location of the study along environmental gradients and on other unknown factors (the unexplained part of the variability). Species mean traits should thus be replaced by populations mean traits (in each environmental conditions) or by a distribution of traits for more realism. This requires, however, that descriptions of trait variances as well as means are given in the trait data bases (Weiher *et al.* 1999; Garnier *et al.* 2001). Second, concerning multi-species studies, our results suggest that further analyses are required to test the relative influence of intraspecific versus interspecific variability.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1. Intraspecific functional variability: extent, structure and sources of variation.

Table S1. Data from data bases and literature.

Table S2. Means and coefficients of variance comparison.

Figure S1. Variation of LNC along the altitudinal gradient.

Table S3. Proportion of total variability at population-level explained by climatic gradients.