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Biogeography of intraspecific trait variability in matgrass (*Nardus stricta*): High phenotypic variation at the local scale exceeds large scale variability patterns

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ABSTRACT

Intraspecific trait variability (ITV) is an important component of functional ecology studies. While functional biogeography requires us to understand broad-scale patterns of trait distribution, ITV remains inadequately studied. Due to isolation of ranges, habitat fragmentation and sharp topoclimatic gradients over short distances, temperate mountains offer opportunities to study the ITV in widespread species. In this study, we explored ITV in geographically separated populations of matgrass (*Nardus stricta*), a clonal dominant plant species of mountain pastures. We measured plant height and leaf traits in 55 populations distributed across six European mountain ranges (from the Pyrenees to the Carpathians) to examine the structure of ITV. We hypothesised that the leaf economics spectrum (LES) and plant size exhibit a strong convergence across a prevailing gradient of growing season length (GSL). We then partitioned the ITV into between ranges, between populations nested in ranges and between individuals nested in populations, and examined the role of climate and local variables on the spatial variation and covariation of traits. Traits showed large ITV and higher between- than within-population variability. Leaf traits displayed a high dependency on height reflecting the growing phase climate and other local factors, while range identity alone (differentiation between ranges) explained a low to moderate amount of the variability in the traits. The strength of coordination between several of the LES traits was higher under shorter GSL due to late snowmelt, suggesting higher phenotypic integration. In the context of functional biogeography of mountain ecosystems, allometric effects and growing phase climate are both key to understanding ITV. We discuss the implications of these findings as they relate to future comparative studies of ITV in seasonal environments.

1. Introduction

There is a growing interest in understanding the role of plant intraspecific traits variability (ITV) in ecological processes (Albert, 2015). Yet studies at large scales are insufficient to draw general conclusions (e.g. Debussche and Thompson, 2002; Niinemets, 2015; Fajardo and

Piper, 2011). Distribution of traits across large spatial scales is a fundamental tenet of functional biogeography (Violle et al., 2014). While research has advanced our understanding of ITV at local scales, functional biogeography aims to shed light on its distribution across broader scales.

Given the paucity of empirical studies, little is known about the

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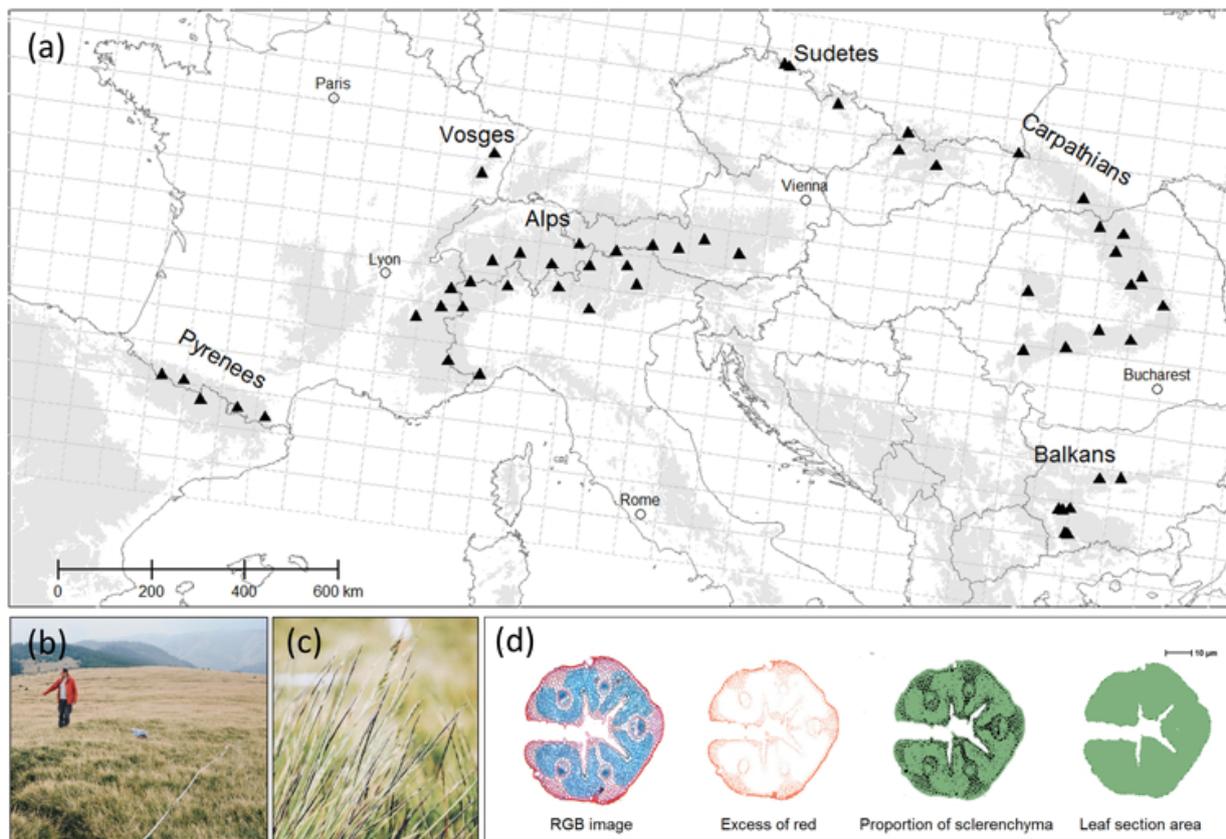


Fig. 1. a) Locations of *Nardus stricta* populations sampled in the temperate European mountains. b) Example of a subalpine pasture dominated by *N. stricta* in the Southern Carpathians. c) Close-up view of a *N. stricta* tussock with flowering heads. d) The histological traits derived from a stained leaf cross-section (RGB image).

extent and drivers of ITV across wide geographical regions. The main sources of ITV are genetic variability and phenotypic plasticity (Violle et al., 2012). Since individuals within populations are expected to be genetically related, we hypothesize that trait variability between distant populations is greater than within-population variability. ITV could also be regionally structured unless local factors override the variability between distant regions (e.g. Moreira et al., 2012). By elucidating the drivers of ITV across broad geographical areas, functional biogeography can help to predict the consequences of global changes on ecosystem functioning.

The effects of environmental pressures on plant species are mirrored in the correlation patterns among traits. The leaf economics spectrum (LES) is a trade-off between leaf traits indicative of fast acquisition vs. efficient conservation of resources (Wright et al., 2004). However, the existence of the LES at the intra-specific level across spatial scales is still debated (Messier et al., 2017; Niinemets, 2015; Wright and Sutton-Grier, 2012), and large-scale studies on this topic are scarce (Sartori et al., 2019; Asner et al., 2016).

Separated mountains constitute natural experiments that are replicated across latitudes (Körner, 2003) and offer opportunities to study ITV in widespread species. The control of bioclimatic factors on ITV has been examined in subalpine and alpine areas (e.g. Albert et al., 2010), as well as their effects on trait correlation (Boucher et al., 2013; Kichenin et al., 2013). However, studies across multiple mountain ranges are scarce (Midolo et al., 2019; Read et al., 2014 for global meta-analyses). Therefore, the potentially structured ITV due to isolated mountains and response to temperature gradients is of particular relevance to the analysis of large-scale patterns of trait distribution, but little is known about the total amount of ITV and its spatial partitioning.

Elevation gradients are often used to study ITV in mountain plants (Pfennigwerth et al., 2017). Growing season length (GSL) is an important component that might not be significantly correlated with

elevation but can be a key driver of ITV (Chalmandrier et al., 2017; Kudo, 1996), along with phenology (Coleman et al., 1994; Fajardo and Siefert, 2016). There is an overall tendency of a reduction in plant height with decreasing GSL. At the interspecific level, the maximum plant height stands as an independent dimension from the LES within the global plant trait spectrum (Westoby, 1998; Díaz et al., 2016). However, under the strong gradient of growing season length occurring in mountain ecosystems, we expect covariations between height and other traits of the LES that are modulated by the thermic regime (Midolo et al., 2019). For example, rapid growth rates occur in late snow melting sites on deep soils, and short-stature is associated with trait values reflecting fast acquisition of nutrients (Choler, 2005; Björk and Molau, 2007).

In this study, we focused on matgrass (*Nardus stricta* L.), a widespread grass commonly dominating subalpine pastures in the temperate mountains of Europe (Kissling et al., 2004). Because *N. stricta* is the major contributor to the biomass of these plant communities, one can expect an overwhelming effect of its trait diversity on ecosystem processes (Grime, 1998). Moreover, as this is a clonal species mostly with agamosperous reproduction that shows little or no genetic variation at the European level (Zorić, 2013), we expect that variation in traits represents phenotypic response to local conditions, rather than a to genetic differentiation (Miller and Cummins, 2014; Kissling et al., 2004, 2006). Therefore, we hypothesized low ITV within populations.

We measured several leaf traits and height to explore ITV and examine whether the LES traits and plant size are coordinated across gradients of growing conditions. Leaf nitrogen content (LNC) and specific leaf area (SLA) are associated with maximum photosynthetic rate and rapid acquisition of resources (Cornelissen et al., 2003; Wright and Westoby, 2000), while leaf tensile strength is a proxy for leaf toughness and tolerance to physical hazards (Wright and Westoby, 2002), indicating conservation of resources. Leaf histological traits have also

been related to LES (Pilote and Donovan, 2016). Here, we examined the importance of leaf sclerenchymatous tissues and questioned whether it brings further evidence to the existence of a LES at the intraspecific level. The sampling was conducted in six major mountain ranges of Europe, covering a significant part of the natural geographic distribution of *N. stricta* in European mountains. We addressed the following questions: (i) how is ITV partitioned among ranges (ITVrange), between-populations within a range (ITVpop) and between individuals within a population (ITVind)? (ii) how much of the ITVpop can be explained by the environment and how strong is the coordination between the LES traits and height along gradients of growing conditions? and (iii) does growing season length modulate the covariation between traits?

2. Materials and methods

2.1. Study design

The study area covers the Pyrenees, the Alps, the Sudetes, the Vosges, the Carpathians and some of the highest mountains from the northern Balkan Peninsula, i.e. Stara Planina, Rila and Pirin (Fig. 1a). The field sampling campaign was part of a long-term monitoring program initiated within the ODYSSEE project (<http://odyssee.granturi.ubbcluj.ro/>). We based our analysis on a systematic sampling design using a grid composed of cells measuring 80 × 80 km each, defined by the European Terrestrial Reference System (ETRS 89). Within each grid cell containing extensive subalpine pastures above treeline, we established at least one 100-m² site in a community dominated by *N. stricta* (cover > 25 %; Fig. 1b; Table A.1). Specialized habitats, such as extremely humid pastures, were not included in the sampling. We sampled a total of 55 sites.

2.2. Soil parameters

From each site, five soil cores were randomly collected, pooled together, sieved and air-dried prior to analysis. Total carbon and nitrogen content were measured on ground material using an elemental analyser (Flash EA1112, Thermo Scientific). Total phosphorus was extracted using the sodium hydroxide fusion technique and quantified in duplicate at 880 nm on a Cary 50 UV/Vis spectrometer (Varian Inc., Palo Alto, CA, USA). Soil pH was measured in a 1:4 (wt/wt) mixture of soil and distilled water with a pH-meter (inoLab 7110, WTW, Germany). Organic matter content was determined by loss on ignition analysis performed for 4 h at 550 °C. Soil parameters used in this study are summarized in Table 2.

2.3. Climate

Several summer climatic predictors were estimated for the June–August period. These included sum of the daily mean air temperature (GDD.JJA), precipitation (Prec.JJA) from the E-OBS dataset at a resolution of 0.1° (1985–2005; Haylock et al., 2008) and surface solar irradiance (SRad.JJA; J/m²) from the HelioClim-1 database at a resolution of approx. 20 km (1985–2005; Blanc et al., 2011). Although the available products differed in spatial resolution and might not fully reflect the local conditions determined by the topographical heterogeneity of mountain landscapes, they fully covered the study area and both provide daily estimates, which are essential for quantifying the accumulation of temperatures or precipitation following the snowmelt day, as estimated by remote sensing time series. To estimate the growing phase climate, we defined a period spanning from the snowmelt date of the sampling year and the sampling date. From this period we calculated the growing degree days (GDD.gro) and the sum of daily precipitation (Prec.gro) (Table 2, Fig. A.2). The accumulated radiation during the same period (SRad.gro) was calculated as the yearly average for the period of 1985–2005. The day of snowmelt was derived from the

MODIS 6 collection at a resolution of 250 m (Moderate Resolution Imaging Spectroradiometer; Myneni et al., 2002). The MODIS product had a high temporal resolution (8 days) which allowed an increased accuracy in the estimation of indices. Temperatures from the E-OBS dataset (2014–2015; corresponding to the two field campaigns) were used to calculate the sum of daily temperatures (Table 2).

2.4. Productivity

We used the integrated Normalized Difference Vegetation Index over the growth phase (NDVIint) as a remotely sensed proxy of primary productivity (Table 2). NDVIint was calculated by summing all NDVI values from the snow melting date to the sampling date. We calculated the NDVI using the moderate resolution data provided by MODIS, a sensor aboard the Terra satellite. Collection 6 of the MOD09Q1 products was downloaded from the Land Processes Distributed Active Archive Center (<https://e4ftl01.cr.usgs.gov/>). The 8 days composite at 250 m resolution, covering the period from 18 February 2000 to 27 December 2016, was processed following a protocol from Choler (2015). We also estimated the above-ground biomass (Table 2) per m² by clipping the vegetation within three random 50 × 50 cm plots (Fig. 1b). The green material was separated from the senescent leaves, oven-dried at 60 °C and weighed to obtain its proportion from the total.

2.5. Trait selection and measurement

We randomly selected 20 individuals of *N. stricta* (Fig. 1c) from each 100 m² plot. The collected leaf material was stored for maximum one day in wet tissues for laboratory analyses. (i) Vegetative height, distance from the ground to the top of the last photosynthetic part, was measured *in situ* for the 20 individuals. (ii) Leaf nitrogen and leaf carbon content (LNC, LCC) per unit of dry leaf mass (mg g⁻¹) were determined on dried leaf samples from 5 individuals in each population grid with an oscillating ball mill and the same elemental analyser used for soil. (iii) SLA, the ratio of leaf area to leaf dry mass (cm² g⁻¹), was measured from 10 individuals. (iv) Leaf tensile strength (N mm⁻¹) or resistance to tearing (Hendry and Grime, 1993) was measured for 10 individuals. We used a portable lab-made tearing device on which each leaf sample was first secured and then gradually stretched using a handcrafted dynamometer until breaking. Similar construction principles for the tearing device were described by Graça et al. (2005). (v) Three histological traits, namely leaf section area, proportion of sclerenchyma and excess of red in stained leaf cross sections were determined for five individuals with two replicates per individual. Middle part of the last two fully mature leaves were collected and preserved in a mixture of formaldehyde:ethanol:acetic acid (final concentration 10 %:50 %:5 %). Leaf segments were first dehydrated in sequential series of increasing concentration of ethanol and butanol, then embedded in paraffin. A microtome was used to cut cross-sections of 15 µm, which were then mounted on microscope slides. Staining with safranin and alcian blue (Sigma) was performed to distinguish between cellulose and lignified or cutinized cell walls. Lignified and cutinized cell walls were stained red, whereas cellulose cell walls were stained blue. Red-green-blue (RGB) images of the same resolution were taken using an Olympus BX51 microscope equipped with a CoolSnap Pro-color camera and used to automatically compute the anatomical traits. The first histological trait was leaf cross-section area, representing the total number of pixels from the whole leaf cross-section area (Fig. 1d, converted in µm²). The second histological trait was the proportion of sclerenchyma, estimated by counting the number of pixels exhibiting red colour, virtually representing tissues with lignified cell wall material (Fig. 1d). This was divided by the total number of pixels within the leaf cross-section area (Zhang et al., 2013). The third histological trait was an excess red index based on the RGB channel values. This was calculated using the formula $2 \times R - G - B$ for each pixel (Meyer et al., 1999), and averaged over the whole image (Fig. 1d). This trait was based on colorimetric information

Table 1

Overview, summary and variability of functional traits measured in this study. Abbreviations: CV, coefficient of variability.

Trait	Description	No. of samples (populations)	Mean	Range	CV (%)
Height	Vegetative height (cm)	1010 (51)	16.17	5.00 – 35.00	39.60
Leaf section area	Leaf cross-section area (μm^2)	248 (52)	1766.17	1136.70 – 2700.53	15.82
SLA	Specific leaf area ($\text{cm}^2 \text{g}^{-1}$)	519 (53)	7.88	5.19 – 12.05	13.64
Leaf strength	Leaf tensile strength (N mm^{-1})	525 (53)	1024.68	475.00 – 2360.00	25.76
LNC	Leaf nitrogen concentration on a dry-mass basis (%)	270 (54)	1.64	0.96 – 2.49	19.87
LCC	Leaf carbon concentration on a dry-mass basis (%)	270 (54)	45.59	42.53 – 48.75	2.08
Proportion of sclerenchyma	Ratio of sclerenchymatic tissue area to total leaf cross-section area (%)	248 (52)	15.45	7.04 – 25.13	20.88
Excess of red	Intensity of red channel from leaf cross-section area	248 (52)	18.72	7.55 – 37.79	31.06

expressing the lignification with a red intensity grade (Legland et al., 2017). For the automatic imagery analyses, we used ‘raster’ (Hijmans et al., 2019), ‘rgdal’ (Bivand et al., 2019) and ‘tiff’ (Urbanek, 2013) R packages.

2.6. Data analysis

To determine the percentage of variability of each trait attributed to ranges (ITVrange), populations within ranges (ITVpop) and individuals within populations (ITVind), we fitted linear mixed effect models for each trait with populations within range as random effects on the intercepts. We extracted variances using the *VarCorr* function from the ‘lme4’ R package (Bates et al., 2015). To estimate the total variability, we calculated the coefficient of variation (CV %) for each trait across populations.

We then performed a Principal Component Analysis (PCA) using population means (ITVpop) to investigate the multivariate trait covariation and how it related to environmental variables. To remove the effect of allometry in each trait, we fitted simple linear models of the population mean trait values in the function of height. The sum of model residuals and intercept were considered as the trait values adjusted for the allometric effect. To evaluate the trait variability within populations in the multivariate space, we used the CV of traits in each population (ITVind). Vectors of explanatory variables were fitted onto the PCA ordination using the environmental fitting method (*envfit* function; 10,000 permutations) in the ‘vegan’ R package (Oksanen et al., 2019).

The effect of height on each leaf trait was described using Standardized Major Axes (SMAs) regressions using population mean trait values in the ‘smatr’ R package (Warton et al., 2012). Pairwise leaf trait covariation was also determined using SMAs.

To estimate the proportion of variability explained by environmental factors and mountain range differentiation (identity) on ITV, we used linear regression with population means. For the LES traits, height was introduced as a covariate in all models. Multicollinearity was

minimized by the exclusion of highly correlated variables (Pearson correlation coefficient > 0.75) and checking the variance inflation factors (VIF) in the best linear models, using the *vif* function in the ‘car’ R package (Fox et al., 2020). A VIF value exceeding 5 indicated strong multicollinearity. Corrected Akaike’s Information Criterion (AICc) was used to select the best models from the set of candidate models constructed from all possible combinations of predictors, using the *dredge* function in the ‘MuMIn’ R package (Barton, 2019). We report all models with $\Delta\text{AICc} < 2$ (difference from the best model), as they have substantial support (Burnham and Anderson, 2002). To test for the effect of mountain range after accounting for the role of height and environmental factors, we used a likelihood ratio test (ANOVA) that compared each selected model with the one including mountain range. To evaluate the unique effects of height and environment, mountain range and their joint contribution on ITV, a redundancy analysis (RDA) in the ‘vegan’ R package (Oksanen, 2019) was applied on the best model ($\Delta\text{AICc} = 0$) for each trait.

To test for the role of geographical distance on ITV, we used Mantel tests (Oksanen, 2019) between the pairwise trait dissimilarity matrix and spatial distances (Euclidean distances; 9999 permutations). Two continuous and most extended mountain ranges were separately considered for comparison: the Alps and Carpathians.

To gain further insights on the modulation of leaf trait relationships by GSL, we stratified the onset of greening into two domains (intervals) based on the mean and distinguished early-melting sites (longer GSL) and late-melting sites (shorter GSL). An SMA was fitted to each domain for each combination of traits. Then, we tested for the significance of slopes in each domain and the homogeneity of slopes (common slope) and ‘elevations’ (intercept) between the two domains. All statistical analyses were performed using R version 3.5.1 (R Development Core Team, 2011).

3. Results

We found that the ITV was large for most traits. Height and excess of

Table 2

Summary and descriptive statistics for the full set of environmental factors used to explain ITV. Abbreviations: SD, standard deviation.

Variables	Description	Mean \pm SD	Range
GDD.JJA	Growing Degree Days; June – August ($^{\circ}\text{C} > 0$)	769.6 \pm 267.0	292.8 – 1486.7
Prec.JJA	Accumulated summer precipitation; June – August (mm)	274.6 \pm 107.6	92.2 – 573.6
SRad.JJA	Accumulated summer radiation; June – August (J/m^2)	177241.5 \pm 25278.2	120784.0 – 216765.0
GDD.gro	Growing Degree Days; snowmelt – sampling date ($^{\circ}\text{C} > 0$)	349.5 \pm 249.7	0.0 – 975.3
Prec.gro	Accumulated precipitation; snowmelt – sampling date (mm)	153.2 \pm 104.8	0.0 – 346.7
SRad.gro	Accumulated radiation; snowmelt – sampling date (J/m^2)	92029.4 \pm 52253.7	14716.0 – 231051.0
Onset	First snow-free date / onset of greening (Julian days)	150.8 \pm 16.2	110.0 – 177.0
AGB	Above-ground live biomass (g/m^2)	196.6 \pm 92.5	49.2 – 386.4
NDVIint	Integrated Normalized Vegetation Index; snowmelt – sampling date	87.7 \pm 28.1	35.1 – 163.2
NDVImax	Maximum Normalized Vegetation Index	0.7 \pm 0.1	0.4 – 0.9
Soil.OM	Soil organic matter content (%)	28.5 \pm 13.5	9.9 – 75.2
Soil.CN	Log soil C:N	3.9 \pm 0.2	3.5 – 4.3
Soil.NP	Log soil N:P	-0.5 \pm 0.5	-1.5 – 0.4
Soil.PH	Soil pH	4.2 \pm 0.3	3.7 – 4.8
Elevation	Height above sea level (m)	1951.1 \pm 377.6	1075.0 – 2543.0

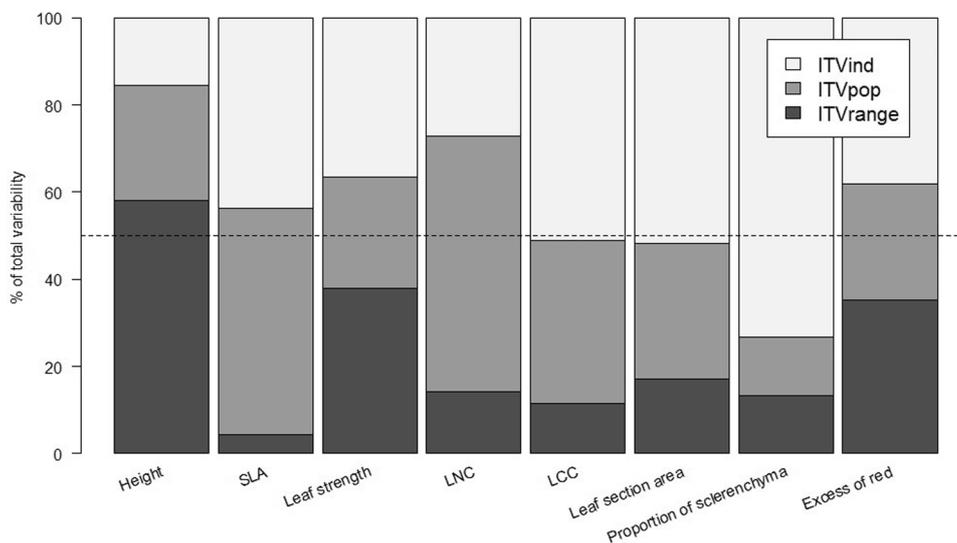


Fig. 2. Relative variance decomposition at the range, population (nested in range) and individual (nested in population) levels for each trait.

red were particularly variable with CVs of 39.6 % and 35.1 %, respectively. LCC was the least variable trait, with a CV of 2.08 % (Table 1). Results for variance partitioning strongly varied between traits (Fig. 2). For most traits, ITVrange was relatively small, contributing to less than half of the total variability, except for height (58.2 %), which showed relatively higher values in the northern ranges (Vosges and Sudetes) (Fig. A.1). The highest between-population ITV (ITVpop) was found for LNC (58.6 %) and SLA (52.0 %) (Fig. 2). Substantial contribution of variability within populations (ITVind) was found for the proportion of sclerenchyma (73.3 %).

In the PCA of environmental factors and height (Fig. 3a), the first axis was related to a gradient of growing phase climate and productivity proxies, while the second axis of variation was associated with summer climate and soil properties. The PCA of the population mean trait values displayed a strong co-variation structure along the first axis related to height, which was collinear with the proxies of primary productivity (above-ground biomass, NDVIint) and the climate of the growing phase (onset of vegetation, GDD.gro; Fig. 3b). When trait population means were adjusted for the allometric effect, the influence of these variables vanished and soil C:N became determinant (Fig. 3c). The

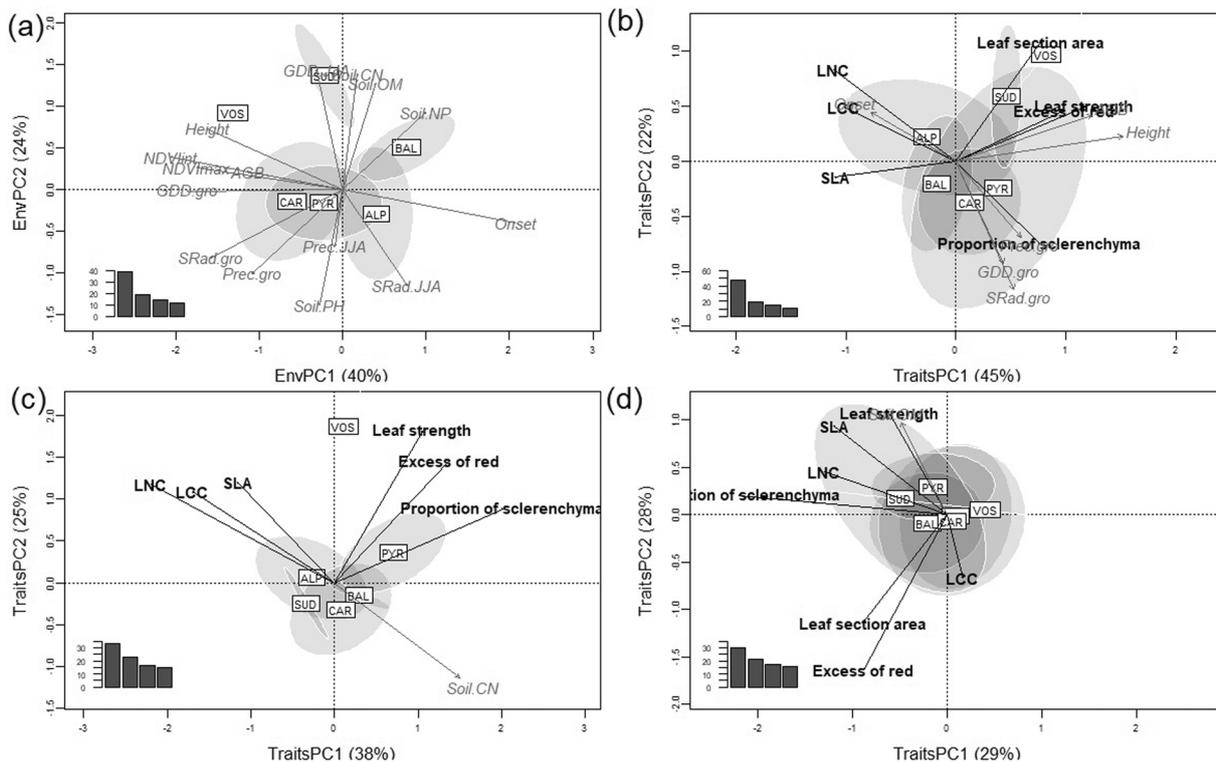


Fig. 3. Principal component analysis (PCA) of (a) environmental factors used in the study and plant height, (b) population trait means values, (c) population trait means adjusted for the allometric effect and (d) within-population coefficient of variability. The environmental variables and height were plotted as explanatory variables onto the trait ordinations using the environmental fitting method (*envfit*), while only significant correlations (P -value < 0.05) are indicated. Ellipses represent the distribution of mountain ranges across the multivariate space (confidence limit: 50 %). Barplots indicate the explained variance of the first four axes. Abbreviations: ALP, Alps; BAL, Balkans; CAR, Carpathians; PYR, Pyrenees; SUD, Sudetes; VOS, Vosges.

Table 3Evaluation of allometric effects on functional traits (SMA slopes with 95 % confidence intervals, R^2 and P -values between functional traits and height in \log_{10} base).

Type	Trait	SMA (95 % CIs)	R^2	P -value
Size	Leaf section area	3.11 (2.35,4.11)	0.18	0.004
Structural	SLA	-0.27 (-0.35, -0.21)	0.34	< 0.001
	Leaf strength	2.01 (1.54,2.61)	0.27	< 0.001
Chemical	LNC	-0.45 (-0.59, -0.35)	0.25	0.001
	LCC	-0.04 (-0.06, -0.03)	0.12	0.023
Histological	Proportion of sclerenchyma	2.77 (2.04,3.75)	0.01	0.445
	Excess of red	1.60 (1.19,2.15)	0.06	0.113

PCA of trait variability within populations (Fig. 3d) displayed no clear pattern. The effect of mountain ranges was less clear in the multivariate space, regardless of the measured trait.

We tested the allometric effects on population mean trait values by SMAs regressions (Table 3). Height-dependency was revealed for all traits except for the proportion of sclerenchyma and excess of red. The strongest height-dependency (negative) was for SLA (SMA $R^2 = 0.34$, P -value < 0.001).

The best fit models for height included predictors related to growing season length (Onset) and productivity (AGB). For the LES traits, the environmental factors differed among models, although some appeared to be common, e.g., GDD.JJA. The highest proportion of explained variability was for height (mean $R^2 = 0.71$), LNC (mean $R^2 = 0.62$) and SLA (mean $R^2 = 0.58$), while the lowest proportion of explained variability was for excess of red (mean $R^2 = 0.06$). Mountain range significantly increased the variability explained in some traits: the explained part of variance increased on average with 10 % for height, 16 % for leaf strength, 18 % for LCC, 13 % for the proportion of sclerenchyma and 26 % for the excess of red (Table A.2). None of the two partial Mantel tests indicated an influence of the geographical distance on the multiple trait dissimilarity (Carpathians: P -value = 0.096; Alps: P -value = 0.247).

The results of the RDA showed that the sources of variation differed across traits. The highest proportion of variability explained by variables (including the shared variability with range) was in height, SLA and LNC (> 50 %). Range alone explained around 10–25 % of the total

variability in traits, while no effect was observed for LNC (Fig. 4).

Overall, we found strong covariation between traits (Table 4). Adjusting trait values for the allometric effect generally weakened the relationships, and many of the significant bivariate relationships were eliminated. The SMA slopes of trait relationships did not significantly differ across the two domains of the onset of greening (non-heterogeneity of slopes; P -value > 0.05). However, for many significant trait relationships, the SMA slope remained significant only in late-melting situations (Fig. 5; Table A.3.). Shifts in ‘elevation’ between domains occurred in the case of several trait-relationships (P -value < 0.05; Table A.3.).

4. Discussion

Although the sources of variation differed across traits, ITV was in general higher between- than within-populations, and the differentiation between mountain ranges (range identity) alone explained a limited part of the total variability in traits after accounting for environmental differences. The population mean trait values significantly depended on height, which, as a measure of productivity, strongly depended on ITV. This suggests the overwhelming role of local conditions on ITV. Bivariate leaf trait relationships were also modified by GSL, with stronger trait coordination in late-melting sites, indicating selective pressures associated with different local thermal regimes. We thus highlight the pivotal role of growing conditions in shaping the distribution of ITV at broad scales, which brings significant insights into

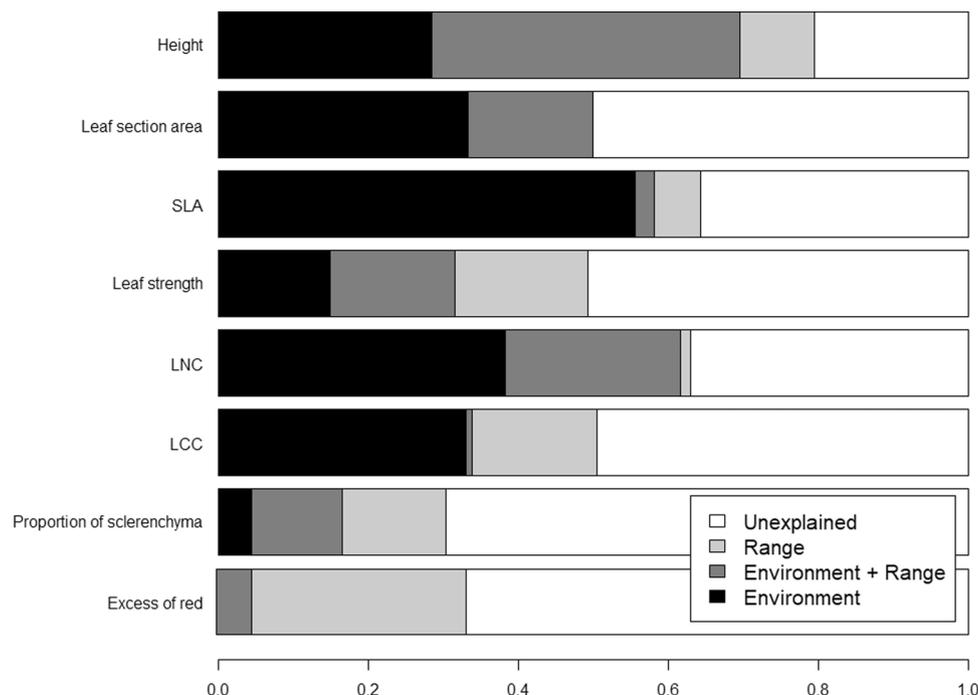


Fig. 4. Partitioning of trait variation at population level. The picture depicts the explained variability (adjusted R^2) of traits by environmental factors (with height as a covariate in the case of leaf traits) and mountain range, as calculated using RDA analysis. Both unique and shared contributions are shown.

Table 4

Bivariate relationships (SMA slope and significance level) among the functional traits using population mean trait values (\log_{10} scale). Corresponding relationships after adjustment for allometric effects are given in parentheses. Significant relationships are shown in bold. Levels of significance: *, P -value < 0.05; **, P < 0.01; ***, P < 0.001; ns, not significant.

	Leaf strength	LNC	SLA	LCC	Excess of red	Proportion of sclerenchyma
LNC	−0.94* (0.38 ^{ns})					
SLA	−0.58** (−0.36 ^{ns})	0.61*** (0.93*)				
LCC	−0.09 ^{ns} (−0.07 ^{ns})	0.09*** (0.17*)	0.15 ^{ns} (0.18 ^{ns})			
Excess of red	1.31* (1.33^{ns})	−1.43* (−3.47 ^{ns})	−2.41 ^{ns} (−4.17 ^{ns})	−15.07 ^{ns} (−20.39 ^{ns})		
Proportion of sclerenchyma	0.75* (0.66^{ns})	−0.81* (−1.72*)	−1.37 ^{ns} (2.07 ^{ns})	−8.57 ^{ns} (−10.12 ^{ns})	0.54 ^{ns} (0.50 ^{ns})	
Leaf section area	0.64** (0.57^{ns})	0.70 ^{ns} (1.49 ^{ns})	−1.18* (−1.79 ^{ns})	−7.36* (−8.76 ^{ns})	0.48 ^{ns} (0.43 ^{ns})	−0.89 ^{ns} (−0.87 ^{ns})

the functional biogeography of mountain plants.

We found higher between-population than within-population variability for several traits (height, SLA and LNC) that also appeared to have stronger responses to environmental factors. This is in line with our expectation that trait variation of this species, which exhibits low genetic variation across Europe (Zorić, 2013), is the result of an environmentally-based change in the phenotype. Albert et al. (2010) measured intraspecific traits for several alpine plants and found that the average ITVpop was 47 % for height. In contrast, Bergholz et al. (2017) showed that most trait variability existed within populations (ITVpop; 51–98 %) in two Mediterranean annuals. The higher ITVind found in our study for the proportion of sclerenchyma and slightly for LCC may have resulted from a high degree of small-scale environmental heterogeneity, which could be fostered by land-use intensity caused by grazing (Herz et al., 2017) or edaphic heterogeneity (Ravenscroft et al., 2014). On the other hand, we hypothesise that the higher ITVpop found in height, SLA and LNC is related to their better response to environmental factors (63–71 % of the variance at the population-level explained), as opposed to 0.07–52 % for traits with lower ITVpop. While most of the LES traits co-varied with height, the responses to environmental factors were largely idiosyncratic. This was similar to the findings from other studies on alpine species, which did not detect any general pattern in trait responses to gradients (Albert et al., 2010).

We found no clear evidence of a regional (range) discrimination in the multivariate trait space defined by the PCA, which seemed to be due to the overwhelming influence of local conditions. Although for some traits, differentiation between mountain ranges significantly improved the models, the proportion of explained variability was rather modest, except for the excess of red (around 26 %). A certain amount of the explained ITVpop was shared by allometry (height), environment and mountain range. The results of the Mantel test further indicated that geographical distance did not significantly influence trait dissimilarity within the Alps and Carpathians. These findings are consistent with studies showing that local factors can override the role of regional differentiation or global environmental drivers at interspecific (Bruehlheide et al., 2018) or intraspecific levels (Moreira et al., 2012). We suggest that low functional trait differentiation across ranges mirrors the similar local climatic conditions of the investigated sites. A higher level of trait differentiation may arise with the inclusion of more contrasting situations, such as lowland *N. stricta* grasslands from Northern Europe.

We found evidence of a correlation between leaf traits of the LES, which is consistent with patterns reported at interspecific level (Freschet et al., 2010; Bai et al., 2015). For example, there was a decrease in the proportion of sclerenchyma with increasing SLA and LNC on a mass basis, which may be the result of a reduction in leaf density (Garnier et al., 1997). The investment in denser tissues such as sclerenchyma was also reflected in the negative association between leaf strength and LNC. Therefore, our results support the hypothesis that the costs associated with greater investments in cell walls underpin the LES (Onoda et al., 2017).

We explain the observed allometric effect in *N. stricta* by the presence of a steady growth rate gradient driving the convergence of the

LES and plant height. To reach maturity with flowering heads, individuals from sites with a shorter growing phase exhibit an increased growth rate and a smaller final stature. Although the LES traits have been generally shown to have little interspecific variation with maximum plant height (Price et al., 2014), prominent gradients such as soil resources were also shown to determine coordinated responses of multiple independent leaf traits (Jager et al., 2015). Leaf intraspecific morphological and biochemical traits were shown to change with height, but most of the studies were conducted on trees (e.g., Kenzo et al., 2006). In our study, predictors such as the growing season length factors (GDD.gro, onset of vegetation) or productivity (above-ground biomass) were retained in the best models. Similar spectra in trait variability were observed at the interspecific level in alpine environments along a snow-melt gradient, where higher SLA and LNC were found in plant communities characterized by a short growing period, as opposed to taller communities across a meso-topographical gradient (Choler, 2005). Our results are also consistent with intraspecific level studies on tundra and alpine species, which demonstrated that reduced plant stature and higher LNC and SLA were the result of a quick acquisition strategy at the cold end of the species niche (Kudo, 1992, 1996; Boucher et al., 2013). Although histological traits like the proportion of sclerenchyma and excess of red were not associated with plant height, they showed positive relationships with LES traits indicative of conservative strategies (e.g., leaf strength) and negative with LNC. Potential drivers of the variability in histological traits like grazing intensity remain to be elucidated for such keystone species of pastures.

Growing season-related bioclimatic variables such as snow cover duration and GSL are key drivers of ecosystem functions (Choler, 2015). We found that accounting for the season length, using the derived time of vegetation onset and GDD from remote sensing data and climate variables is essential for understanding ITV patterns in *N. stricta*. Notably, these variables explained a large part of the variability in plant height, which in turn proved to be essential in modelling variability of the LES traits. This should prompt us to pay more attention to these variables to improve our models of functional diversity in seasonally snow-covered ecosystems (Carlson et al., 2015). More accurate estimations of growing phase bioclimatic parameters, like information derived from temperature data loggers (Choler, 2018), could provide a better understanding of the distribution of ITV.

Substantial evidence exists for climate-related trends in the relationships among traits (Wright et al., 2005), but less attention has been given to the effects of conditions during the growing phase (Geng et al., 2014). We demonstrated that the onset of greening is a key determinant of ITV and also had the potential to modulate relationships between traits. Although we found that the slopes of trait scaling relationships were virtually constant across the two domains defined by onset of greening, we showed that most of the significant bivariate trait relationships were stronger in late-melting sites (shorter GSL). This suggests that strength of correlations between traits (i.e. the intensity of phenotypic integration; Boucher et al., 2013) is higher in very specialized habitats where species richness is low (Björk and Molau, 2007). The SMA slopes found significant within both domains in some cases

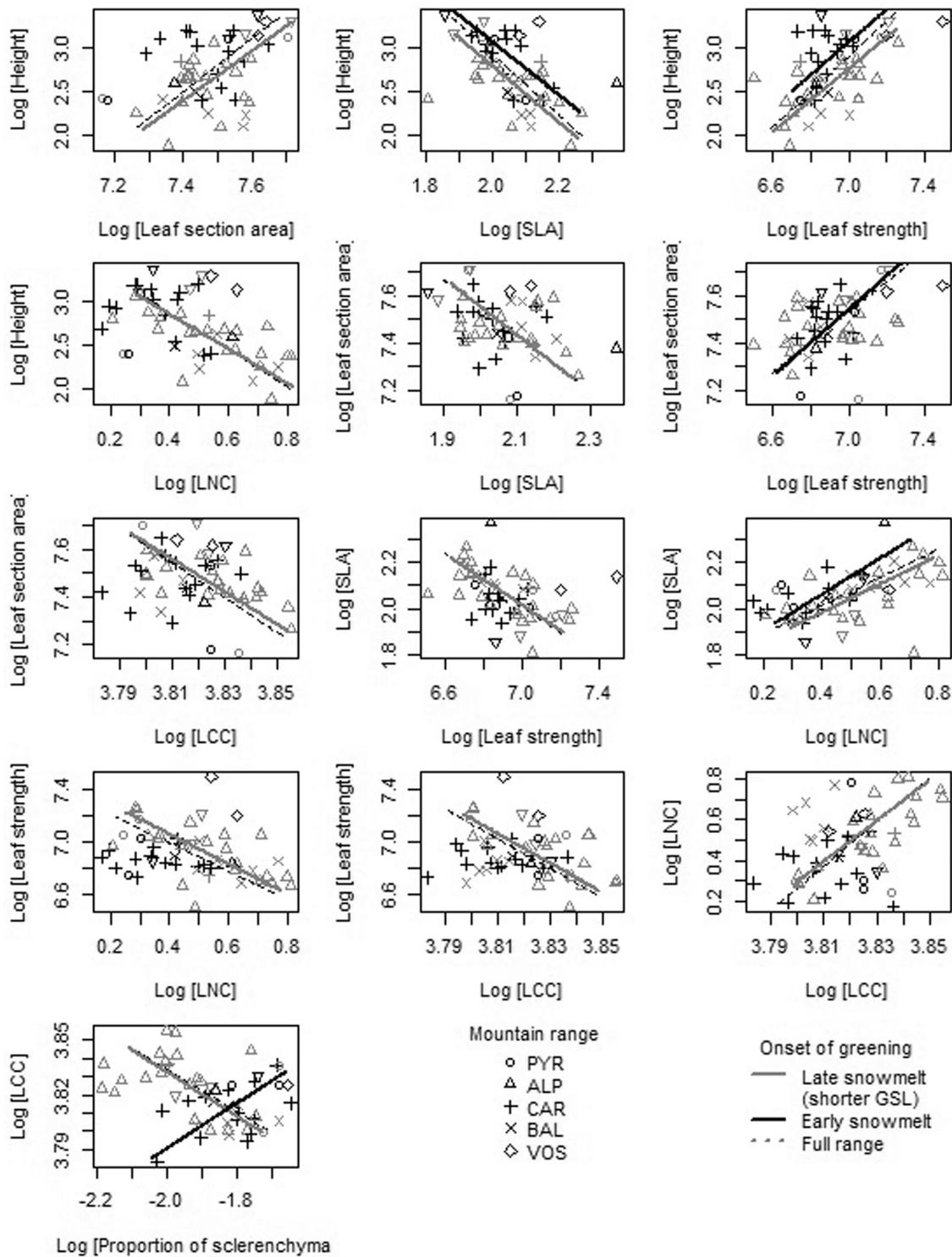


Fig. 5. Modulation of the bivariate traits relationships by the onset of greening. Only significant relationships are shown (P -value < 0.05). Associated statistics are given in Table A.3.

suggest that GSL has no implications for trait integration. However, significant shifts in ‘elevation’ (intercept) suggest greater investment in one trait, for example in height at a given level of SLA or leaf strength, to increase competitiveness in more productive sites under more extended growing season.

5. Conclusion

We provide evidence for a large amount of ITV within clonal *N.*

stricta dominating subalpine mountain pastures in Europe. The LES traits show strong allometric effects in relation to the climate of the growing phase. Overall, our findings emphasize consideration of local soil and bioclimatic drivers when biogeographic studies are conducted. Although traits do not exhibit a generally marked differentiation among the mountain ranges, a certain amount of the variability in some of the traits could be attributed to the pure regional effect. Moreover, we show that short GSL can strengthen the integration between traits and modulate trait relationships. This highlights the importance of

accounting for snow melting patterns to predict intraspecific functional diversity in temperate mountains. To gain further insights on the ITV of dominant species in mountain habitats, genetic analysis will have to be carried out in addition to trait measurements. This will be pivotal to better assess the relative importance of local adaptation and phenotypic plasticity in ITV patterns across scales.

Author's contributions

P.C. and M.P. planned and designed the research. P.C., M.P., P.D.T., A.S., B.I.H., J.S. and S.B. conducted the fieldwork. Z.R.B. and D.P. performed the leaf cross-sections and the measurements for the anatomical traits. A.S. and P.D.T. prepared the samples for chemical analyses. P.D.T. and P.C. performed the statistical and imagery analyses, with contributions from C.B., W.T. and J.R. P.D.T. and P.C. wrote the manuscript, and all authors contributed critically to the drafts and gave final approval for publication.

Data accessibility

ODYSSEE project database are available at <https://odyssee.osug.fr/login>.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ppees.2020.125555>.

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