A multi-trait approach reveals the structure and the relative importance of intra- vs. interspecific variability in plant traits

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Summary

1. Functional traits have been extensively used to describe, group and rank species according to their functions. There is now growing evidence that intraspecific functional variability, as well as interspecific variability, can have significant effects on community dynamics and ecosystem functioning. A core hypothesis for the use of functional traits expressed as species means, that their intraspecific variability is negligible compared with their interspecific variability, has however been too rarely tested empirically. We then addressed four questions: Is intraspecific functional variability across species ranges negligible compared with interspecific variability? Are the major resource economics trade-off and functional strategies robust to individual trait variability? Are species rankings or ordination robust across species ranges once considering intraspecific variability? Can species be discriminated by their leaf traits?
2. Using an environmentally stratified sampling design within an alpine catchment, we collected five functional traits for 13 common plant species with contrasting life histories and traits. Several populations from a range of environmental conditions were then sampled for each species across their ranges.
3. With an original combination of single-trait and multi-trait analyses, we highlighted a non-negligible contribution of intraspecific variability to overall functional trait variability ($\sim$30%). Although not affecting general and well-known functional trade-offs and strategies, intraspecific functional variability had the potential to alter species ordination and produced a functional continuum rather than a clear-cut species classification.
4. Deciding whether intraspecific functional variability can be considered as negligible – species being represented by mean trait values –, or not – species being represented by multivariate trait distributions –, is an essential question for multiple ecological issues. However, this decision cannot be generic, but will depend on the studied system and selected traits and species, as well as on study objectives.

Key-words: alpine ecosystems, environmental gradients, functional strategies, interspecific and intraspecific variability, leaf economics spectrum, linear mixed models, plant functional traits, species ranking

Introduction

The long-lasting quest for general principles simplifying the diversity and complexity of the plant world (Van Humboldt & Bonpland 1807) has led to the emergence of functional

ecology (Calow 1987). This conceptual domain suggests describing species not by their taxonomic identity alone but by their biological characteristics through the measurement of functional traits – any measurable features at the individual level affecting its fitness directly or indirectly (Lavorel *et al.* 1997; Violle *et al.* 2007). Individuals of taxonomically distant species might thus have similar trait values. The set

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of trait values (or levels) of an individual – its trait syndrome – results from functional trade-offs between different plant functions (Díaz & Cabido 1997) and from adaptive and plastic responses to its biotic and abiotic environments. According to the similarities in the trait syndromes of their individuals, species can be grouped into plant functional types (PFT) representing distinct functional strategies (Lavorel *et al.* 1997). For instance, the major leaf economics trade-off (Grime *et al.* 1997; Diaz *et al.* 2004; Wright *et al.* 2004), which can be captured by the trade-off between leaf dry matter content (LDMC) and specific leaf area (SLA), describes resource dynamics within plant leaves and discriminates between two of the main functional strategies: (i) exploitative species, that have high SLA (low LDMC) corresponding to low-density leaf tissues, high photosynthetic rates and high growth rates, have a high resource acquisition capacity and are dominant in fertile areas (Grime *et al.* 1997; Reich *et al.* 1999); (ii) conservative species, that have low SLA (high LDMC) corresponding to dense leaf tissues and low growth rates, have a high resource conservation and are dominant in low fertility areas.

Functional traits and strategies have been investigated extensively during recent decades to understand plant adaptations through the study of trait–gradient (Thuiller *et al.* 2004; Wright *et al.* 2005) or trait–trait (Reich *et al.* 1999; Fonseca *et al.* 2000; Diaz *et al.* 2004) relationships. Functional traits have also been used to understand and predict community and ecosystem functioning (e.g. net primary productivity, biochemical cycles) through the study of community-weighted mean traits or functional diversity indices (Garnier *et al.* 2004; Diaz *et al.* 2007), and also to understand patterns of diversity and species co-existence along environmental gradients (Ackerly & Cornwell 2007; De Bello *et al.* 2009). Finally, plant functional traits are the basis of modelling tools to predict vegetation dynamics and biochemical cycles through the parameterization of PFT in dynamic global vegetation models (Sitch *et al.* 2003), landscape models (Albert *et al.* 2008) and plant individuals in trait-based models (Norberg *et al.* 2001; Savage, Webb & Norberg 2007).

In most of the previous studies, species have been described by mean functional trait values, functional traits being assumed to be robust, i.e. to have intraspecific variability negligible compared with the interspecific variability. This 'robustness assumption' leads to species classifications along leading axes of variation based on unique trait values for each species, implying consistent and meaningful species rankings (Garnier *et al.* 2001; Roche, Diaz-Burlinson & Gachet 2004; Al Haj Khaled *et al.* 2005). However, to the best of our knowledge, this 'robustness assumption' has been too rarely tested empirically (but see Reich *et al.* 1999; Wilson, Thompson & Hodgson 1999; Garnier *et al.* 2001; Hulshof & Swenson 2010), while there is growing evidence that intraspecific functional variability, as well as genetic diversity, can have significant effects on community dynamics and ecosystem functioning (Booth & Grime 2003; Boege & Dirzo 2004; Crutsinger *et al.* 2006; Lecerf & Chauvet 2008). Such intraspecific functional variability could also influence community assembly (Ackerly *et al.* 2007), and stability (Fridley, Grime & Bilton 2007) and is essential to the fundamental processes of natural selection and speciation (Ridley 2003). As such it needs to be considered for trait-based research in community ecology (McGill *et al.* 2006; Ackerly *et al.* 2007).

The relative importance of functional intraspecific and interspecific variability remains poorly known and we consequently addressed the four following questions: (i) Is intraspecific functional variability across species ranges negligible compared with interspecific variability (robustness assumption)? Answering this question then justifies or not the use of functional mean trait values to adequately represent all populations and individuals of a species in multi-species studies; (ii) Are the major resource economics trade-off and functional strategies robust to individual trait variability? (iii) Are species rankings (single traits) or ordination (multi-traits) robust across species ranges when considering intraspecific variability? (iv) Given objectives of trait-based community ecology, are species well discriminated by their leaf traits? To answer these questions, we proposed an original approach through the analysis of an extensive data set of five functional traits collected on 13 species of an alpine catchment. Alpine systems are characterized by steep climatic gradients over small spatial scales that constrain species functional make-up (Körner 1999). We selected species from different life-forms (graminoid, forb, legume and shrub) to best represent the diversity of subalpine grasslands and alpine meadows, and species that were sufficiently common and widely distributed to be found in contrasting conditions. The resulting trait data set, collected with a specifically designed stratified and hierarchical strategy, includes for each species a large sample of individuals (between 63 and 153), and populations from contrasting environmental conditions and across species ranges. Trait values from this data set were largely variable within species with large discrepancies between traits and species (Albert *et al.* 2010). Between populations, the overall variability was partly explained by environmental gradients, and within populations, it did not appear to be structured (Albert *et al.* 2010). Moreover, we addressed our research questions by focusing not only on intraspecific vs. interspecific trait variability for single traits, as done previously, but by analysing with innovative multivariate techniques the robustness of trait syndromes and species classifications to intraspecific variability for multiple traits.

Materials and methods

FIELD SITES

This study was conducted in the upper catchment of the Guisane River, which is located in the central French Alps (44°5′ to 45°4′ N; 6°21′ to 6°40′ E, 1200–2600 m a.s.l.). This valley is characterized by mean annual temperatures ranging from 0 to 6·3 °C and annual precipitations from 600 to 1200 mm, mainly on calcareous and schist substrates. It encompasses a heterogeneous range of representative mountain vegetation types (e.g. coniferous forests, shrub heaths, subalpine grasslands and alpine meadows).

SPECIES SELECTION

To represent a large proportion of the vegetation diversity of the site we selected 13 common and perennial species of different life-forms (shrub, graminoid, legume and forb). These species are widely distributed and are encountered over contrasting conditions along the valley. They were chosen to cover a set of 11 plant families in order to limit phylogenetic effects in our analyses and to maximize interspecific variability. Herbaceous species were represented by four graminoids (FP: *Festuca paniculata* Schinz & Thell.; DG: *Dactylis glomerata* L.; SC: *Sesleria caerulea* (L.) Ard.; CS: *Carex sempervirens* Vill.) covering the conservative to exploitative spectrum (Gross *et al.* 2007; Quetier *et al.* 2007), four forbs (PV: *Polygonum viviparum* L.; GM: *Geum montanum* L.; SN: *Silene nutans* L.; LV: *Leucanthemum vulgare* Lam.) and one legume (TA: *Trifolium alpinum* L.). Shrub species were represented by two dwarf shrubs from higher altitude (DO: *Dryas octopetala* L.; SH: *Salix herbacea* L.), one evergreen subalpine shrub (RF: *Rhododendron ferrugineum* L.) and one deciduous shrub (VM: *Vaccinium myrtillus* L.).

SAMPLING STRATEGY

The sampling strategy and its rationale are presented in detail in Albert *et al.* (2010). In order to select, for each species, contrasting populations covering the widest environmental heterogeneity in the valley and species ranges (from 7 to 17 populations per species), we first stratified the site along two orthogonal interpolated topo-climatic variables from the meteorological model AURELHY at 50×50 m (Benichou & Le Breton 1987): mean minimal temperature in winter, ranging from -12 °C to -3 °C (mean $= -7 \cdot 5$ °C) and solar radiation in August, ranging from 155 to 785 MJ m^{-2} (mean $= 627$ MJ m^{-2} and quantile$_{0 \cdot 05} = 434$ MJ m^{-2}). We then applied the environmental stratification presented above to the known occurrences of this species in the study valley (data set from the National Alpine Botanical Conservatory, http://cbn-alpin.org/). For each species the range in temperature between the sampled populations spanned between $1 \cdot 7$ and $4 \cdot 2$ °C (on average $3 \cdot 2$ °C) and the range in solar radiation between 204 and 340 MJ m^{-2} (on average 292 MJ m^{-2}).

For each species and within each population (scaled to individual size: 50×50 cm or 1×1 m for herbaceous species, and 10×10 m for shrubs) nine sexually mature and unshaded individuals (tussock or ramets) were randomly selected and measured. We collected non-senescent, non-grazed, non-frozen leaves for trait measurements. One leaf per individual was collected for herbaceous species (standard protocol, Cornelissen *et al.* 2003) and 10 per individual for shrub species, to capture the variability within individuals (means per individual were then used in the analyses). We tracked the growing season according to altitude, aspect and field observations in order to sample all individuals of a given species at a similar phenological stage. This was done to reduce as far as possible measurement error, as traits are known to vary during the growing period (Garnier *et al.* 2001). Additional sources of errors in measurements of inter-individual variation were minimized by the use of standardized protocols.

TRAIT SELECTION AND MEASUREMENTS

Five functional traits reflecting the ecological strategies of the studied species were measured. (i) Maximum vegetative height (H_{max}) is the distance between the top of photosynthetic tissue and the ground, which is associated with plant competitive vigour and tends to be allometrically correlated with above-ground biomass (Cornelissen *et al.*

2003); measurements were standardized for each species, although it has been shown that plant height estimates can be influenced by sampling procedures (Gaucherand & Lavorel 2007). (ii) SLA is the one-sided area of a fresh leaf divided by its oven-dry mass; SLA is usually well correlated with relative growth rate for herbaceous species (Cornelissen *et al.* 2003). (iii) LDMC is the oven-dried mass of a leaf divided by its water-saturated fresh mass (Cornelissen *et al.* 2003), here expressed in mg g^{-1}; it was measured using the partial rehydration method, which has been proved to give results similar to the full rehydration method (Vaieretti *et al.* 2007); LDMC is related to the average density of leaf tissues and tends to scale with 1/SLA (Cornelissen *et al.* 2003). (iv) Leaf nitrogen concentration (LNC) is the total amount of nitrogen per unit of dry leaf mass (in mg g^{-1}), which is closely linked to the mass-based maximum photosynthetic rate (Cornelissen *et al.* 2003). (v) Leaf carbon concentration (LCC) is the total amount of carbon per unit of leaf dry mass (in mg g^{-1}) and represents investment into structural tissues. For both LNC and LCC, dried and marble-ground leaves samples of 3–5 mg were analysed with a FlashEA 1112 elemental analyser (Thermo Fisher Scientific Inc., Milan, Italy) at the individual level or with three individuals pooled together for species with too small leaves (*C. sempervirens*, *L. vulgare*, *P. viviparum*, *S. herbacea*, *S. caerulea*, *S. nutans*, *T. alpinum*).

STATISTICAL ANALYSIS

The first goal of this study was to determine how trait variability was structured between and within species and to test the robustness assumption. To do this, we decomposed the trait variability according to different levels of variation in both single-trait analyses (linear mixed models) and multi-traits analyses [between principal component analyses (PCA)]. To assess whether the variance structures were robust independently of life-form, we performed these variance decompositions for the entire data set as well as for data reduced to herbaceous species.

Single-trait analyses

We used linear mixed models (Bolker *et al.* 2009), which are appropriate for representing hierarchical data structures. Models were calibrated for each functional trait using individual trait measurements and included either no fixed effects (written as fixed~1, m_0) or a species fixed effect (fixed~Species, m_1) and a random intercept population effect (random~1| Population). Estimated standard deviations at the population level (σ) were used to determine the percentage of variability attributed to each level (interspecific, intraspecific between populations and intraspecific within populations). As populations were represented as a random effect, we determined the between-population part of variance as the ratio between σ^2 and total variance. As species were represented as a fixed effect, we calculated a measure of explained variation based on the variances at population level for the different models following (Xu 2003): $R^2 = 1 - \frac{(\sigma_{m1})^2}{(\sigma_{m0})^2}$, where σ_{m0} and σ_{m1} are the estimated error standard deviations at population level estimated under models m_1 and m_0 respectively. Variances were estimated by maximizing the restricted log-likelihood (REML).

Multi-trait analyses

We used between-species PCA (Dodélec & Chessel 1991) on the five traits set measured at the individual level. Between PCA is close to a classical PCA; it uses the correlation matrix based on species' means (weighted by their sample size). Hence, between PCA finds linear

combinations of variables maximizing the between-species variance instead of the overall variance. The inertia calculated in a between PCA represents the part of the total variance due to the differences between species (Dodélec & Chessel 1991). As different numbers of populations – and then individuals – were sampled for each species, we used a re-sampling procedure to balance the data sets (equal number of individuals). First, we determined the smallest number (SN) of individuals measured within species (SN = 63). We then subsampled the original data set by drawing randomly 1000 balanced data subsets with SN individuals per species and then ran the between PCAs (BPCA$_{between}$) over these data subsets. The resulting distribution of between-species variance was tight enough to assume that the re-sampling procedure did not affect variance decomposition. Finally within each species, the variance decomposition between populations was assessed with between PCAs (BPCA$_{within}$).

The second goal of this study was to determine whether the major resource economics trade-off and functional strategies are robust to individual trait values. To do this, we successively ran PCAs on data at the individual level (PCA$_{ind}$), on data averaged by populations (PCA$_{pop}$) and on data averaged by species (PCA$_{sp}$) to observe whether the structures were maintained across these different levels. We then determined the correlations between traits at the interspecific (axes discriminating species in the trait space) and intraspecific (axes discriminating individuals within species in the trait space) levels with both between- and within-PCA analyses performed using the re-sampling procedure described above (BPCA$_{between}$ and WPCA$_{between}$). Within PCA decomposes the structure within species, by considering the data centred on species means (Dodélec & Chessel 1991). To observe trait correlation within species, we also ran for each species a PCA on individual data (PCA$_{within}$).

The third goal of this study was to assess whether species rankings and ordinations are robust across species ranges when considering intraspecific variability. To do this, we ran a PCA on our data set aggregated at the population level (PCA$_{pop}$). We extracted two subsets of populations, one corresponding to populations living in 'cold' conditions (winter temperature < −7·91 °C, 30% quantile) and another corresponding to populations living in 'warm' conditions (winter temperature > −6·86 °C, 70% quantile). We used such subsets because in our data set different species could rarely be sampled in communities where they co-occur and therefore not in exactly the same environmental conditions. We then projected both groups along the first two axes of the trait space. We also calculated Spearman rank correlation coefficients to test whether the ranking of species along both axes of the PCA$_{pop}$ was conserved between 'cold' and 'warm' conditions.

The fourth goal of this study was to assess whether species are well discriminated by their leaf traits. First, we determined whether a given individual was closer to individuals from its own species than from other species. To do so, we calculated a 'functional distance' using the Mahalanobis metric (Mahalanobis 1936) and quantifying the differences between all pairs of individuals within the trait space (four leaf traits). Small (respectively large) distances thus indicate pairs of individuals with similar (respectively contrasting) functional strategies. We represented this functional distance as a density of Mahalanobis distances between individuals from a species 'A' and individuals from a species 'B'. Secondly, we determined the probability of correctly attributing a given leaf to the correct species from its leaf traits. This was done by performing a linear discriminant analysis (LDA), which finds the linear combination of continuous explanatory variables best separating two or more classes of a categorical variable (Venables & Ripley 2003). LDA is similar to between PCA but maximizes the ratio of the between-groups variance to the total variance, and can be projected onto new observations. For each measured leaf or 'mean' leaf for

shrubs (i.e. 1374 data), we calibrated an LDA with the four leaf traits (LDMC, SLA, LNC, LCC) on a data set containing all the other measurements. The LDA was then used to predict to which species that leaf belongs. This then allowed us to calculate over the entire set of LDAs the probability P_{ij} to attribute a leaf from the species i to the species j.

All statistical analyses were computed with R 2.9.1 (R Development Core Team 2008), libraries: nlme, stats, ade4, MASS.

Results

VARIANCE DECOMPOSITION

Single- and multi-trait analyses led to similar results with a partition around 70% vs. 30% for interspecific vs. intraspecific
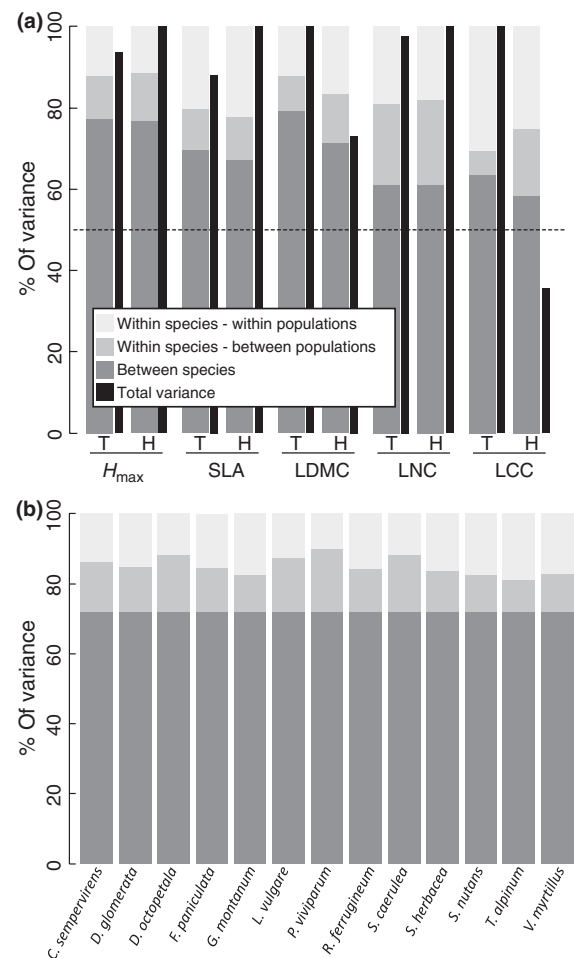

Fig. 1. Variance decomposition in interspecific and intraspecific contributions for single-trait and multi-trait patterns. Variance decomposition into the different levels: species, populations and within populations with: (a) Single-trait analyses: decompositions, resulting from mixed models, are given for each of the five measured traits, for the complete data set (T) and for data reduced to herbaceous species (H). The black bars are the observed variance given as percent of the highest variance observed per trait; and (b) Multi-traits analyses: decomposition takes into account the five traits together. A between PCA (BPCA$_{between}$, mean results after a re-sampling procedure) on the whole data set gave the relative importance of within- and between-species variances, while between PCAs on each species (BPCA$_{within}$) data gave the decomposition into between and within populations.

variability (Fig. 1a,b). There were small differences in this partition between traits, with LNC and LCC showing relatively more intraspecific variability (up to 42%, Fig. 1a). Concerning the multi-trait analyses, the variance decompositions using between PCAs (BPCA$_{between}$) led to very narrow resampling distributions of interspecific contributions (mean = 0·70, SD = 0·007), individuals from each species having equivalent contributions to the variance. Single- and multi-trait analyses (BPCA$_{within}$) also led to similar partitions of the variance within species, with around 50% of this variance due to differences between populations and 50% due to variance within populations (Fig. 1a,b). Similar results were obtained with the data restricted to herbaceous species.

MAIN AXES OF VARIATION

PCAs on data at the individual (PCA$_{ind}$), population (PCA$_{pop}$) and species levels (PCA$_{sp}$) produced the same leading axes of variation and general structure of the trait space (results of these PCAs are not shown as they are similar to the ones given by the between PCA BPCA$_{between}$). From the between PCA (BPCA$_{between}$), we showed that the data set was structured by a strong first axis (53% of the variance) mainly

explained by LDMC and SLA, and a second axis (29% of the variance) explained by H_{max} (Figs 2a and 3). This general structure was stable when the analysis was restricted to herbaceous and/or shrub species alone. Within the trait space, life-forms remained segregated as well as fast-growing exploitative species (e.g. *D. glomerata*) and slow-growing conservative species (e.g. *F. paniculata*, Fig. 3b).

Within species, multi-trait variation had a less repeatable structure (WPCA$_{between}$, Fig. 2b,c, axis 1: 31% of the variance, axis 2: 27%, axis 3: 20%). The PCAs conducted on each species separately (PCA$_{within}$) showed that the functional structures were not fully consistent across species: the first axis (from 33% to 42% of the variance) was mainly driven by SLA and LDMC for all species, but the second axis was driven by LNC and LCC and/or H_{max} and the correlations between these traits and SLA and LDMC were not consistent (Fig. 2b,c for three contrasting examples).

RANKING AND TEMPERATURE GRADIENT

Only nine species were present in both cold and warm conditions. The way population mean trait syndromes were spread around the species mean trait syndromes were only partly
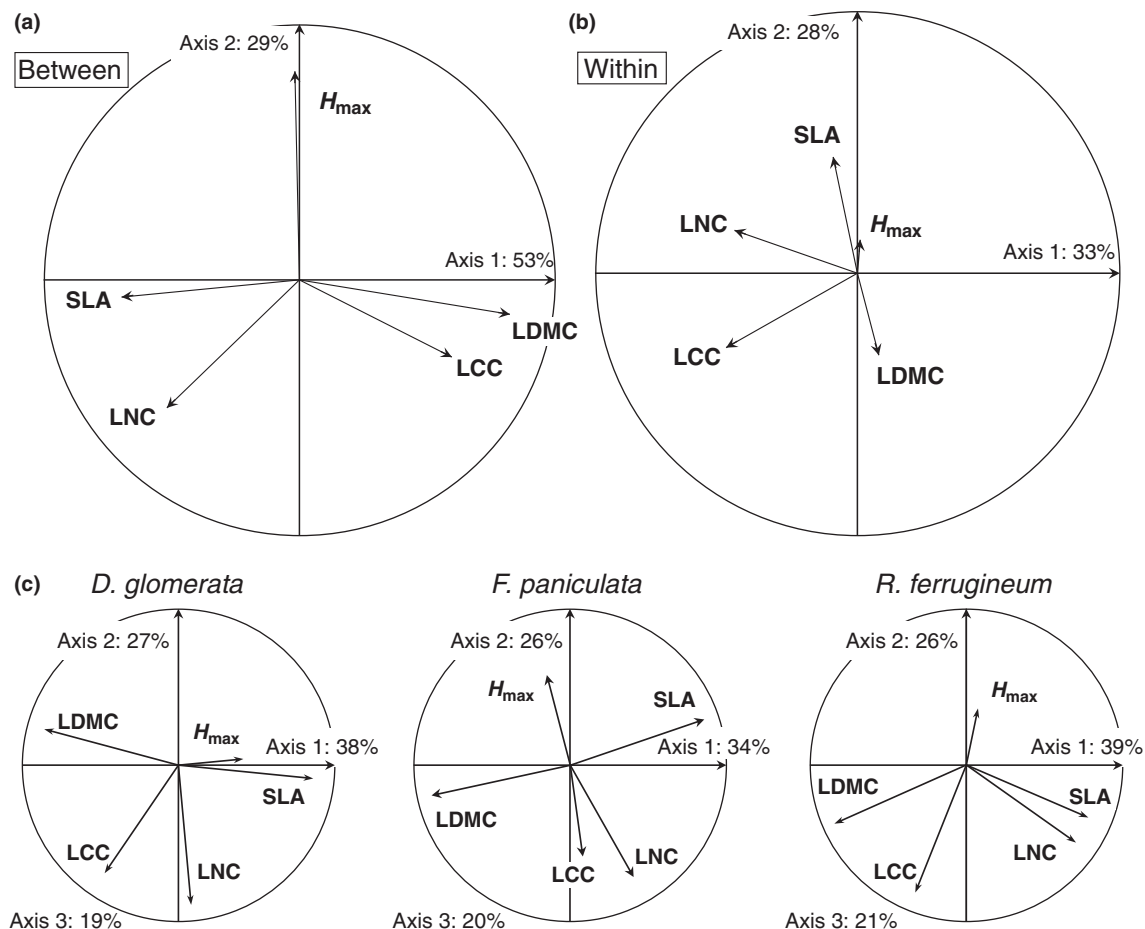

Fig. 2. Multidimensional structure within the trait space: intraspecific and interspecific trade-offs. Between and within principal component analysis using the fives measured traits (mean results after a re-sampling procedure). The correlation circles and the two-first PCA axes at (a) the interspecific level (BPCA$_{between}$); (b) at the intraspecific level (WPCA$_{between}$) with all data; and (c) at the intraspecific level within three example species.
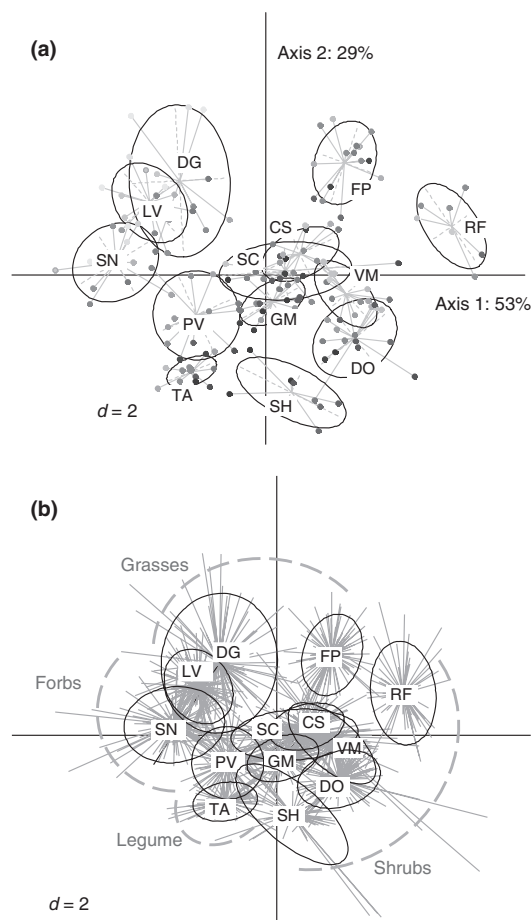
Fig. 3. Dispersion of species, populations and individuals in the trait space. Between PCA within the trait space and projection of the different levels of complexity (BPCA$_{between}$): (a) trait syndromes averaged at the species level (stars centres) are spread around the general mean and trait syndromes averaged at the population level (grey dots) are spread around the trait syndromes averaged at the species level, (b) individual trait syndromes (grey segments) are spread around the trait syndromes averaged at the population level. (a) Trait syndromes averaged at the population level are represented by dots, whose colour depends on the winter temperature. The darker dots represent the 'coldest' populations and the lighter dots the 'warmest' populations. (a, b) The ellipsoids of inertia are encompassing 65% of the individuals of each species. (b) The grey half circles define the different life-forms and their place in the trait space. (a, b) Species are named as follows: CS, *Carex sempervirens*; DG, *Dactylis glomerata*; DO, *Dryas octopetala*; FP, *Festuca paniculata*; GM, *Geum montanum*; LV, *Leucanthemum vulgare*; PV, *Polygonum viviparum*; RF, *Rhododendron ferrugineum*; SC, *Sesleria caerulea*; SH, *Salix herbacea*; SN, *Silene nutans*; TA, *Trifolium alpinum*; VM, *Vaccinium myrtillus*.

explained by the environment. Along the first axis of the PCA$_{pop}$, representing 48% of the variance, the mean coordinate of each species varied between cold and warm environmental conditions, as did their ranking (Fig. 4). The same phenomenon occurred on the second axis (29% of the variance). Ordination of species in the five-trait space was thus not consistent between cold and warm conditions (Fig. 4). Surprisingly, in spite of these observed changes, Spearman coefficients on species coordinates along each axis were very high (0·92) and largely significant ($P = 5 \times 10^{-4}$).

Functional distance

Distributions of distances between individuals showed that individuals from the same species were sometimes further (i.e. leaves were less similar) than two individuals from different species. For instance some individuals of *R. ferrugineum* had leaves more similar to leaves of *D. octopetala* or *F. paniculata* than to leaves of other *R. ferrugineum* individuals (Fig. 5a). Figure 5 presents two contrasting examples of distributions: (i) either the species was functionally very different from all other species (e.g. *R. ferrugineum*) and only few marginal individuals were similar to marginal individuals from other species (Fig. 5a); (ii) or a major number of individuals were functionally very close to those of another species (e.g. *C. sempervirens* close to *S. caerulea*, Fig. 5b).

Attribution probability

The probabilities of attributing leaves to the correct species from its trait values were high overall (mean = 74%; min = 36%, Fig. 6) in spite of some unexpected misclassifications. For instance *T. alpinum* which was the only legume, characterized by high and slightly variable LNC, was well predicted (96%), while the conservative grass *S. caerulea* for which LDMC and LNC were strongly variable was predicted accurately at only 36% and was sometimes predicted as a completely different life-form (10% attributed to *D. octopetala*, a deciduous dwarf shrub).

Discussion

RELATIVE IMPORTANCE OF INTERSPECIFIC VS. INTRASPECIFIC ON THE OVERALL TRAIT VARIABILITY

Our results on variance decomposition warrant discussion of the robustness assumption (Wilson, Thompson & Hodgson 1999; Garnier *et al.* 2001; Roche, Diaz-Burlinson & Gachet 2004; Al Haj Khaled *et al.* 2005). Intraspecific variability in our data set remained generally lower than interspecific variability but it was certainly not negligible, as both single- and multi-trait analyses on herbaceous and all species identified a relatively strong component of intraspecific variability (around 30% of the total variability). These results on alpine ecosystems corroborate observations on dry tropical forest of intraspecific variation of the same order of magnitude for leaf traits including SLA and leaf water content, the complement to LDMC (Hulshof & Swenson 2010). In contrast, Roche, Diaz-Burlinson & Gachet (2004) observed 36·5% of intraspecific variability for SLA but only 9% for LDMC in Mediterranean vegetation. For this study we specifically focused on selecting contrasted species within the alpine flora in order to maximize interspecific variability; however, the relative importance of interspecific and intraspecific variability obviously strongly depends on the species and traits considered. Depending on the subset of species considered (among 769
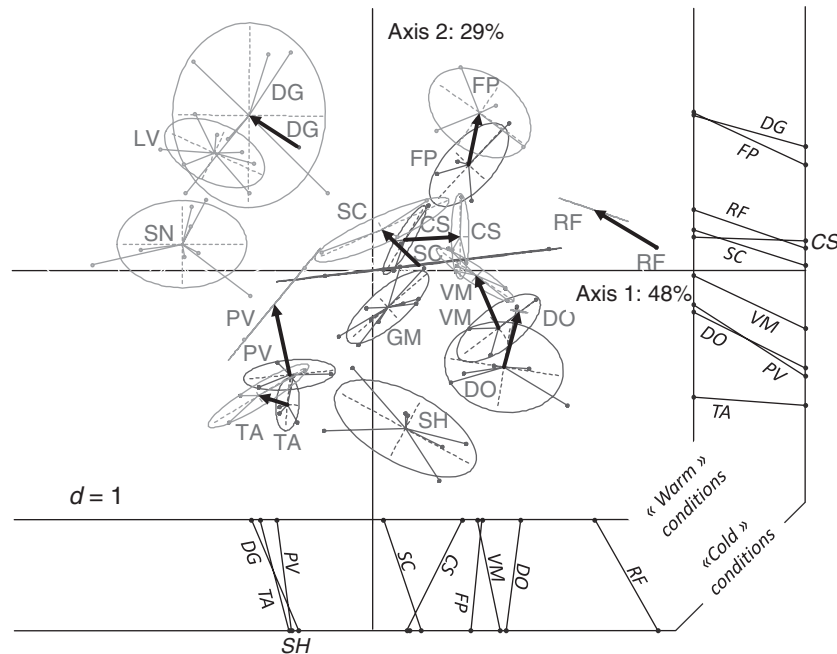
Fig. 4. Ranking and ordination changes between cold and warm conditions. The central panel is the first plane of a principal component analysis on the five measured traits averaged at the population level (PCA$_{pop}$), with a projection of two subsets of the data corresponding to cold (winter temperature < -7.91 °C, dark grey) and warm (winter temperature > -6.86 °C, light grey) conditions. Only nine species were present in both conditions. Black arrows show the relative change in the species mean syndrome position. The lateral panels represent the projections of the species mean values on the first (bottom) and second (right) axes for warm conditions and cold conditions. The black segments between the two sets of conditions illustrate the changes and inversions in species rankings. Species are named as follows: CS, *Carex sempervirens*; DG, *Dactylis glomerata*; DO, *Dryas octopetala*; FP, *Festuca paniculata*; GM, *Geum montanum*; LV, *Leucanthemum vulgare*; PV, *Polygonum viviparum*; RF, *Rhododendron ferrugineum*; SC, *Sesleria caerulea*; SH, *Salix herbacea*; SN, *Silene nutans*; TA, *Trifolium alpinum*; VM, *Vaccinium myrtillus*.

observed species, but few replicates per species), Wilson, Thompson & Hodgson (1999) found 8% (all plants), 32% (angiosperms) of intraspecific variability for SLA and 6% (all plants), 14% (angiosperms) for LDMC. Thus, deciding whether intraspecific variability can be or not be considered as negligible will depend on the studied system and on the selected traits and species. For instance, when partitioning variance with a random selection of species from a regional pool, the importance of intraspecific variability will depend on the strength of the environmental gradients. Due to habitat filtering (Keddy 1992), one might expect differences between species to be reduced in a homogeneous (respectively heterogeneous) environment, leading to a strong (respectively weak) relative importance of intraspecific variability. This hypothesis cannot be tested with our data, as species were not selected randomly but rather to favour species with broad distributions along the environmental gradients.

INTERSPECIFIC FUNCTIONAL STRATEGIES ARE ROBUST TO INTRASPECIFIC VARIABILITY…

Our results show that the well-known leaf economics trade-off, and thus the definition of two of the main ecological strategies (acquisitive vs. exploitative species, Diaz *et al.* 2004; Grime *et al.* 1997) are robust to intraspecific variability. We also identified a second main axis of variation determined by plant height, consistent with previous results (Diaz *et al.*

2004; Gross *et al.* 2007). Interestingly, this pattern was repeatable when restricting our data on each of the different life-forms and for a twin data set with similar sampling at another site in the Swiss Alps (results not shown). Trait trade-offs within species, which to the best of our knowledge had not been studied so far, were broadly similar but less pronounced than between species.

…, BUT ORDINATION AND THUS RANKING MIGHT NOT BE

Ranking (single trait) and ordination (multi-trait) of species are usually established using trait values or syndromes available for each species either from a limited number of points or using mean values across more numerous measurements. Thanks to our stratified sampling strategy, several contrasting populations were sampled throughout each species' range (Albert *et al.* 2010). We thus showed that the variability of mean trait values between populations in response to climate and soil conditions led for some species to changes in ranking (or ordination) along gradients and across species ranges. This is questioning the generality of former results on the stability of rankings for single traits (Garnier *et al.* 2001; Roche, Diaz-Burlinson & Gachet 2004), as we showed that instabilities may not be detected by Spearman coefficients. However, the ranking changes we observed did not affect large contrasts. In the two-dimension trait space, species ordination changes only
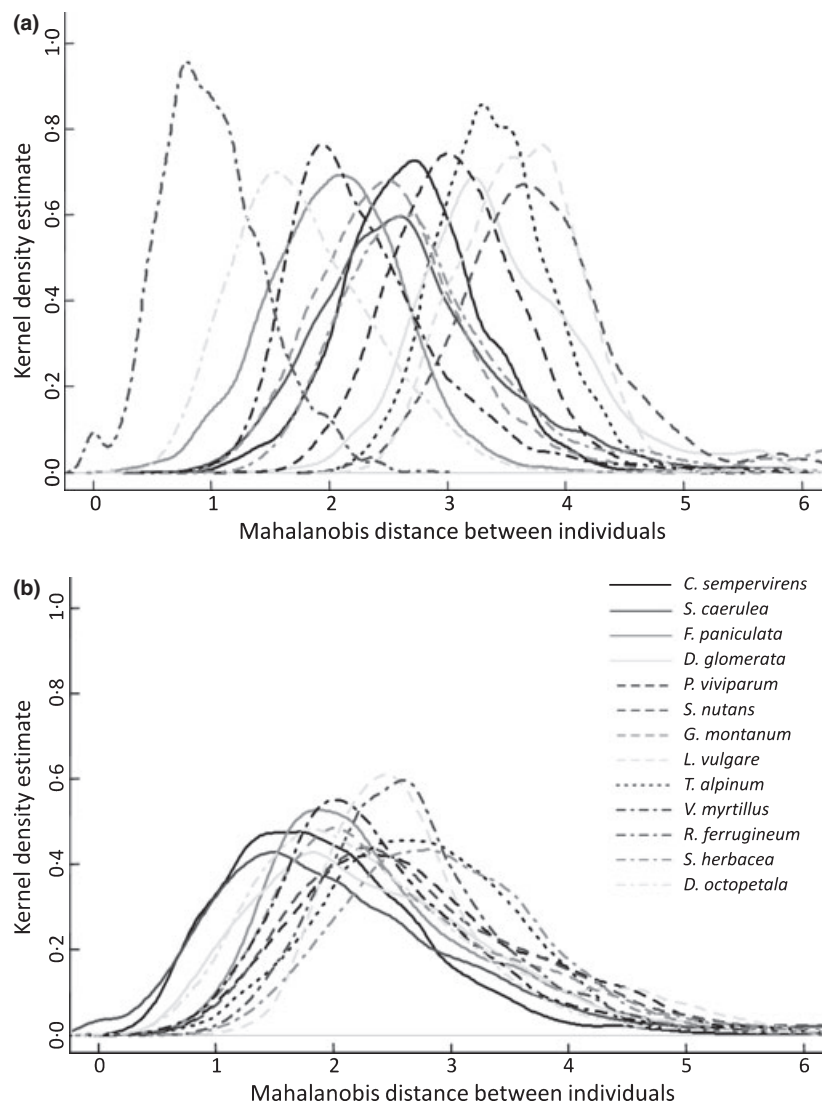
(a)

(b)

Legend:
— C. sempervirens
— S. caerulea
— F. paniculata
— D. glomerata
- - P. viviparum
- - S. nutans
- - G. montanum
- - L. vulgare
···· T. alpinum
-·- V. myrtillus
-·- R. ferrugineum
-·- S. herbacea
-·- D. octopetala

Fig. 5. Functional distances between individuals. Functional distance (calculated on the four measured leaf traits), represented as densities of Mahalanobis distances between individuals from a species 'A' and individuals from other species, for (a) A = *Rhododendron ferrugineum* and in (b) A = *Sesleria caerulea*. Densities are estimated with a kernel density function, a nonparametric way of estimating the probability density function of a random variable.

occurred between species from the same life-form (e.g. small graminoids or small deciduous shrubs). In contrast, species ordination changes along each axis separately occurred between species that were not necessarily expected to be functionally close (e.g. a graminoid and a deciduous shrub), supporting the idea that several independent functional axes of variation are necessary to describe functional strategies (Westoby *et al.* 2002). Given our rough temperature classes (cold vs. warm), the observed patterns are not a generic quantification of how rankings vary along environmental gradients. A more comprehensive quantification would require data specifically collected on co-existing species. This would probably also require a quantification of species environmental requirements, as functional traits might be constrained more by these requirements than directly by gradients (Thuiller *et al.* 2010).

FUNCTIONAL CONTINUUM VS. SPECIES CLASSIFICATION

The intraspecific variability we observed led to strong overlaps between species trait distributions in the trait space

resulting in continuums of trait values across individuals rather than in discrete distributions driven by interspecific differences. Could such a continuum challenge the possible discrimination of a species by its functional traits? On a data set composed of few contrasted species, our results showed a relatively strong discrimination, individual leaves being well identified overall. Yet, we observed some erroneous attributions between species that were expected to be rather different (e.g. between one graminoid and one shrub species). Such poor attribution was mainly due to large intraspecific functional variability producing functional distances that could be larger between individuals from the same species than between individuals from different species.

IMPLICATIONS FOR FUNCTIONAL ECOLOGICAL RESEARCH

How should we represent the functional characteristic of species: mean trait values *or* trait value distributions? Progress on this question is essential for multiple ecological issues such as quantifying functional diversity (Mouillot *et al.* 2005; Leps
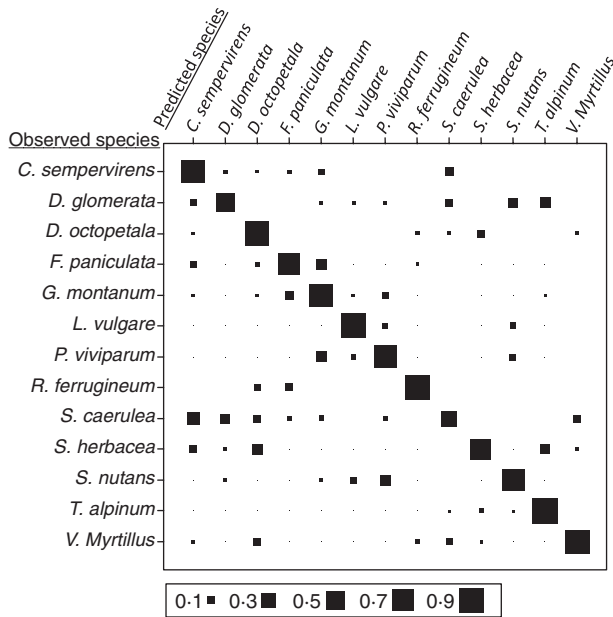
Fig. 6. Probabilities of attributing a leaf of species A to species B from its traits. Probabilities of correctly predicting the origin of a leaf from its traits are given for the different observed and predicted species. All squares that are outside the diagonal are errors of prediction.

et al. 2006), parameterizing species within vegetation models (Norberg *et al.* 2001; Kleidon, Fraedrich & Low 2007) or improving the understanding of the links between the dynamics of individual plants, communities and ecosystem functioning (Ackerly *et al.* 2007; Diaz *et al.* 2007; Suding *et al.* 2008). On this basis we believe that deciding whether intraspecific variability can or cannot be considered as negligible will depend not only on the studied system and on the selected traits and species but also on the study objectives. While studying ecosystem functioning will probably tolerate representing species by their mean trait values, because only the overall trait distribution is required and mean community properties show low sensitivity to intraspecific variation (Lavorel *et al.* 2008); studying community assembly patterns or evolutionary processes will probably require prior knowledge of the trait distribution for each of the coexisting species. Then, further quantifications such as this study and also further tests on the effects of intraspecific functional variability for key ecological questions are required to be able to decide when this intraspecific variability can be neglected.

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