



Ecole de Biologie

**SPATIAL VARIATION IN FUNCTIONAL TRAITS ALONG
ENVIRONMENTAL GRADIENTS: A CASE STUDY WITH ALPINE
PLANTS.**

Master of sciences in Evolutionary and Conservation Biology (BEC)

by

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February 2008

Abstract

Functional traits are measurable plant features useful to understand and describe relationships between plant and their environment. As a result, a series of functional traits measured on a species can theoretically give indications on its ecology, life strategy and capability to cope with a particular environment. By extension, we assume that they should also be useful to predict the performance of a species in a changing environment, for example along a gradient of environmental conditions or following climatic changes. Yet, still very few studies have investigated how plant functional traits vary intra-specifically along environmental gradients.

We studied the variation of five functional traits: leaf dry matter content (LDMC), specific leaf area (SLA), maximal height, biomass and C:N ratio in leaves, along two direct gradients (temperature and solar radiation) in two study sites, in the French and the Swiss Alps. We then assessed the variation in trait values along a gradient of habitat suitability predicted by niche-based models calibrated for each species. Here, we present results for five species from the Swiss study site: *Carex sempervirens*, *Dactylis glomerata*, *Dryas octopetala*, *Sesleria sempervirens* and *Vaccinium myrtillus*.

We observed that intraspecific variation in functional trait along gradients was species specific, with plant height and biomass being the two most responsive traits and temperature being the most influential gradient. As each species seems to show a specific pattern of traits behavior, traits variation measured on one species along one environmental gradient cannot necessarily be extrapolated to another species along the same gradient. In addition, the sparse responses of functional traits to modeled probability of occurrence seem to indicate that predictions by niche-based models may not allow prediction of plant fitness or performance.

Résumé

Les traits fonctionnels sont des caractéristiques mesurables qui peuvent être employées pour comprendre et décrire les relations entre une plante et son environnement. Une série de traits mesurés sur une espèce peut, théoriquement, donner des indications sur sa stratégie de vie dans un certain environnement. Ces traits sont utilisés pour prédire les performances d'une espèce, par exemple le long d'un gradient de conditions environnementales ou au cours un changement climatique. Actuellement, peu d'études se sont penchées sur la façon dont ces traits varient à l'intérieur d'une espèce le long d'un gradient environnemental.

Nous avons étudié la variation de cinq traits fonctionnels : la teneur en masse sèche des feuilles, leur surface spécifique, le rapport entre leur contenance en carbone et azote, la hauteur maximale de la plante et sa biomasse ; le long de deux gradients directs, à savoir la température du site d'échantillonnage et la quantité de radiations solaires potentielles reçues par ce même site. L'échantillonnage a été conduit parallèlement dans deux zones d'étude, dans les Alpes suisses et françaises. La variation des valeurs de traits a ensuite également été mise en rapport avec un gradient d'adéquation de l'habitat prédit par un modèle de niche calibré pour chaque espèce. Dans ce travail, nous présentons les résultats pour les cinq espèces de la zone d'étude suisse : *Carex sempervirens*, *Dactylis glomerata*, *Dryas octopetala*, *Sesleria caerulea* and *Vaccinium myrtillus*.

On observe que la variation des traits fonctionnels est spécifique à chaque espèce. Les traits répondant le mieux sont la hauteur et la biomasse, le gradient qui a le plus d'influence est la température. On en conclut que, comme chaque espèce semble avoir un patron de réponse particulier, la variation d'un trait mesuré sur une espèce le long d'un gradient ne peut pas être extrapolée à une autre espèce même le long du même gradient. De plus, même si on avait pu penser que les traits répondraient mieux à un optimum de conditions environnementales décrit par le modèle de niche, la réponse éparse des traits aux probabilités d'occurrence semble montrer que les prédictions des modèles de niche ne permettent pas de prédire une quelconque valeur de performance pour une espèce dans un certain environnement.

Introduction

Destruction and fragmentation of habitats (Pitelka et al. 1997), pollution (Emberson et al. 2001) or biological invasions (Walther et al. 2002) are drivers of global change that have a direct and fast impact on the distribution of species and biodiversity. In contrast, climate change is one component of global change that will have a long term impact on ecosystems and the geographic distribution of species, particularly for sessile organisms like plants. Therefore, understanding how species will respond to projected future climate change is of fundamental importance for effective management and conservation of biodiversity (Hannah et al., 2002).

A large number of mathematical techniques are available for modeling species' distribution and predict probable climate change impacts. Models can be empirical, like niche-based models (also called species distribution models, habitat suitability models or envelope models; Guisan & Thuiller 2005). These models statistically relate geo-referenced presences and absences (or abundances) of species, to predictors (usually topo-climatic variables). They also give information on the habitat suitability of a site for a particular species or a group of species (Guisan & Zimmermann 2000, Ferrier & Guisan 2006). This kind of technique is broadly used in varied studies (Guisan & Thuiller 2005), not only to study plant distribution in changing environment but also to help making decisions in applied environmental management of rare or invasive species, for example (Engler et al. 2004, Broennimann et al. 2007).

Models can also be dynamic, such as cellular automaton (Carey 1996, Dullinger et al. 2004) or GAP models (Bugmann 2001). The latter are individual based mechanistic models, simulating forest evolution through establishment, growth and mortality of each individual tree. Dynamic global vegetation models (Sitch 2003) combine vegetation dynamic and other ecological processes, such as global carbon balance and biogeochemical cycles.

Landscape model are another type of dynamic models. They simulate the changes in the landscape structure according to changes in the vegetation under influence of disturbances like fire or grazing (Cousins et al. 2003). The vegetation is modeled as plant functional types (PFT), which are groups of plants with similar response to environment and / or common effects on ecosystem processes (Lavorel & Garnier 2002). A strategy between others to form PFT is to group the plants showing similar roles in ecosystem (Lavorel 1997). These roles are evaluated for example, through the values of a set of functional traits.

According to Violle (2007), a trait is any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole organism level, without reference to the environment or any other level of organization. A plant functional trait is every trait, as defined above, which impacts fitness indirectly via its effect on growth, reproduction or survival of the plant. Traits are known to vary between species and to be plastic within a species, influenced by environmental conditions. For example, alpine species are known to have different growing forms (smaller leaves) than species occurring at lower altitude (Körner 2003), but on the same species the leaf size is not absolutely constant and could vary with elevation.

Predicting the ecological impact of global and climate changes requires understanding how species are affected by a change in the environment. This could be easily understood by investigating variation of vegetation, particularly functional traits, along gradients of environmental conditions (McGill et al. 2006).

An environmental gradient is a continuous change of environmental conditions. Several studies and publications have already linked functional traits and environmental gradients (Ryser & Aeschlimann 1999, Hultine & Marshall 2000, Fabbro & Körner 2003, Luo et al. 2004). But the gradients used in these previous studies are in general indirect environmental gradients (*sensu* Austin 1984) such as elevation, latitude or longitude. These complex gradients are substitute for a set of more direct ones (*sensu* Austin 1984), for example: temperature, precipitations or solar radiations (resources and energy). The problem of complex gradients is that they are difficult to interpret, since their composition is not exactly known. For the same reason, they cannot be used for future projections or changes predictions in other sites. It is thus interesting to find some direct gradient along which the functional traits will be measured.

Some important and recent studies have still demonstrated the variation of a plant functional trait of communities along an environmental gradient (Luo et al. 2004, Wright et al 2004, Luo et al 2005, Choler 2005). Some other studies have been conducted to investigate these traits variations inside a species along an altitudinal gradient (Morecroft 1996, Suzuki 1998, Hultine 2000). However, to our knowledge, no study has yet investigated the relationship between functional traits at an intra-specific level and direct gradients. Consequently it would be informative to investigate in this direction. To go a step further, niche-based models could be seen as another more global approach of environmental conditions to which plants could be exposed. Therefore it could be interesting for a given species, to link its values of functional traits to a habitat suitability gradient predicted by a niche-based model on the location of the measurement of the trait. Thus a significant relationship between variation of

trait and habitat suitability gradient may give an estimation of the ecological significance of the model.

To choose traits to investigate in a consistent context, we propose to follow the logic of some authors (Westoby 1998, Grime 1977) who suggest summarizing plant strategy through three major axes: resource exploitation, competition ability and response to disturbance. In this work focused on some often-studied functional traits relevant to these three axes. Leaf traits represent resource use, plant height stand for competitive ability and seed number and mass embody the response to perturbations (Wilson 1999, Westoby 1998). In addition, we present here some expectations about traits behavior along gradients of environmental conditions.

First specific leaf area (SLA) is a growth and resource acquisition indicator, good correlate of the potential relative growth rate of the plant (Cornelissen et al. 2004, Körner 2003). We hypothesize that in favorable sites the SLA will be higher than in sites with harsher and unsuitable conditions (Wilson 1998).

Second leaf dry matter content (LDMC) is a growth and acquisition indicator and gives an indication of the leaf water content corresponding to the leaf content in mesophyll, the photosynthetic tissue (Cornelissen et al. 2004). The hypothesis is that in suitable conditions the LDMC will be lower than in difficult conditions. LDMC and SLA are expected to be strongly negatively correlated.

Third, since C:N ratio in leaves is an assimilation indicator, it provides an indication of the leaf protein content. A high nitrogen concentration makes possible an increased investment in leaf chlorophyll and RUBISCO. We expect low C:N ratio in favorable conditions.

Fourth, the maximum vegetative height of a focused plant is associated with its competitive vigor and tends to be correlated with aboveground biomass (Cornelissen et al. 2004). We expect it to be maximal in the most favorable sites.

Fifth, number of seeds and seed mass can be seen as indicators of dispersal ability and resistance to perturbations. We expect the seeds to be bigger and more numerous at favorable sites.

To study the variation of these traits and test following hypotheses at an intra-specific level, a field campaign of functional trait measurement along direct gradients was planned by the Laboratoire d'écologie alpine (Grenoble) and ECOSPAT (Lausanne). This campaign took place in two study sites, in alpine ecosystems, in the French and the Swiss Alps. The following work aims to present the results of the Swiss part of the study, for five species.

Here we first assessed whether measured traits vary intra-specifically along the direct gradients following a quadratic response. If there is a significant variation, traits values should indicate the best plant efficiency on the sites with the most favorable conditions. Then we controlled for potentially confounding factors that may explain the variation in the measured traits. Finally, we also tested the relationship between plant traits values and niche-based models predictions on each site of measurement.

Material and methods

Study area

Our study site is located in the Canton Vaud, Switzerland, in the Avançon Valley between Gryon and Col du Pas de Cheville ($7^{\circ}03'$ to $7^{\circ}12'$ E ; $46^{\circ}16'$ to $46^{\circ}18'$ N, figure 1). The study zone is about 12 kilometers long, about 180 km². The lowest study site is at an altitude of 1076m and the highest at 2313m above sea level. The soil parent material is mainly calcareous. The climate is relatively humid, due to West Atlantic perturbations hitting directly the Diablerets massif. Mean annual precipitations vary between 1309.3 and 2390.1 mm (mean for the years 1961 to 1990), and mean annual temperature between -3.58 and 8.17 degrees (mean for the years 1961 to 1990).

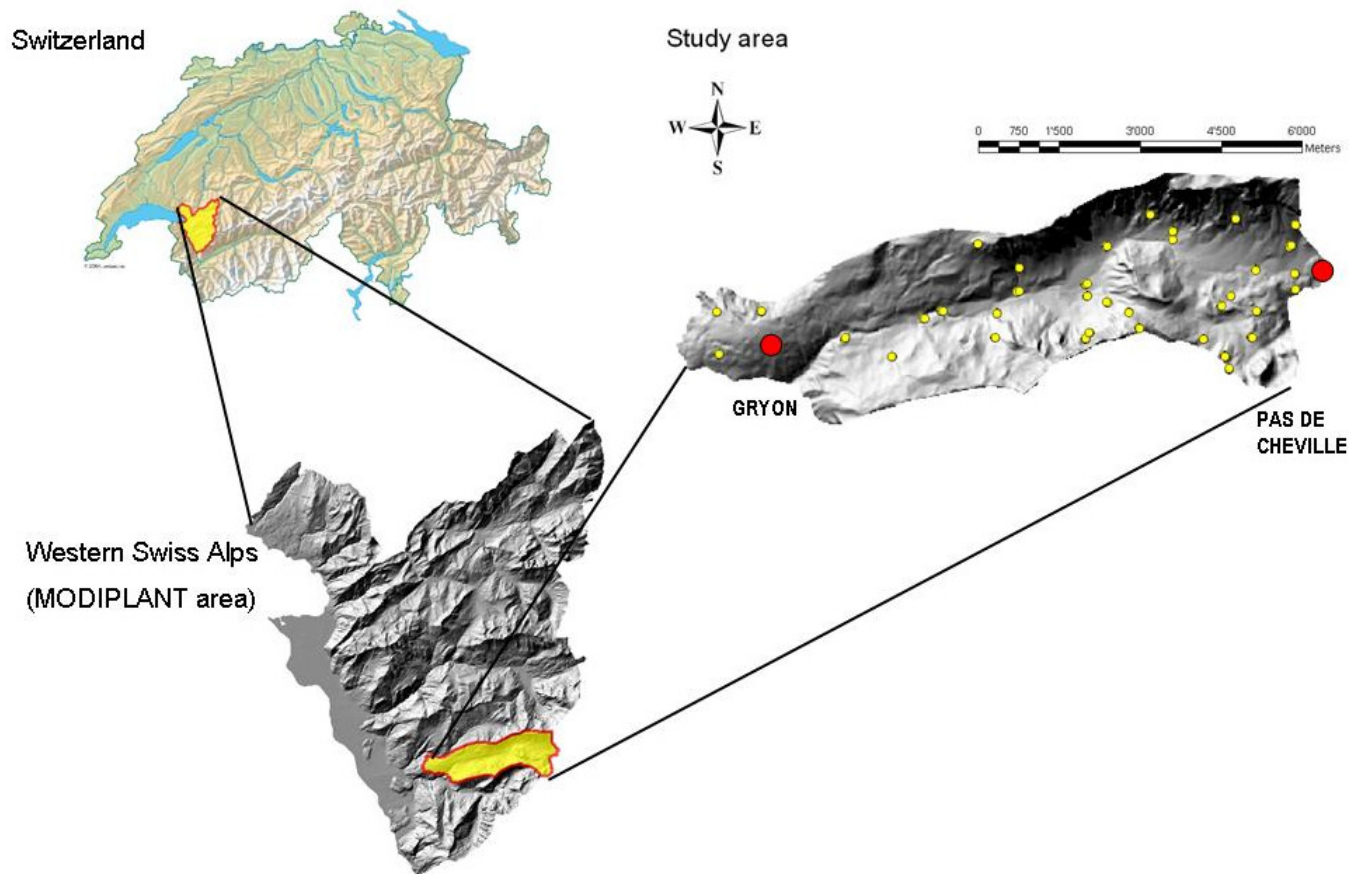


Figure 1: Study area situation in the western Swiss Alps between Gryon and Col du Pas de Cheville. The yellow points correspond to the sampling sites.

Selected species

We worked with a subset of five between the thirteen species chosen for the French part of the experiment.

We focused on common, non-woody species: three herbaceous species *Carex sempervirens* (cyperaceae), *Dactylis glomerata* and *Sesleria caerulea* (poaceae), one shrub *Vaccinium myrtillus* (ericaceae) and one dwarf shrub *Dryas octopetala* (rosaceae). This diversity reflects different plant growing forms. These plants have different ecological needs and altitudinal amplitudes (see table 1).

Table 1: Studied species, their families and preferred habitat according to Lauber & Wagner 2003

Species	Family	Vegetation level	Amplitude
<i>Carex sempervirens</i>	cyperaceae	(montane-) subalpine-alpine	mean
<i>Dactylis glomerata</i>	poaceae	colline-subalpine-(-alpine)	mean
<i>Dryas octopetala</i>	rosaceae	subalpine-alpine	mean
<i>Sesleria caerulea</i>	poaceae	(colline-) montane-alpine	broad
<i>Vaccinium myrtillus</i>	ericaceae	colline-alpine	broad

Vegetation data

We used two vegetation databases containing species occurrences for the Western Alps of the Canton Vaud. The MODIPLANT database consists in 550 8 x 8 meters vegetation plots, following a random-stratified sampling strategy restricted to non-woody vegetation (grassland, rock and scree). Stratification was done by elevation, slope, aspect and simplified classes of geology. The PHYOT-VD database consist in 3077 vegetation surveys in forested zones approximately on a regular 400 x 400 meters grid.

Environmental data

Topographical and climatic data were used at a 25 meter resolution. Topographical variable are derived from the Swiss digital elevation model (MNT25), climatic data are derived and interpolated from the Swiss network of meteorological stations. The used environmental data come from the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL).

Sampling strategy

We carried out a principal component analysis (PCA; see results in figure 1 in annex 2) on 17 continuous environmental descriptors for the study zone (table 1 in annex 1). This allowed, after removing indirect and correlated variables, choosing two direct and orthogonal variables: the mean temperature for January that nearly follows the altitude, and the August radiations that reflect orientation, to stratify the sampling. Orthogonal variables have the advantage to maximize environmental heterogeneity while allowing statistical analysis and separation of both factors effects. Along these two gradients, between 8 and 10 sites were chosen for each species, among the MODIPLANT and PHYTO-VD sites. Sites were selected to represent each combination of mean, high and low temperature and radiation conditions.

Field sampling and field measurements

The field sampling took place between June and August 2007. The selected sites were localized with a Trimble Geoexplorer XT GPS (allowing sub-meter accuracy after differential correction). We tried to be synchronized as much as possible with vegetation development. Once on a site, three subplot of one square meter were chosen randomly, constraint by the species' presence, and in each subplot three randomly chosen plants were harvested.

Following measures were noted down: for herbaceous species: maximal vegetative height, mean vegetative height, inflorescence number, inflorescence height, occupied surface on the foot of the plant, maximal leaves length and width. For shrubs: maximum and mean vegetative height, inflorescence number, percentage of cover in 10 cm² square for *Dryas* and 15 cm² for *Vaccinium*. Then the plant was cut, stored in a plastic bag with water and kept at cool temperature in an icebox, until measurement in the lab (Vaieretti et al. 2007).

To further describe the site, we listed: bare soil percentage and percentage of cover for high, median and low strata. Latitude, longitude, altitude, orientation, slope and soil depth were measured for each sub-site and a 15 centimeter deep soil (removing the surface layer of humus) sample was taken.

When it was possible, three inflorescences per plot were bagged in mesh to keep their seeds for later counting and weighting.

Trait and soil measurements

Plants were dried with a towel, their total green biomass was weighted (with a Mettler-Toledo AL54/01 analytical balance), then for herbaceous species, one entire, well developed, and

not eaten leaf was chosen. The selected leaf (without its petiole) was scanned, weighted fresh, then dried for 72 hours at 60 degrees and weighted dry. For the shrubs ten leaves were measured to give an idea of the probable intra-individual variations.

After scanning, the leaf surface was measured with the software “Medición de Objetos” (V. 4.2 © 1999-2000). Carbon, nitrogen and hydrogen contents were determined by combustion method with a CHN analyzer.

Soil samples were air dried, sifted at 2 mm, their pH was determined with a pH meter and organic matter by burning the soil for 4 hours at 550 degrees.

Measured traits

Measured traits are listed in table 2.

Table 2: Name, definition and unit of measured traits.

Trait		Definition	Unit
SLA	specific leaf area	the area of one leaf divided by its dry weight	mm ² /g
LDMC	leaf dry matter content	the dry mass of one leaf divided by its fresh mass	unitless
Biomass		the total fresh mass of the green part of the plant	g
Hmax	maximal height	the maximal vegetative height of the chosen plant.	cm
C:N	carbon nitrogen ratio	the carbon content of one leaf divided by its nitrogen content	unitless

Niche qualification and modeling

We used the computation framework BIOMOD (Thuiller 2003), running in R (V.2.4.1, 2006), to implement niche based models for the five chosen species for all Western Swiss Alp (MODIPLANT area). The used environmental predictors are mean annual temperature (mean for the years 1961 to 1990), moisture index (difference between precipitation and evapotranspiration, expressing the amount of soil water available at a site), the mean annual sum of radiation, slope and topographic position that are derived from the MNT25. These variables are expected to be of greatest ecophysiological significance (Pearson et al. 2002, Körner 2003).

An ensemble modeling approach was used. BIOMOD computed 7 different kinds of models (GLM, GAM, GBM, CTA, MDA, MARS and RF, for references see table 3).

Table 3: Names and key references for the seven models implemented in BIOMOD

		Key references
GLM	generalised linear model	McCullagh & Nelder 1989
GAM	generalised additive model	Hastie & Tibshirani 1990
GBM	generalised boosting model	Ridgeway 1999, Friedman et al. 2000, Friedman 2001
CTA	classification tree analysis	Breiman et al. 1984
MDA	mixture discriminant analysis	Hastie et al. 1994, Hastie et al. 1995, Hastie & Tibshirani 1996
MARS	multivariate additive regression spline	Friedman 1991
RF	random forest	Breiman 2001

A sum of the presence probabilities obtained with the seven models was calculated and was averaged over hundred iterations. We obtained a value between 0 (no probability of presence) and 7 (maximal probability of presence) for each study site. The models predictive power was tested on an independent data set (30 percent of the total) using the area under the curve (AUC) of a receiver-operating characteristics (ROC) plot (Fielding & Bell 1997, Elith et al. 2006).

Data analysis

Figure 2 shows a summary of the experimental design. The interrogation marks stand for the relationships investigated in the data analysis. The part presenting the confrontation between functional traits and principal environmental components (question mark number 3 in figure 2) is presented in annex 3 because it do not really bring anything new in the results.

Data were analyzed with R (V.2.6.0, 2007). First, the relationships between traits were investigated with Spearman correlation tests on the mean trait value per plot. After that we worked with the mean trait value per individual when several trait measurements existed for one individual. To know if plots differ form each other in terms of trait, Kruskal-Wallis tests were executed.

To explore effect of gradients on traits (question mark number 1 in figure 2), mixed regression models were implemented with the lme4 library in R. The trait was the response variable, the site temperature or radiation (as simple and quadratic term) the explanatory variable and the sample site was defined as a random effect. When possible, the trait values were transformed to follow a Gaussian distribution (see table 1 in annex 1 for used transformations). When this was impossible, a mixed model with gamma family was used.

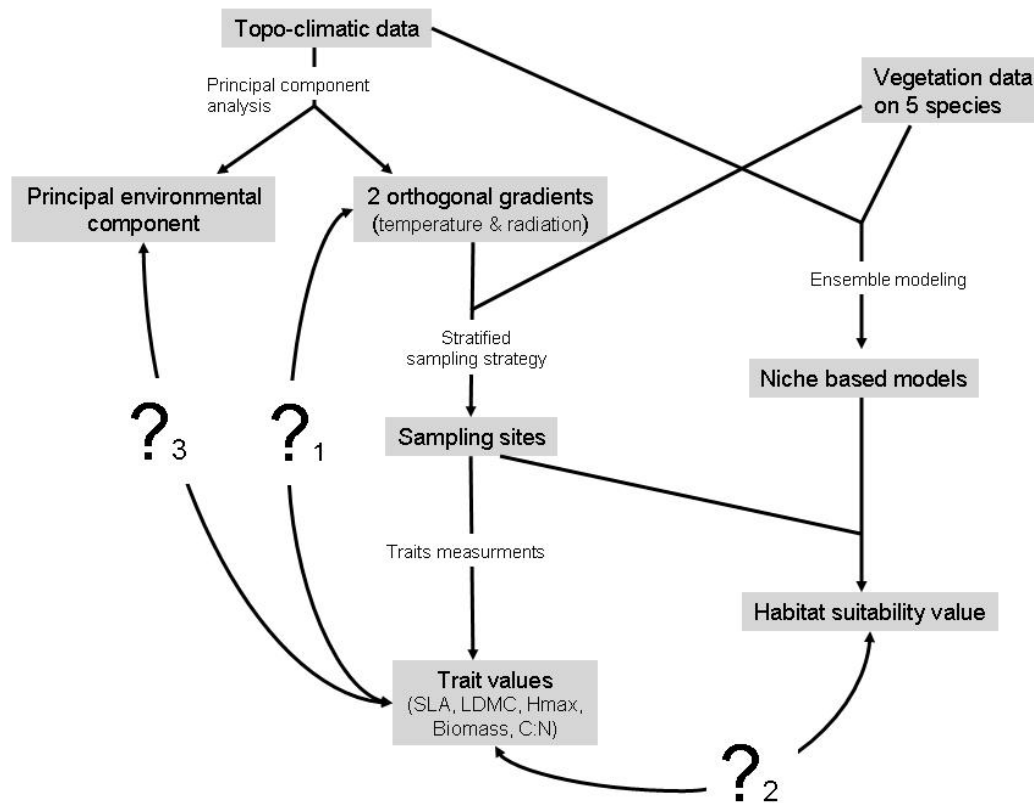


Figure 2: Summary of the experimental design.

To check if variations in traits values could be explained by environmental confounding factors, mixed models were constructed with the residuals of the previous models as response variable and soil pH and organic matter content as explanatory variable. P-values reflecting the importance of explanatory variables were obtained performing an analysis of variance between the regression model with the variable of interest and the model without it.

Finally, to join niche-based models and functional traits (question mark number 2 in figure 2), a set of mixed models was implemented with the BIOMOD probabilities of presence as explanatory variable.

All presented graphs are realized with non-transformed data to represent the biological reality.

Results

Trait measurement and relation between traits

Minimum, maximum, median and mean of traits are presented for each species in table 2 of annex 1.

Results of Spearman correlation tests between traits are showed in table 3 of annex 1. They are consistent with the prediction and previous studies (Cornelissen et al 2004). Maximal height is correlated with biomass for herbaceous species. The C:N ratio is much more correlated with nitrogen content than with carbon content. As expected, LDMC and SLA are negatively correlated for three in five studied species: *Dryas*, *Vaccinium* and *Dactylis*. When not significant, the tendency is negative too (figure 3).

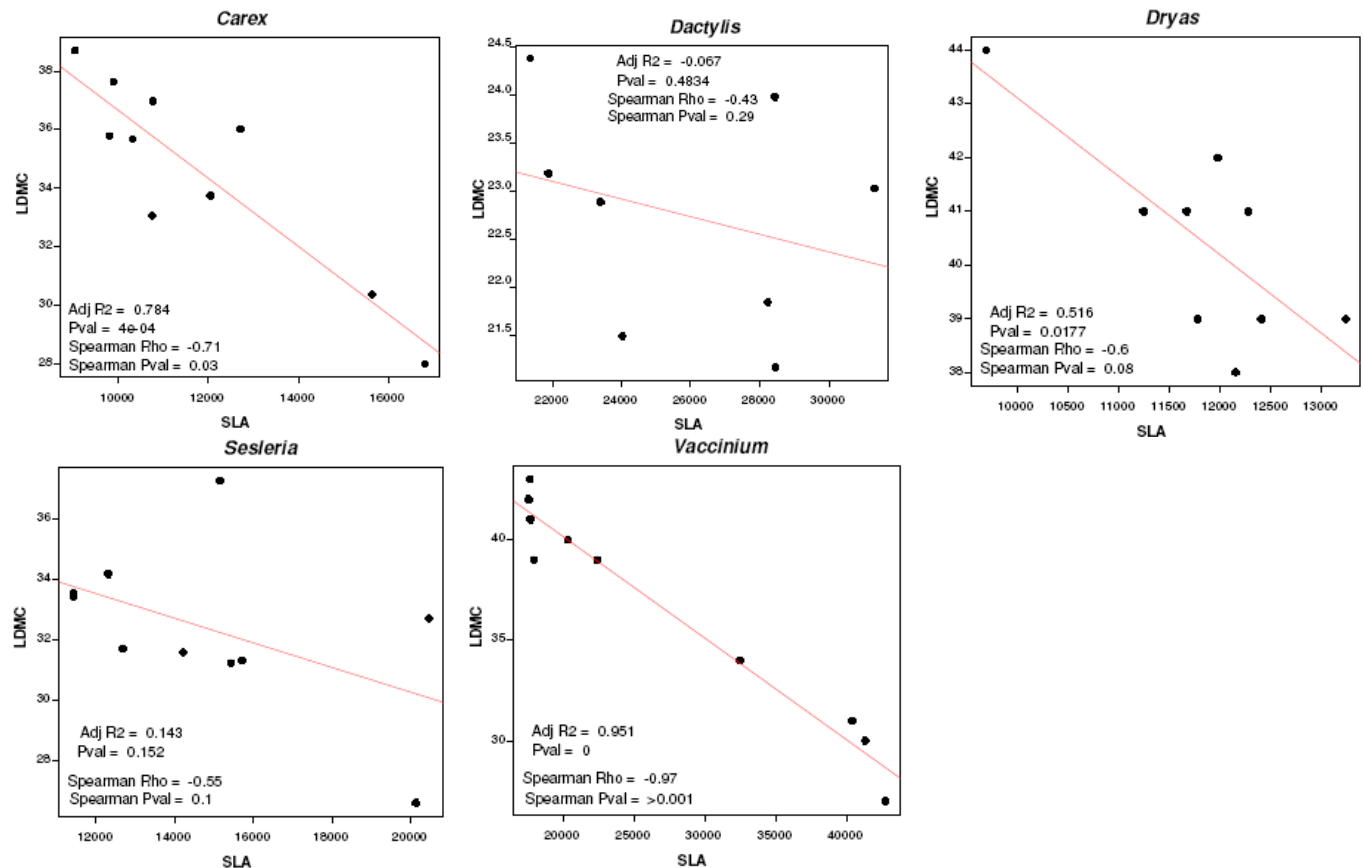


Figure 3: Relation between LDMC and SLA mean values per each plot. Red lines display the linear regression through observations. For each case R² and p-value are displayed as well as value of Spearman Rho and p-value illustrating the correlation between SLA and LDMC values.

The Kruskal Wallis tests demonstrate that, except for *Dactylis* LDMC, at least one plot differs significantly from the others (table 4).

Table 4: P-values of the Kruskal Wallis tests on the trait values per plot

	LDMC	SLA	Hmax	C_N	Biomass
<i>Carex</i>	>0.001***	>0.001***	>0.001***	>0.001***	>0.001***
<i>Dactylis</i>	0.445	0.007**	>0.001***	>0.001***	0.015*
<i>Dryas</i>	>0.001***	>0.001***	>0.001***	>0.001***	>0.001***
<i>Sesleria</i>	>0.001***	>0.001***	>0.001***	>0.001***	0.029*
<i>Vaccinium</i>	>0.001***	>0.001***	>0.001***	>0.001***	0.002**

Analysis on seed traits were not achieved due to a lack of statistical power.

Ecological niche modeling

Spatial projections of the obtained models for the five study species are presented in annex 2, figure 2. The mean AUC for each type of modeling algorithm are presented in table 5 of annex 1. On average, *Dactylis* obtained the best model as *Dryas* and *Carex* obtained the worst.

Response to direct gradients

All p-values of models for trait to gradient interaction are displayed in table 5. The maximal height is the trait responding best to the temperature gradient. We can show a positive relationship between temperature and height for all species except *Dactylis*: the warmer the site, the higher the plant (figure 4). The models with quadratic term for temperature are significant but not significantly better than those with simple term. Maximal height responds to radiations only for *Dryas*; the less the amount of radiation, the higher the plant. The quadratic term for radiation is never significant.

Biomass is the second best reacting trait. It shows a significant response for two species (*Dactylis* and *Vaccinium*) to temperature and for *Dryas* to radiation. LDMC shows a quadratic response to temperature for *Carex* and a positive response to temperature for *Sesleria*.

Ratio between carbon and nitrogen content in the leaves responds positively to the temperature gradient only for *Dactylis*, the other species do not show any reaction.

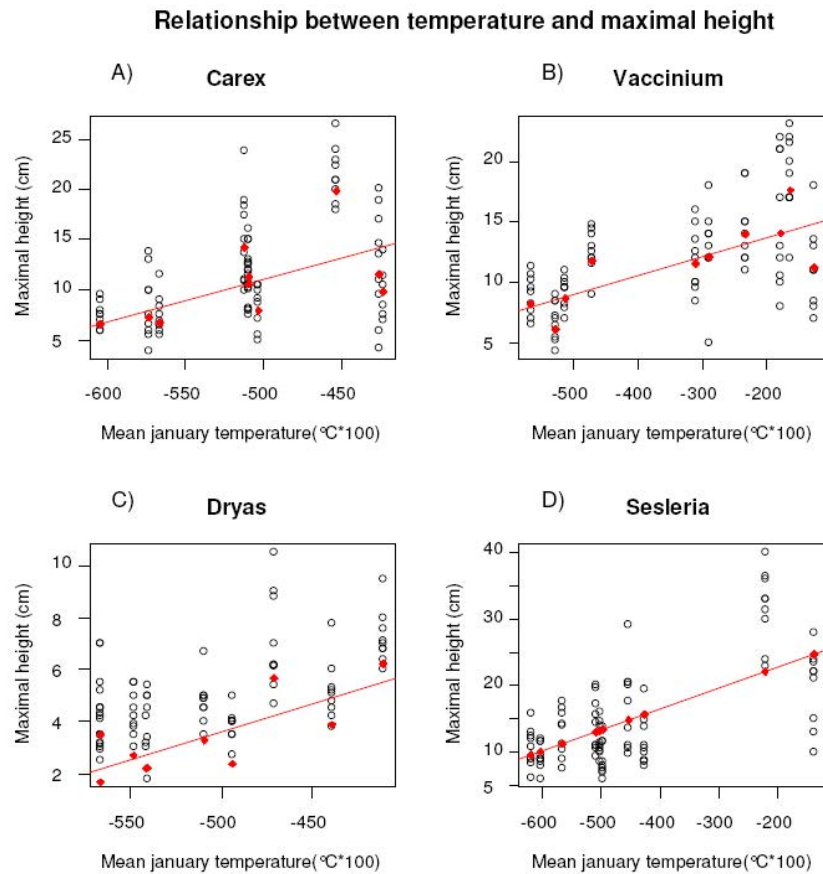


Figure 4: Plot of the maximal height against the mean January temperature for *Carex* (A), *Vaccinium* (B), *Dryas* (C), and *Sesleria* (D). Black points are observations, red points are mixed models fitted values and red lines, the linear regression between fitted values to illustrate the trend. Graphs A to C shows mixed models implemented with a Gaussian distribution, the relation in graph D was calculated with Gamma distribution, which explains all the fitted points being on the same line.

Table 5: P-values of the relationship between gradient values and traits

		LDMC	SLA	Hmax	Biomass	C:N
<i>Carex</i>	temp	0.793	0.808	0.045*	0.096	1
	rad	1	0.859	0.734	0.225	0.369
	temp+temp ²	0.028*	0.513	0.07	0.394	0.27
<i>Dactylis</i>	temp	0.432	0.887	1	0.031*	0.002**
	rad	0.2209	1	0.525	0.502	0.368
	temp+temp ²	0.284	0.448	0.738	>0.001***	0.002**
<i>Dryas</i>	temp	1	1	0.011*	1	1
	rad	1	0.251	0.007**	0.002**	0.447
	temp+temp ²	1	1	0.04*	1	1
<i>Sesleria</i>	temp	0.044*	0.525	0.003**	0.834	0.946
	rad	0.483	0.795	0.423	0.383	0.967
	temp+temp ²	0.153	0.774	0.011*	0.947	0.891
<i>Vaccinium</i>	temp	0.226	0.002**	0.005**	0.034*	0.092
	rad	0.686	0.128	1	0.168	0.134
	temp+temp ²	0.418	0.004**	0.012*	0.051	0.117

Finally SLA do not shows any reaction except for *Vaccinium*, but for this species, the trait distribution is bimodal (figure 5), so the p-value cannot be considered.

We tested the soil characteristics influence: pH and organic matter content. These two factors do not have a significant influence on leaf traits (see table 6 in annex 1).

Response to modeled habitat suitability

The traits responding to habitat suitability are globally the same than those reacting to gradients. Maximum height responds negatively for *Sesleria*, and *Vaccinium*, biomass positively for *Dactylis* and *Vaccinium*, and negatively for *Dryas*, and C: N ratio positively only for *Dactylis*. The relationships are linear. Obtained p-values are displayed in table 6.

Table 6: P-values of the relationship between habitat suitability values and traits

	LDMC	SLA	Hmax	Biomass	C:N
<i>Carex</i>	0.772	0.935	0.087	0.075	0.646
<i>Dactylis</i>	0.608	0.255	0.540	0.015*	0.008**
<i>Dryas</i>	1	0.260	0.513	0.036 *	0.32
<i>Sesleria</i>	0.714	0.316	0.017*	0.517	0.813
<i>Vaccinium</i>	0.284	0.003**	0.003**	0.018*	0.085

Discussion

Two important findings result from this study. First, no single trait responded similarly for all five species along the two gradients tested, suggesting that trait variation along environmental gradients is species specific. Secondly, probabilities of presence predicted by niche models (fitted on presence-absence data) was not correlated with trait variation, suggesting that predictions by these models do not reflect plant fitness or functional variation and can only inform on the likelihood of species presence, given the habitat.

Trait variation along environmental gradients

No trait responded equally for all species. Leaf dry matter content (LDMC) showed a response only for small herbaceous species (*Carex* and *Sesleria*) but it did not show any response for the taller *Dactylis* and for the shrubs. Maximum height, the best responding trait did not respond either for *Dactylis*. These results, and the fact that the C:N ratio only showed a response for *Dactylis*, is an indication that traits behavior cannot be extended as a generality from one species to the others. Each species has its own pattern of traits behavior. This can be confirmed with the study of Gerdol (2005) who demonstrated that two species with close life form, which would have been included in the same PFT; *Vaccinium myrtillus* and *V. uliginosum*, do not show the same growth response to soil nutrient availability. Their leaf phosphorus content and net primary productivity suggest the growth of *V. myrtillus* to be phosphorus-limited whereas that of *V. uliginosum* is not.

In the field, *Vaccinium* could be observed as two different ecotypes: one in forest understory and, another in heaths at higher altitude, under harsher wind and temperature conditions. Their leaf morphology is very different, as confirmed by the bimodal distribution of SLA values for *Vaccinium* (figure 4). The leaves are broader in the understory ecotypes, likely due to the shadowy conditions and a relative protection against wind, and smaller in the heath ecotypes due to their greater exposure to wind and radiations. This situation could let us think to the existence of a genetic differentiation between these two kinds of plants. The locations of these two varieties are very different so that a lack of genetic exchange could be hypothesized between them. A similar situation has been underlined for *Poa alpina*. The phenotypes of two ecotypes are maintained after a reciprocal transplant experiment along an altitudinal gradient, the genetic effect being far more important than the environmental one (Hautier et al. submitted).

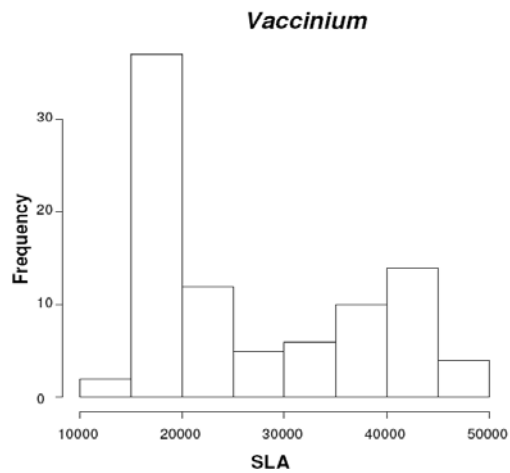


Figure 5: Histogram showing the bimodal frequency distribution of *Vaccinium* SLA. Low values (little leaves) correspond to high altitude plants as higher values (broad leaves) correspond to forest ecotype.

These results show the difficulty to summarize a species' ecology with functional traits only, as the latter are not only influenced by abiotic conditions, but are additionally modulated by complex biotic interactions like competition with other species or through genetic effects. It is an indication that PFT approach cannot fully describe ecosystem relations at the local scale.

In addition, functional traits are subject to measurements errors. SLA is the only traits not displaying any response, except for *V. myrtillus*, where it seems caused by the bimodal distribution of traits between the two ecotypes. Wilson (1999) showed that SLA is difficult to handle because it is linked with both leaf area and thickness and has a very high variation between replicates. Such trait variation could be the consequence of genetic variability or environmental plasticity (e.g. leaves of *Vaccinium* being very sensitive to shading).

We could have expected quadratic relationship between traits and gradients to comply with the theory of a species' niche being limited on one side by abiotic factors and on the other side by biotic interactions (competition), as; this is not truly the case here. The light tendencies are not significant. This could be due to our sampling not covering the entire zone of plant presence, so we see the response only partially.

Traits response to the temperature gradient

Our study demonstrated quantitatively for all species, except for *Dactylis*, that maximum height (plant size) increases with temperature. *Dactylis* showed a positive response of biomass, rather than height, to temperature, but these two traits are usually strongly correlated in plants (Corneliessen 2004).

C:N ratio responded also strongly to temperature, but only for *Dactylis*: the lower the mean temperature (i.e. the higher the altitude), the more nitrogen in the leaves. This pattern is the same as found by Wenger et al. (2003), Suzuki (1998) and Morecroft & Woodward (1996) for ferns in Bolivian Andes, *Sanguisorba tenuifolia* in Japan and *Alchemilla alpina* in England respectively. Temperature is highly correlated with altitude which determines the length of the growing season for plants. Plants enduring short growing seasons are thought to have more nitrogen in their leaves to maximize photosynthesis (Suzuki 1998). Another hypothesis to explain this difference is the low growth rate causing nutrient concentration in the leaves (Morecroft & Woodward 1996). Why is *Dactylis* the only plant showing significant variation of C:N ratio? Perhaps because it grows in habitats that are naturally richer in nutrients than habitats of our other species and nitrogen could have become a limiting element only for *Dactylis*.

Traits response to the radiation and other gradients

Dryas is the only species which height responded strongly to solar radiations, perhaps because it grows mostly on well exposed rocks or bare soils, and thus is particularly sensitive to exposure. More generally it is not surprising that radiations explain less trait response than temperature. This variable is based on a topo-climatic model and thus expresses potential radiation at a site. For this reason, it cannot account for shadowy conditions caused by a forest cover for example. Furthermore, it is calculated on a rough scale, which may not reflect the real variation in radiation caused by the rugged micro-topography observed in mountain landscapes (Lassueur et al. 2006).

As to the other gradients investigated, the soil pH and organic matter content showed no effect on traits, which is consistent with what Roche (2004) found in his study. Further testing is required here, as our sampling was not specifically designed to test these additional gradients (contrary to temperature and radiations, which were used as stratifying factors).

Traits response to modeled habitat suitability

The lack of trait to gradient response or its species-specificity could have been due to the trait not following a direct gradient, but responding to a species-specific optimum of environmental conditions. Such an optimum could be better described with niche-based model. This was tested by relating traits to predicted habitat suitability. Additionally, we aimed at testing whether outputs from niche-based presence-absence models could provide an indication of plant vigor or fitness. Considering the lack of traits response to predicted habitat suitability the hypothesis explaining the non response of traits to direct gradient is not

confirmed. Moreover, it seems that models based on presence-absence, data only indicate the probability of observing a species in a site characterized by a given set of environmental conditions. This means for example that a probability of presence of 0.5 will not necessarily correspond to a site where individuals of the species will have the average or median height and a probability of 0.1 will not indicate that only very small individuals (if any) will be observed. This is likely because no information on vigor (vegetative) or fitness (reproduction), or even species' abundance, was used to fit these models, because most vegetation surveys do not gather, in the field, such additional information. In a presence-absence model, the same weight is given to an occurrence, be it one or a few sterile individuals or a large population of reproductive individuals. Thus, one cannot know if these observations correspond to source or sink populations. Yet it has been demonstrated that plants do not have the same fitness across their distribution range. For example Jump and Woodward (2003) demonstrated that *Cirsium* species produce less seeds in the border than in the center of their distribution area. Similarly, *Hordeum vulgare* has been demonstrated to grow slower and produce smaller seeds in periphery of its range, where its slower growth do not permit seeds to have enough time to desiccate and be able to germinate the next spring (Prince 1976).

If observed abundance would prove to be an adequate surrogate for vigor/fitness measures, then abundance models should show a better relationship with variation in functional traits than simple presence-absence models. We are not aware of a study testing the relationship between abundance and vigor, and results of studies comparing presence-absence to abundance models are contradictory. Randin et al (in revision) demonstrate abundance models to have a better predictive power, when Pearce et al (2001) shows no difference between both type of models, but probably due to the bad quality of abundance data. One could make the hypothesis that, in places where abundance is low, the environmental conditions are less suitable for the species than where abundance is high, resulting in the plant exhibiting suboptimal trait values. But this still needs further investigations.

Limitations of niche-based models

Several additional limitations can be associated with modelled habitat suitability. First, the 25 meter resolution used to implement our niche models may be too rough to embody the subtle variations in species distribution, such as micro-topographical habitat requirements in *Dryas octopetala*. Using very fine scale predictors could be better suited to predict the distribution of species with low stature (Lassueur et al. 2006; Wilhelm 2007). This hypothesis is at least supported by our results, with worst model evaluation being obtained for low-stature species

that are tightly linked with microtopography, (*Carex* and *Dryas*) and best model evaluation being obtained for the tallest species (*Dactylis*).

As mentioned concerning the trait-gradient interaction, the radiation predictor is based on a model that does not take forest shadowing into account and thus has limited value for plants growing both in forested and non-forested area, such as *Vaccinium myrtillus*. Assessing the total variation in plant traits of a species requires considering all its habitats. Yet, forests were not sampled in the MODIPLANT data set used to fit the models. This means that, in our case, the model for *Vaccinium* is only applicable to populations growing outside forests and it would be preferable to consider the two groups of populations separately in further analyses. *Sesleria* has the same problem, although in a less accentuated manner, as it was only observed within two forest plots. This problem does not apply to all three remaining species, as *Carex* and *Dryas* only grow in open habitats and *Dactylis* in open grasslands or in forest borders. Temperature data also suffer from an important shortcoming since they correspond to air temperature measured at some points and interpolated through the Swiss territory. This means they do not represent to temperature sensed by the plant, which is protected by snow in winter or sustain a cool airstream during summer, for example.

Furthermore, the alpine plant distribution is driven by other complex phenomenon, like snow distribution, that are not taken into account in the topo-climatic predictors used to build our niche-based models. It has been demonstrated (Randin et al. accepted) that models fit improved when process-based predictors like a snow accumulation or a geomorphology index are brought in. Snow could have a particularly important influence on functional traits since its accumulation is determinant for soil water content, nitrogen amount (Bowman 1992) and isolation against frost during winter.

Suggestions for further experiments and perspectives

Seeking for relationship between habitat suitability and traits requires testing other kinds of models; for example: abundance models, fine scale models or models containing mechanistic predictors.

Relations between traits and fine scale temperature could be investigated recording temperature at soil level at the sample site during growing season and then to conduct further analysis to see if the obtained data better explain the trait variation.

Further investigations about plants growing in both forested and open environment could be proceeded to compare their niche optimum with multivariate technique. Some exploration should be lead about competition and its influence on intra-specific variation of plant traits.

Finally, having conducted the sampling in two different study areas will allow us comparing the data, and perhaps strengthening or detailing our conclusions thanks to an increased amount of data.

Acknowledgments:

I would like to thank:

- ❖ Antoine Guisan for proposing me this subject, following my progress with interest during all this year and corrections on the manuscript,
- ❖ Christophe Randin for helpful discussions, availability to answer my questions even across the ocean, helping on the field and corrections on the manuscript,
- ❖ Cécile Albert for allowing me sharing a little piece of her PhD project, supervising and commenting my work and answering my question,
- ❖ Wilfried Thuiller for co-supervision and interesting discussions,
- ❖ All the ECOSPAT group and more particularly: Gwenaëlle Le Lay for helping me with soil analysis, Robin Engler for answering questions about R and GIS, Blaise Petitpierre for coffee breaks and help with BIOMOD,
- ❖ David Paulin for his essential contribution on the field during summer, patience, enthusiasm, and good mood,
- ❖ Sébastien Nusslé and Anne Burkhardt for help with statistical problems,
- ❖ François Bonnet and his family, for sharing their chalet with us at Pont de Nant, and supporting our leaves and balances during all summer, Colin Hostein and Bojana for agreeable cohabitation,
- ❖ Franck Chalard for his availability and helpfulness during field work preparation,
- ❖ All my family and friends that listened with patience to my little plants stories.

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Annex 1: Tables

Table 1: Presentation of the set of variable used for the sampling.

Variable	Resolution (m*m)	Definition	Unit
dem25ch	25*25	digital elevation model	meter
asp25	25*25	aspect	degree
topo	25*25	topographic position	unitless
slp25	25*25	slope	degree
flow_acc	25*25	accumulation of flow	unitless
flow_dir	25*25	direction of flow	unitless
twi25	25*25	topographic wetness index	unitless
mind7	25*25	moisture index in July	1/10mm/month
etpt7	25*25	evapotranspiration Turc in July	1/10mm/day
ddegch	25*25	degree day above 0degC	Day* °C
srad1	25*25	mean daily potential exposition to sun in January	kJ /day
srad8	25*25	mean daily potential exposition to sun in august	kJ /day
tave1	25*25	mean of mean temperatures in January (1961-1990)	°C *100
tave8	25*25	mean of mean temperatures in august (1961-1990)	°C *100
gams_t	25*25	continentality index	unitless
prec_ete	25*25	mean of mensual sums of precipitation in June, July and August	1/100mm/period
prec_hiver	25*25	mean of mensual sums of precipitation in Jan., Feb. and March	1/100mm/period

Table 2: Transformation used to reach the normality of variable. Normal means that the trait distribution did not need any transformation, log= natural logarithmic transformation, Gamma means the use of a mixed model with Gamma familiy.

	SLA	LDMC	Hmax	Biomass	C:N
<i>Carex</i>	Gamma	²	log	^{0.5}	log
<i>Dactylis</i>	log	log	log	Gamma	log
<i>Dryas</i>	Normal	Normal	log	^{0.5}	log
<i>Sesleria</i>	Gamma	log	Gamma	Gamma	Gamma
<i>Vaccinium</i>	Gamma	Gamma	log	log	Normal

Table 3: Mean, median,minimal and maximal values for each trait and each study species

	<i>Carex</i>				<i>Dactylis</i>				<i>Dryas</i>			
	Min	Median	Mean	Max	Min	Median	Mean	Max	Min	Median	Mean	Max
LDMC	20.3	35.5	34.74	48.14	14.48	22.01	22.72	43.98	33	41	40.31	47
SLA	4810	10652	11639	33843	13996	25774	25946	46761	8390	11730	11822	15580
Hmax	4	10.5	11.53	26.5	20.5	40.25	46.53	94	1.8	4.5	4.988	10.5
Biomass	0.01	0.4	0.4299	0.94	1.7	4.6	7.137	53.5	0.25	2.98	3.196	6.29
C_N	17.86	27.46	28.24	41.14	9.44	16.75	18.9	39.64	17.4	27.81	28.57	46.3
% C	41.69	44.07	44.06	45.69	40.13	42.84	42.85	44.77	46.6	47.77	47.7	48.89
% N	1.06	1.63	1.634	2.48	1.06	2.5	2.547	4.68	1.02	1.71	1.714	2.7

	<i>Sesleria</i>				<i>Vaccinium</i>			
	Min	Median	Mean	Max	Min	Median	Mean	Max
LDMC	23.66	32.52	32.35	52.04	24	38	36.43	63
SLA	5471	14304	14895	43530	14800	20690	27049	47930
Hmax	6	11.75	14.85	40	4.4	12	12.16	23
Biomass	0.07	0.255	0.2784	0.89	0.21	0.83	1.005	4.07
C_N	16.6	26.41	26.08	34.93	17.52	29.21	29.14	43.04
% C	42.98	44.08	44.2	45.42	45.78	47.18	47.24	49.15
% N	1.26	1.665	1.736	2.64	1.12	1.62	1.671	2.72

Table 4: Spearman correlation coefficients between traits

		LDMC	SLA	Hmax	Biomass	C_N	Pour_C	Pour_N
<i>Carex</i>	LDMC	1.00						
	SLA	-0.43	1.00					
	Hmax	0.35	-0.14	1.00				
	Biomass	0.23	-0.04	0.80	1.00			
	C_N	0.36	-0.41	0.03	-0.20	1.00		
	Pour_C	-0.07	0.22	-0.01	-0.01	-0.04	1.00	
	Pour_N	-0.36	0.41	-0.02	0.21	-0.99	0.06	1.00
<i>Dactylis</i>	LDMC	1.00						
	SLA	-0.64	1.00					
	Hmax	-0.20	0.24	1.00				
	Biomass	-0.26	0.11	0.64	1.00			
	C_N	0.12	0.12	0.00	0.08	1.00		
	Pour_C	-0.11	-0.07	0.32	0.08	-0.46	1.00	
	Pour_N	-0.12	-0.12	0.03	-0.07	-0.99	0.50	1.00
<i>Dryas</i>	LDMC	1.00						
	SLA	-0.61	1.00					
	Hmax	-0.19	0.46	1.00				
	Biomass	-0.05	-0.28	-0.26	1.00			
	C_N	0.34	-0.46	-0.23	0.23	1.00		
	Pour_C	0.04	0.07	0.32	-0.37	-0.04	1.00	
	Pour_N	-0.33	0.46	0.25	-0.25	-0.99	0.10	1.00
<i>Sesleria</i>	LDMC	1.00						
	SLA	-0.33	1.00					
	Hmax	0.37	0.10	1.00				
	Biomass	0.11	-0.02	0.46	1.00			
	C_N	0.27	-0.34	0.11	0.02	1.00		
	Pour_C	0.02	-0.21	-0.08	-0.01	0.15	1.00	
	Pour_N	-0.28	0.34	-0.12	-0.01	-0.98	-0.02	1.00
<i>Vaccinium</i>	LDMC	1.00						
	SLA	-0.76	1.00					
	Hmax	-0.32	0.45	1.00				
	Biomass	0.02	-0.24	0.04	1.00			
	C_N	0.61	-0.61	-0.13	0.04	1.00		
	Pour_C	0.14	0.08	0.27	-0.16	0.19	1.00	
	Pour_N	-0.61	0.63	0.16	-0.04	-0.99	-0.12	1.00

Table 5: AUC values for the models implemented with MODIPLANT presences and absences in 8 meters ²

		GLM	GAM	GBM	CTA	MDA	MARS	RF
<i>Carex</i>	Calibration	0.8702	0.8925	0.9499	0.8497	0.8716	0.9021	1.0000
	Evaluation	0.8474	0.8554	0.8645	0.7717	0.8093	0.8532	0.8516
	Prediction	0.8631	0.8809	0.9259	0.8259	0.8527	0.8874	0.9774
<i>Dactylis</i>	Calibration	0.9599	0.9622	0.9804	0.9387	0.9478	0.9625	1.0000
	Evaluation	0.9622	0.9618	0.9564	0.9117	0.9421	0.9414	0.9622
	Prediction	0.9602	0.9618	0.9730	0.9305	0.9458	0.9561	0.9923
<i>Dryas</i>	Calibration	0.8678	0.9174	0.9630	0.7826	0.8338	0.9067	1.0000
	Evaluation	0.8460	0.8706	0.8588	0.7082	0.7917	0.8447	0.8654
	Prediction	0.8617	0.9025	0.9302	0.7597	0.8193	0.8869	0.9733
<i>Sesleria</i>	Calibration	0.8597	0.8948	0.9508	0.8914	0.8803	0.9077	1.0000
	Evaluation	0.8368	0.8668	0.8725	0.8174	0.8166	0.8631	0.8830
	Prediction	0.8526	0.8865	0.9280	0.8690	0.8613	0.8945	0.9825
<i>Vaccinium</i>	Calibration	0.8884	0.9226	0.9621	0.8698	0.8702	0.9155	1.0000
	Evaluation	0.8679	0.8820	0.8494	0.8001	0.7994	0.8756	0.8552
	Prediction	0.8828	0.9103	0.9291	0.8493	0.8507	0.9042	0.9745

Table 6: P-values of mixed models with soil variables (pH and organic matter content) as explanative values on residuals of a preliminary regression with temperature and radiation as explanative values.

		LDMC	SLA	Hmax	Biomass	C/N
<i>Carex</i>	pH*OM	0.851	0.731	0.887	0.635	0.996
	pH	0.716	0.647	0.575	0.633	0.877
	OM	0.946	0.709	0.504	0.313	0.992
<i>Dactylis</i>	pH*OM	0.224	0.475	0.91	1	0.847
	pH	0.259	0.667	0.522	0.999	0.381
	OM	0.682	0.851	0.954	0.999	0.979
<i>Dryas</i>	pH*OM	0.870	0.832	0.908	0.736	0.79
	pH	0.46	0.496	0.522	0.874	0.387
	OM	0.723	0.763	0.725	0.948	0.981
<i>Sesleria</i>	pH*OM	0.954	0.605	0.849	1	1
	pH	0.721	0.57	0.734	1	0.999
	OM	0.698	0.55	?	1	0.999
<i>Vaccinium</i>	pH*OM	0.314	1	0.434	0.816	0.961
	pH	0.741	0.999	0.722	0.577	0.985
	OM	0.069	0.999	0.851	0.749	0.664

Annex 2: Figures

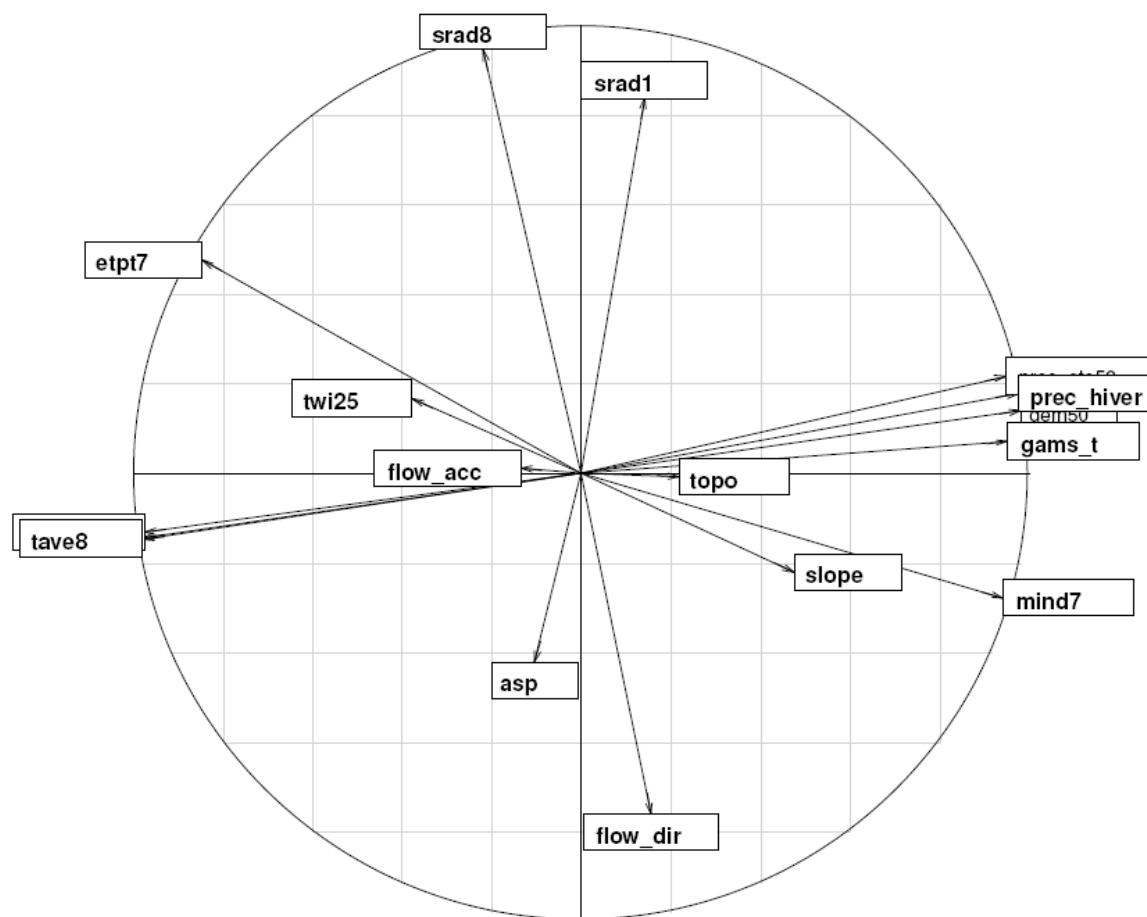


Figure 1 Correlation circle resulting from the PCA on 17 environmental predictors, aiming to choose two direct and orthogonal environmental gradients to stratify the sampling.

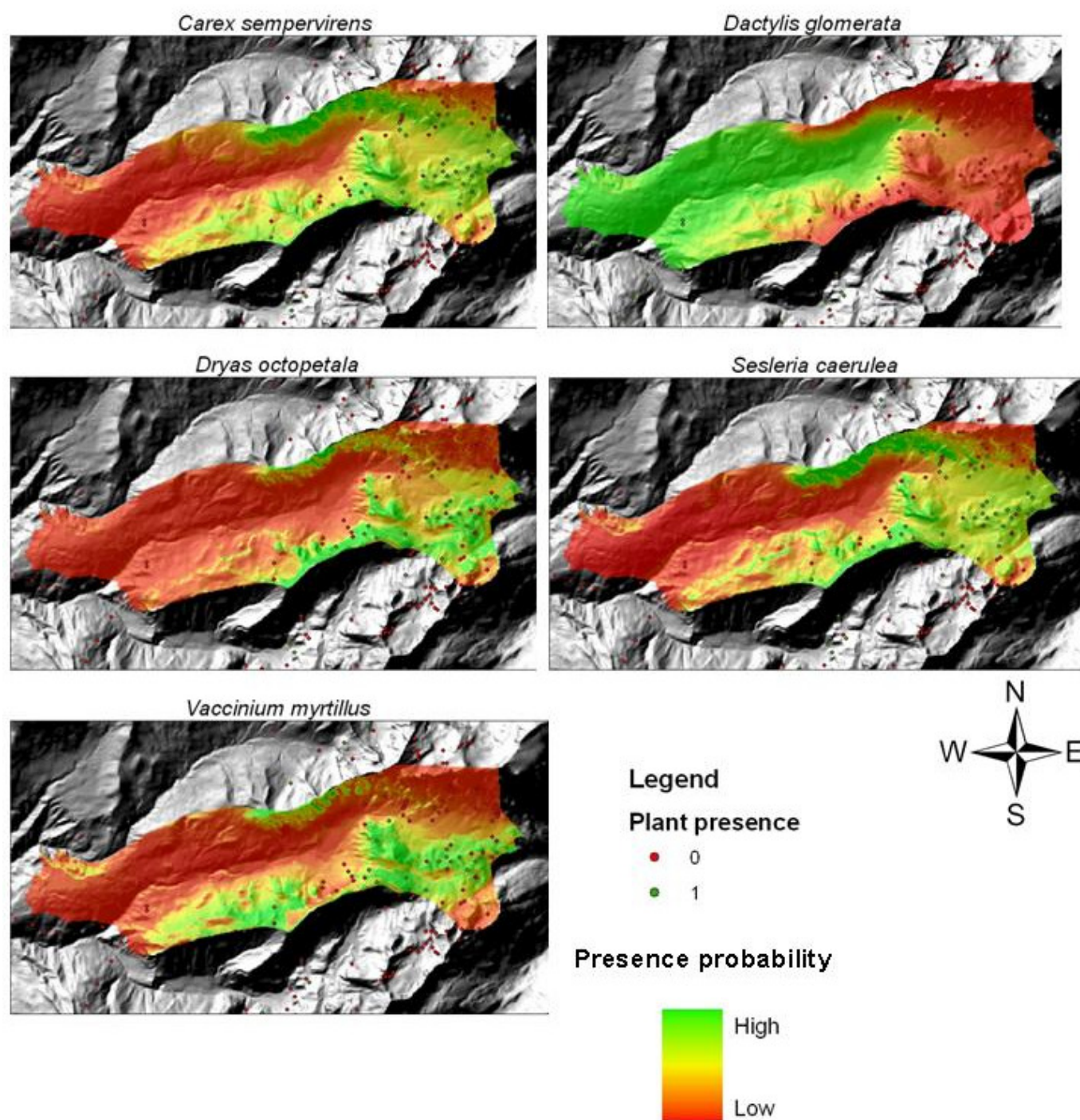


Figure 2: Spatial projections of the models calibrated with BIOMOD for each study species. Red points represent observed absence of the species and green points observed presence of the species.

Annex 3: Traits response to principal environmental component

Method:

To give an idea of more complex life conditions, a principal component analysis was performed on the most direct topo-climatic data available for the study zone: slope, moisture index, continentality index, topographical position, radiation, evapotranspiration, temperature and degree day (for details see table 1 in annex 1). The first two axes of the principal component analysis on environmental factors are approximately associated with temperature and radiation. Together the total axes explain the total variance in the data. A correlation circle indicating the relative contributions of environmental variables to these two axes is displayed in figure 1.

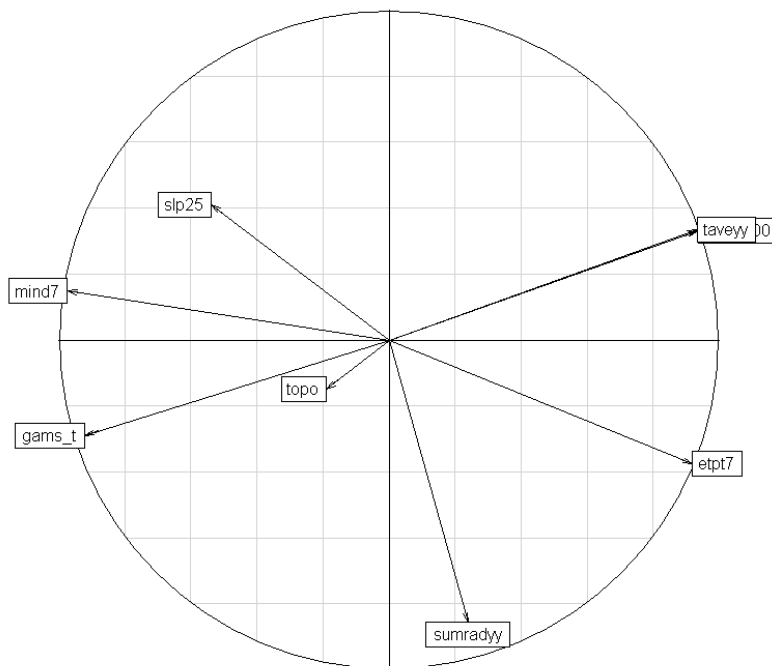


Figure 1 : Correlation circle from environmental topo-climatic data indicating the relative contributions of environmental variables to the two axes for relating to functional traits.

A set of mixed models was implemented with the value of the two first PCA axes as explanatory variable.

Results and discussion:

The responses to PCA axes are poorer for maximal height (only *Sesleria* react to the first axe and *Dryas* to the second) and biomass (*Dryas* to both axes and *Vaccinium* to first axe).

LDMC do not respond anymore. *Dactylis* C:N ratio displays a greater response to first axe.

Table X: P-values of the relationship between principal component values and traits

		LDMC	SLA	Hmax	Biomass	C:N
<i>Carex</i>	axe1	0.699	0.805	0.276	0.092	0.645
	axe2	1	0.945	1	0.538	0.505
<i>Dactylis</i>	axe1	0.333	0.617	1	0.054	0.0002***
	axe2	0.262	1	1	0.868	0.82
<i>Dryas</i>	axe1	1	0.321	0.168	0.099 **	0.335
	axe2	1	0.276	0.001**	0.008 **	0.541
<i>Sesleria</i>	axe1	0.099	0.553	0.005 **	0.419	0.999
	axe2	0.219	0.667	0.089	0.474	0.94
<i>Vaccinium</i>	axe1	0.249	0.003**	0.055	0.013 *	0.044 *
	axe2	0.773	0.446	0.111	1	0.721

By relating traits to principal components integrating the variation in all investigated gradients, we aimed at testing the effect on traits of other gradients when used in co-variation with the two stratifying gradients (temperature and radiation). Contrary to our expectations, none of these additional factors responded nor did they modify the strength of the response to the main gradients. The traits showing a significant response are the same as for temperature gradient and the responses did not differ much in their intensity. This is likely due to temperature and radiation being originally chosen to stratify the sampling, precisely because they proved to be orthogonal (uncorrelated) factors in the PCA analysis.