

1 BIOC (Biodiversity and Conservation) 2013 22(10):2353-2374

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7 **Intraspecific phenotypic variability of plant functional traits in contrasting**  
8 **mountain grasslands habitats**

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28  
29 **Abstract**

30 Empirical studies that link plants intraspecific variation to environmental conditions are  
31 almost lacking, despite their relevance in understanding mechanisms of plant adaptation, in  
32 predicting the outcome of environmental change and in conservation. Here, we investigate  
33 intraspecific trait variation of four grassland species along with abiotic environmental  
34 variation at high spatial resolution (n=30 samples per species trait and environmental factor  
35 per site) in two contrasting grassland habitats in Central Apennines (Italy). We test for  
36 phenotypic adaptation between habitats, intraspecific trait-environment relationships within  
37 habitats, and the extent of trait and environmental variation. We considered whole plant,  
38 clonal, leaf, and seed traits. Differences between habitats were tested using ANOVA and  
39 ANCOVA. Trait-environment relationships were assessed using multiple regression models  
40 and hierarchical variance partitioning. The extent of variation was calculated using the  
41 coefficient of variation. Significant intraspecific differences in trait attributes between the  
42 contrasting habitats indicate phenotypic adaptation to in situ environmental conditions.  
43 Within habitats, light, soil temperature, and the availability of nitrate, ammonium, magnesium  
44 and potassium were the most important factors driving intraspecific trait-environment  
45 relationships. Leaf traits and height growth show lower variability than environment being  
46 probably more regulated by plants than clonal traits which show much higher variability. We  
47 show the adaptive significance of key plant traits leading to intraspecific adaptation of  
48 strategies providing insights for conservation of extant grassland communities. We argue that  
49 protecting habitats with considerable medium- and small-scale environmental heterogeneity is

50 important to maintain large intra-specific variability within local populations that finally can  
51 buffer against uncertainty of future climate and land use scenarios.

52

53 Keywords

54 Clonal growth, Intraspecific trait variation, Leaf traits, Phenotypic adaptation, Phenotypic  
55 plasticity, Seed mass

56

57 Abbreviations

58 SLA Specific leaf area

59 LDMC Leaf dry matter content

60

61 Nomenclature

62 Pignatti (1982) for taxa; Venanzoni & Kwiatkowski (1995) for the syntaxa mentioned in  
63 Table 1.

64

65 Electronic supplementary material

66 The online version of this article (doi:10.1007/s10531-013-0484-6) contains supplementary  
67 material, which is available to authorized users.

68

69

70 Introduction

71

72 Trait-based approaches increasingly contribute to link environmental changes with plant  
73 community variation (e.g. Webb et al. 2010; Wellstein et al. 2011). Studies on interspecific  
74 trait-environment relationships make it clear that plant species are related to environmental  
75 conditions via their functional traits (e.g. Poorter et al. 2009). However, trait variation extends  
76 beyond interspecific differences being affected by intraspecific phenotypic and genetic  
77 variation (Albert et al. 2010; Nicotra et al. 2010). Intraspecific (i.e. within-species) variation  
78 is partly due to phenotypic plasticity, i.e. the production of multiple phenotypes from a single  
79 genotype (e.g. Miner et al. 2005). Phenotypic plasticity gives a plant the ability to adjust its  
80 performance by altering morphology and/or physiology in response to varying environmental  
81 conditions throughout its lifespan. This phenotypic adaptation is also named phenotypic  
82 accommodation (see West-Eberhard 2005; Badyaev 2009) and is considered to confer a  
83 benefit on the organism with regard to its present relationship with its environment (Sultan  
84 1987). Moreover, intraspecific variation can be caused by inheritable differences in gene  
85 expression and function (i.e. epigenetic changes, addressed as microevolution, Bossdorf et al.  
86 2008) and by differences in the genotype which can be induced by local long-term adaptation  
87 to the environment (i.e. evolution).

88

89 Since plants as sessile organisms are known for their functional variation within both  
90 species and individuals (Miner et al. 2005; Hulshof and Swenson 2010), it is likely that finer-  
91 scale trait variation plays an important role in controlling species establishment and  
92 persistence (e.g. Violle et al. 2012). Actually, it has been proved that many plant populations  
93 exhibit significant phenotypic variation for a range of traits within very small areas (Linhart  
94 and Grant 1996), including life-history characters (Linhart 1988), pathogen and herbivore  
95 resistance (Burdon 1987; Simms 1990), and nutrient allocation related to resource capture and  
96 competitive ability (Turkington and Aarssen 1984; Turkington 1989). Intraspecific trait  
97 variation may lead to phenotypic differentiation resulting in different abilities of plants to  
98 cope with environmental change, i.e. phenotypic specialization (e.g. Bolnick et al. 2003). In  
99 this context, the adaptive capacity of traits related to specific ecological processes such as  
dispersal, establishment, competition, regeneration and flowering, might be of particular

100 importance. While these traits have been tested for variation across communities taking into  
101 account relative species cover (community-weighted means (CWM), e.g. Garnier et al. 2004;  
102 Wellstein and Kuss 2011), their intra-specific variation in response to changing environmental  
103 conditions has rarely been tested so far. The knowledge on the mechanisms and of the extent  
104 of plant adaptation is a prerequisite when predicting the outcome of climate and land use  
105 changes. At the same time, there is an increasing demand to incorporate climate change issues  
106 into conservation planning (e.g. Groves et al. 2012; Mawdsley et al. 2009).

107 Since the analysis of trait variation along gradients and under contrasting  
108 environmental conditions provides an approach to quantify intraspecific variation and niche  
109 breadth of individual species (Ackerly and Cornwell 2007), it is of high interest to test for  
110 phenotypic adaptation in this context. We selected key plant traits and environmental  
111 conditions, which are likely candidates to stay in trait-environment relationships according to  
112 evidence in current literature (Table 2). Those traits were sampled by a large amount of  
113 individuals, in order to cover the phenotypic variability of leaf, seed, clonal and whole plant  
114 traits in four representative species of montane nutrient poor dry grassland ecosystems  
115 (*Sesleria nitida*, *Lotus corniculatus*, *Astragalus sempervirens*, *Thymus longicaulis*) in two  
116 habitats with contrasting environmental conditions in terms of soil chemical, physical  
117 parameters and light availability.

118 We hypothesize that phenotypic variation of leaf, seed, clonal and whole plant traits is  
119 adaptive at small geographical scales (i.e. few 100 m to few centimetres). In detail, we  
120 hypothesize that trait attributes of species significantly differ between habitats with  
121 contrasting environmental characteristics (H1), and that trait attributes are linked to  
122 environmental gradients within a habitat (H2). Furthermore, we explore the relationship  
123 between trait and environmental variation, searching for conservative, regulated versus highly  
124 plastic traits. Finally, we discuss the implications of our findings for nature conservation and  
125 climate change adaptation.

## 128 Materials and methods

### 130 Study area and site selection

131  
132 The Nature Reserve ‘Montagna di Torricchio’ (Central Apennines, Italy, online resource  
133 ESM 1a) provides areas of montane grasslands under different environmental conditions. The  
134 ones considered in this study are located in contrasting habitats, on north and on south-facing  
135 slopes along the SW–NE orientated valley (Val di Tazza), between 1,100 and 1,200 m a.s.l.  
136 Mean annual precipitation reaches 1,250 mm and mean annual temperature is around 11 °C  
137 (Halassy et al. 2005). Jurassic-Cretaceous limestone (scaglia rosata) prevails in the area. The  
138 Reserve is under protection regime since 1970; consequently the grazing activities are  
139 forbidden since that time. Previously, the grasslands were grazed mostly by sheep and cows,  
140 sometimes also by small groups of horses. We selected two study sites with an area of about 1  
141 ha each representing the contrasting environmental conditions of the north and south slope  
142 (online resource ESM 1b; Table 1). The north-facing slope (habitat N) is covered with a dense  
143 grassland assigned to the association *S. nitidae*–*Brometum erecti*, here a semi-mesic  
144 secondary community originated by the destruction of a former beech forest. The south-facing  
145 slope (habitat S) hosts open grassland with a more scanty cover, assigned to *Asperulo*  
146 *purpureae*–*Brometum erecti*, a more xerophilic, mostly secondary community replacing both  
147 a beech forest as well as the local uppermost fringe of a mixed sub-Mediterranean open forest  
148 dominated by *Ostrya carpinifolia* and *Quercus spp.* Paleobotanical and archaeological  
149 evidence (see Branch 2012; Barker et al. 1991, for reviews) suggest the onset of the

150 deforestation of the beech forest at higher elevations close to the summits in most of the  
151 northern and central Apennines, to date back to the time of the spread of pastoralism and the  
152 establishment of the altitudinal transhumance in the area, at ~ 4,700 cal. years BP, a period  
153 with warmer and drier conditions than at Middle Holocene.

154 Nevertheless, rocky outcrops, poorly developed, shallow, skeletal soils or solifluction  
155 on steeper slopes occur all over the study area, due to its geological assessment (Kwiatkowski  
156 and Venanzoni 1994). These sites, located far below the climatic treeline at this latitude  
157 (1,900–2,000 m a.s.l.) could hardly carry a close canopy, if any, of forest trees, even in  
158 absence of human disturbance. They can therefore be suggested as primary stands of  
159 "permanent" communities of more or less xerophilic grasslands, already growing "in situ"  
160 before the Copper- and Bronze Age deforestation, from which the present day, widespread,  
161 secondary grasslands developed. This grassland/forest mosaic close to summits and its  
162 changes induced by a long history of grazing can be considered as a general pattern all over  
163 the study area.

164

#### 165 Study species

166

167 From a list of species occurring in both habitats we selected four perennial representatives of  
168 montane grasslands: *S. nitida* ssp. *nitida* (Poaceae), *T. longicaulis* (Lamiaceae), *L.*  
169 *corniculatus* L. (Fabaceae), *A. sempervirens* (Fabaceae). Due to their more or less accentuated  
170 degree of orophytism, they can be assumed as persistent component of these grasslands  
171 during the late-glacial/holocene. The frequency of each species in each habitat is given in the  
172 online resource ESM 1c. These species represent different life forms in the community, i.e.  
173 grass, woody forb, legume, and dwarf shrub. The grass *S. nitida* is an efficient colonizer on  
174 scree and rocky outcrops; it has a secondary root system which is restricted to the upper soil  
175 layer. The species exhibits a high capacity of clonal growth by epigeogenous rhizomes. *S.*  
176 *nitida* is very abundant and dominant in the habitat N and is abundant but scattered as thick  
177 tussocks in the habitat S. The woody forb *T. longicaulis* develops a tap root and forms a dense  
178 mat with thin stems, often prostrate and creeping (Pignatti 1982). It has a high capacity of  
179 vegetative spread by horizontal above ground stems. *T. longicaulis* is very abundant in habitat  
180 S but is also widespread in habitat N where it is scattered in small patches on rocky outcrops  
181 and on shallow soil. *L. corniculatus*, one of the most important legume species in dry and  
182 nutrient poor grasslands, is less abundant on both slopes but shows a similar distribution  
183 pattern. The species can grow clonally by hypogeogenous rhizomes (own observations from  
184 the present study), it develops tap roots and has a high capacity to withstand soil erosion and  
185 is a highly efficient accumulator of nitrogen, for which it is used also in agricultural  
186 management (Carter et al. 1997). The dwarf shrub *A. sempervirens* is a spiny, long-living  
187 species (over many decades) growing in summit grasslands on limestone (Pignatti 1982). It is  
188 not capable of clonal growth; it produces deep-reaching tap roots which enable the species to  
189 withstand erosion and to reach deeper water reserves in the soil. *A. sempervirens* is quite rare  
190 and has the same density on both slopes; it grows on patches with deeper soil or alternatively  
191 uses cracks in the rocks for the development of its tap root.

192

#### 193 Sampling of plant traits and environmental parameters

194

195 For each species in each habitat we randomly selected thirty robust, well grown and adult  
196 individuals without symptoms of pathogen or herbivore attack. All field sampling of plant and  
197 environmental parameters was done from June 26th to 30th in 2010. In each selected  
198 individual we measured the following traits that are associated with different life-history  
199 processes (see Table 2): (i) leaf traits (specific leaf area [SLA]; leaf dry matter content

200 [LDMC]), (ii) whole plant traits (height; horizontal stem length), (iii) clonal traits (no. of  
201 bifurcations/nodes per cm; distance between bifurcations/nodes) (see Table 2). As an  
202 exception, seed traits (iiii) (i.e. seed mass and seed germination), were measured from a  
203 random rate of seeds collected from all individuals in each habitat. The trait sampling  
204 followed standard procedures (Cornelissen et al. 2003; Kleyer et al. 2008). In particular, leaf  
205 traits were measured on three leaves per individual; after the measurement of the fresh weight  
206 and area, the leaves were oven-dried (80 °C for 48 h) to measure their dry weight. The plant  
207 height and horizontal stem length were measured in the field, while the clonal traits were  
208 determined in the laboratory on the harvested individuals. Seeds were air-dried and manually  
209 cleaned; seed mass was calculated using a balance with accuracy 0.0001 g. Germination tests  
210 were carried out at *Germoplasm Bank for ex situ conservation of anfiadriatic plant species*  
211 (ASSB), according to the international seeds germination protocols (ISTA 1999). Seeds were  
212 sown in Petri dishes with 1 % of bacteriological agar and germination was tested at 20 °C  
213 constant temperature and 12/12 h photoperiod. We tested four replicates of 25 seeds for each  
214 species per habitat; this represents a commonly used amount of seeds in ecological studies.  
215 The germination tests were completed after 30 days.

216 For the functional traits, only 9 % of the values are lacking. This originated from the  
217 absence of seeds in *L. corniculatus* in the habitat N during the sampling season. In habitat S  
218 seeds were usually present.

219 Additionally, in order to adjust analyzes for plant size, we sampled the biomass of all  
220 individuals of *L. corniculatus* and *T. longicaulis*. We collected the entire individual, i.e. above  
221 and belowground biomass; samples were oven-dried at 80 °C for 48 h and weighted  
222 (accuracy: 0.001 g). In case of *S. nitida*, it was not reasonable to adjust for plant size, since  
223 the species does not develop huge individuals because clonal offspring disintegrates after  
224 some years resulting in no differences appearing with age. In case of *A. sempervirens* we were  
225 not allowed to collect the plant specimen for biomass measurements in the nature reserve.

226 We used a sample size (30 individuals per species per habitat) 3 times larger than the  
227 one required by standard protocols (e.g. Cornelissen et al. 2003) for measuring plant traits in  
228 order to take into account the effects of environmental heterogeneity of the habitats. Soil  
229 physical and chemical parameters and light availability were measured at the exact position of  
230 each sampled plant individual. Soil samples were taken only after the measurements of traits  
231 in the field.

232 The sunlight reaching the canopy [Photosynthetic Active Radiation (PAR)] was  
233 measured using a PAR/LAI Ceptometer (LP80 AccuPAR—Decagon Devices, inc.). The  
234 percentage of soil moisture and the soil temperature (°C) were measured using a humidity and  
235 temperature probe meter (Tr 46908). For the analysis of soil chemical parameters, a soil  
236 sample (3 cm diameter, usually 10 cm depth; on rocky outcrops the soils were more shallow)  
237 was taken at the exact position of each plant individual. We determined the pH value and  
238 conductivity (in water), the total nitrogen (Nt) and total carbon (Ct) content (CNA analyzer  
239 FlashEA 1112, Thermoquest) and the CaCO<sub>3</sub> (Calcimeter 08.53, Eijkelkamp) according to  
240 Scheibler (Hoffmann1991). The C/N ratio was calculated considering that total carbon minus  
241 the CaCO<sub>3</sub>-carbon gives the organic carbon in the soil.

242 Samples were extracted with calcium-acetate-lactate (CAL) for the determination of  
243 plant available phosphorus (P<sub>CAL</sub>) and potassium (K<sub>CAL</sub>) (Hoffmann 1991) as well as  
244 nitrate(NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) (FIA, flow injection analysis, Firma MLE). The  
245 elements K, Mg and P were determined with the ICP-OES (Vista-Pro) spectrometer (Varian).

246  
247 Data analyses  
248

249 Significant differences in environmental variables between species and habitats (at the place  
250 of growth of individuals of each species) were evaluated by two-way ANOVA, followed by  
251 post hoc test (Tukey HSD). Main effect of habitat, species and their interactions are given in  
252 Table 3.

253 For *S. nitida* and *A. sempervirens*, ANOVA was used to test for the significance of  
254 differences in functional trait values between the two habitats. For *L. corniculatus* and *T.*  
255 *longicaulis*, ANCOVA was used to adjust for the effect of plant size (biomass) and to test for  
256 the significance of differences in functional trait values between the two habitats (e.g.  
257 McCarthy and Enquist 2007).

258 For seed germination, ANOVA was used since no biomass data were available for  
259 plants from which seeds were collected. Prior to statistical analysis, the traits values were log  
260 transformed when conditions of normality were not met or in order to improve homogeneity  
261 of variances. In all analyses, the level of significance was  $p < 0.05$ .

262 We applied linear regression and hierarchical variance partitioning to evaluate the  
263 intraspecific trait-environment relationship for each trait per species and habitat. For each trait  
264 (response variable), we fitted the full model of all measured environmental parameters,  
265 leading to 12 distinct models per species. In case a model revealed a significant trait-  
266 environment relationship we applied hierarchical variance partitioning and subsequent  
267 bootstrapping (package *relaimpo*, Grömping 2006) in order to evaluate the relative  
268 importance of explanatory environmental variables on a certain trait (Grömping 2006; Murray  
269 and Conner 2009). Prior to analyses, data were log, square root or arcsin transformed when  
270 necessary. For linear regression, ANOVA and ANCOVA we used the package *nlme*.

271 Additionally, we use the coefficient of variation (CV; SD divided by the mean) as a  
272 relative measure of phenotypic variability in order to assess and compare the degree of trait  
273 and environmental variation within habitats. The CV has frequently been used in the context  
274 of environmental and trait variation (e.g. Lemke et al. 2012). We calculated the CV for each  
275 trait of a species and for each environmental parameter (measured at the place of growth of  
276 the individuals of each species) within a habitat (Table 5) as well as throughout species and  
277 habitats (Table 6). We used quartiles based on data of all parameters for all species within and  
278 throughout habitats (data not shown) to categorize four classes of variation (CV) for  
279 environmental parameters as well as for traits, i.e. low, medium, high, and very high CV  
280 (online resource ESM 2).

281 Our further investigation of the relationships of trait- and environmental variation  
282 (CV–CV graphs, Fig. 1) is based on the results of linear regression and hierarchical variance  
283 partitioning as we investigated the most important, significant trait-environment relation-ships  
284 (Table 5). Each CV–CV graph contains a reference line which allows distinguishing if a trait  
285 varies more than expected from the null model. The null model is that in evolution the trait  
286 variability follows the variability of the environment. Values of traits that lay above the  
287 reference line indicate higher trait variability, values below the line indicate lower variability  
288 than expected (Fig. 1).

289 All statistical analyses were performed using R (Version 2.13.1) (R Development Core  
290 Team 2010).

291

292

## 293 Results

294

### 295 Phenotypic differences between habitats

296

297 All tested environmental factors, i.e. light availability (PAR), water availability (soil  
298 moisture), nutrient availability (plant available phosphorous, plant available potassium,

299 magnesium, nitrate, ammonium, C/N ratio, soil pH, soil conductivity), and soil temperature,  
300 were significantly different between the two habitats (Table 3). This contrast was mirrored by  
301 significant differences in mean values of leaf traits, whole plant traits, clonal traits and seed  
302 traits within species between the two habitats (Table 4). The grass *S. nitida* and the dwarf  
303 shrub *A. sempervirens* exhibited significantly different trait attributes for all tested traits. The  
304 legume *L. corniculatus* showed significant differences in 2/3 of the traits, but no  
305 differentiation in the clonal traits (number of nodes and distance between nodes) was  
306 observed. The woody forb *T. longicaulis* differed in half of the traits but no differentiation in  
307 LDMC, in clonal traits (number of nodes and distance between nodes), and in germination  
308 rate was observed (Table 4).

309

#### 310 Intraspecific trait-environment relationships within habitats

311

312 Based on linear regression and the results of hierarchical variance partitioning, throughout  
313 habitats and traits the most important, significant drivers of trait-environment relationships in  
314 sequence are soil temperature, NO<sub>3</sub>, light, NH<sub>4</sub>, magnesium and potassium (Table 5, online  
315 resource ESM 3).

316

317 In at least one of the study species, these parameters strongly and significantly affected  
318 at least one trait. Twice as much trait-environment relationships were seen in the north-facing  
319 slope compared to the south-facing slope. The SLA showed an increase with increasing  
320 nitrate availability in *A. sempervirens* on the south-facing slope; the LDMC showed an  
321 increase with increasing magnesium availability but a decrease with increasing potassium  
322 availability in *T. longicaulis* on the north-facing slope. Height growth was negatively  
323 associated with soil temperature in *A. sempervirens* on the south-slope, in *L. corniculatus* on  
324 both slopes, and positively influenced by light and the C/N ratio in the later species on the  
325 south-slope. On the north-facing slope, the horizontal stem length of the clonal *S. nitida* was  
326 positively associated with the availability of magnesium, but negatively associated with the  
327 availability of potassium and phosphorus, and with conductivity; in the clonal *T. longicaulis*,  
328 stem length was negatively associated with the availability of ammonium. On the north-facing  
329 slope, in *T. longicaulis*, the number of nodes per cm responded positively to light and  
330 negatively to nitrate, potassium and soil moisture; the distance between the nodes was  
331 positively associated with the availability of potassium, phosphorous and magnesium. On the  
332 south-facing slope the distance of nodes in *L. corniculatus* exerted a positive association with  
333 soil temperature and negative one with the pH (Table 5 ).

334

#### 334 Environmental and intraspecific variability within habitats

335

336 Detailed information on the plasticity of traits and environmental variables (measured as the  
337 CV) is provided in the online resource (ESM 2). Generally, the plasticity of LDMC was very  
338 low, of SLA and horizontal stem length was intermediate, and of number of  
339 bifurcations/nodes and distance between bifurcations/nodes was very high (Table 6).  
340 Variability of nitrate and phosphorous was very high; variability of the C/N ratio, pH, and soil  
341 temperature was very low; the other environmental variables showed intermediate variability  
342 (Table 6).

343

344 We assessed the relationships between the variation of traits (CV-traits) and of the  
345 environmental factors (CV-environmental parameter) emerging as the most influential based  
346 on results of linear regression and hierarchical variance partitioning (Table 5, online resource  
347 ESM 3). As the variable soil temperature showed a very low variation (Table 6, online  
348 resource ESM 2) it was not considered. Consequently, CV-CV graphs are displayed for light,  
nitrate (NO<sub>3</sub>), ammonium (NH<sub>4</sub>), magnesium (P) and potassium (K) which exhibited

349 intermediate to high variation (Fig. 1). From the CV-traits to CV-environment comparison  
350 (Fig. 1) it appears that the variable traits SLA, LDMC, and height growth have CV values that  
351 are similar to or smaller than the CV values of the environmental factors. The highly variable  
352 clonal traits, by contrast, appear to have largely higher CV values than the related  
353 environmental factors.

354

355

356 Discussion

357

358 In our study, significant intraspecific differences in plant functional traits between the  
359 contrasting habitats indicate phenotypic functional adaptation to in situ environmental  
360 conditions, supporting hypothesis H1 for all traits investigated. While species adapted their  
361 trait attributes between the two habitats in the same way, they showed species-specific  
362 responses to environmental factors within habitats. Environmental differences within habitats  
363 were less pronounced. However, soil temperature, light, nitrate, ammonium, magnesium and  
364 potassium emerged to be important drivers of intraspecific trait-environment relationships  
365 even at the scale of few meters. Leaf traits, horizontal stem length and clonal traits responded  
366 significantly positively or negatively to the availability of nutrients, while plant height  
367 responded negatively to soil temperature and positively to light availability, supporting H2 for  
368 these traits. The variation of leaf traits and plant height was lower than the environmental  
369 variation indicating that those traits are more regulated by the plant than clonal traits. The  
370 latter, i.e. horizontal stem length, number of bifurcations/nodes and distance between  
371 bifurcations/nodes, exhibited much larger trait variation than environmental variation.  
372 According to Grassein et al. (2010, see review of Schellenberg and Pontes (2012)) species  
373 strategy is defined by both trait values and trait plasticity. Our findings confirm to some  
374 extent the conclusions of Grassein et al. (2010) on LDMC as we found the same trait to be  
375 controlled compared to other traits. However, in our study, the plasticity of this trait was still  
376 high enough to enable adjustment to environmental factors. Our findings encourage  
377 investigating trait control at the intraspecific level.

378

379 In more detail, our results show that SLA and plant height are not only characterized  
380 by considerable variation between communities and species, as already assessed (Westoby  
381 1998; Grime 2002; Poorter et al. 2006; Pierce et al. 2007), but that ecologically significant  
382 variation is also occurring at the intraspecific level (Tables 4, 5). In line with our results,  
383 Wilson et al. (1999) found in a survey of 769 herbaceous species of the British flora that SLA  
384 exhibits strong variation within populations. With respect to phenotypic adaptation, all four  
385 study species showed significantly higher values of SLA and significantly higher height (with  
386 the exception of height variation in *T. longicaulis*) in the north-exposed habitat. Our findings  
387 support the view that higher plant height and higher SLA in the species in the north-exposed  
388 habitat might be related to the significantly higher availability of all investigated nutrients (P,  
389 K, Mg, NH<sub>4</sub>, NO<sub>3</sub>) along with higher soil moisture and conductivity. Within the south-facing  
390 habitat, the SLA proved to be significantly positively influenced by nitrate availability in *A.*  
391 *sempervirens* (Table 5). This finding is in line with experimental results of Al Haj Khaled et  
392 al. (2005) which demonstrated that nitrogen availability positively affects SLA attributes  
393 within species.

393

394 Literature evidence points out that high SLA values are positively correlated with  
395 rapid leaf turnover, potential relative growth rate and photosynthetic efficiency (Cornelissen  
396 et al. 2003; Kleyer et al. 2008); fast turnover of plant parts allows also a more flexible  
397 response to the spatial patchiness of light availability (Grime 1994), which is a characteristic  
of the habitat on the north-facing slope for all species (except *T. longicaulis*). The higher



398 density of the canopy in this habitat causes competition for light, which might modulate light  
399 availability giving advantage in having a higher SLA.

400 With respect to the second investigated leaf trait, i.e. the leaf dry matter content  
401 (LDMC) we found the inverse response as SLA (Table 4). The LDMC was significantly  
402 higher in the south-facing habitat for all species (with the exception of *T. longicaulis* whose  
403 higher values were not significant). The LDMC of this species, however, showed variation in  
404 response to variation in magnesium and potassium availability within the north-facing habitat  
405 (Table 5). High values of LDMC correspond to a low turnover rate (Cornelissen et al. 2003;  
406 Kleyer et al. 2008) and thicker leaves, better withstanding physical constraints. Both  
407 characteristics help to store nutrients, representing a big advantage in unproductive  
408 environments (Ryser and Urbas 2000) such as the south-facing slope. Following Gross et al.  
409 (2007), this trait appears to be a better predictor for plant responses to nutrient stress than  
410 SLA. Earlier literature confirms that LDMC is less variable than SLA (Garnier et al. 2001)  
411 which is confirmed by our data showing lower intraspecific and interspecific variation (CV)  
412 of LDMC.

413 The second investigated whole-plant trait, i.e. horizontal stem length of clonal species,  
414 responded in the same way to the differentiated environmental conditions than plant height.  
415 Horizontal stem length represents the diameter of individuals and thus their maximum range  
416 of space occupancy. All investigated species showed significantly higher space occupancy in  
417 the north-facing habitat (Table 4). These data were not sampled for *A. sempervirens*, but this  
418 is the species with the longest horizontal stems of all examined species. Due to this, it exhibits  
419 a much higher capacity of space occupancy in the N-facing habitat (several meters long)  
420 compared to the S-facing ones (shorter than 1 m). The results of all study species might be  
421 explained by two mechanisms. First, the higher availability of nutrients and water allowing  
422 for higher growth potential and second, the space-occupancy advantage under competitive  
423 conditions with lower light availability. This is in line with the findings of Tissue and Nobel  
424 (1988) and Grime (2002) who state that in a dense community, the horizontal stem length  
425 provides higher ability to explore new space, increasing the possibility to exploit new  
426 resources and to allocate them within the organism.

427 Both clonal traits 'number of bifurcations/nodes per cm' and 'distance between  
428 bifurcations/nodes' showed the highest variation in all study species. This might to some  
429 extent be fostered by the high variation of light-, water- and nutrient-availability within  
430 habitats. Apart from being an alternative to sexual reproduction, clonality allows rapid  
431 colonization of open habitats, pre-emptive occupation of space by forming dense patches,  
432 avoidance of competition implicit in fugitive growth and better foraging for resources in a  
433 heterogeneous soil matrix (Stöcklin 1992; Oborny and Bartha 1995).

434 In both investigated clonal traits, phenotypic adaptation to the contrasting environ-  
435 mental conditions of the north- and south-facing slope were found only in *S. nitida*. This  
436 dominant grass showed a higher capacity of multiplication (number of bifurcations) at the  
437 south-facing slope but higher capacity of space occupancy (distance between bifurcations) at  
438 the north-facing slope. The space occupancy might be confined by the lower nutrient and  
439 moisture availability of the habitat S while higher availability of these resources allows for  
440 higher general growth capacity, which is reflected also in the horizontal stem length. This trait  
441 of *S. nitida* was significantly positively influenced by an increased availability of magnesium  
442 within the north-facing habitat. Within this habitat, *T. longicaulis* was seen to produce fewer  
443 nodes but longer internodal stem-segments with higher nutrient availability while higher light  
444 availability led to the production of more nodes.

445 In our study, the seed mass showed significant intraspecific differences between the  
446 two contrasting habitats. This is surprising because seed mass differences were found to be

447 often conservative between genera or families (Hodgson and Mackey 1986; Mazer 1989; Peat  
448 and Fitter 1994; Westoby 1998).

449 Our results confirm phenotypic adaptation of seed mass in all tested species. Their  
450 seed mass was significantly higher in the south-facing habitat compared to the north-facing  
451 one. As summarized by Pakeman et al. (2008), it has been shown that larger seeds offer an  
452 advantage (e.g. Buckley 1982) or are more common in drier environmental conditions  
453 (Wright and Westoby 1999), since the seedlings of larger seeds better withstand  
454 environmental hazards being reserves needed for drought-resistance mechanisms (Leishman  
455 and Westoby 1994). However, Pakeman et al. (2008) found substantially more evidence that  
456 seed size was higher at warmer sites. The significant differences found in our study are  
457 possibly related to differences in both, temperature and water availability. However, lower  
458 competition in the south-facing habitat might foster a higher investment in sexual  
459 reproduction. The north-facing habitat, in contrast, exhibits higher levels of competition since  
460 *S. nitida* forms an extraordinarily dense vegetation carpet.

461 Despite the fact that germination rate observed in our study was very low, the results  
462 on the intraspecific differences of the germination rate also support our findings on seed mass.  
463 Significantly higher intraspecific germination rate of seeds produced by plants in the south-  
464 exposed habitat are possibly linked to the higher seed mass of these seeds. Thus, the  
465 production of larger seeds in the stressful south-facing habitat provides more reserves for  
466 germination, in agreement with the advantages of larger seeds reported by Westoby et al.  
467 (2002) and the subsequent higher survival rates of seedlings (Moles and Westoby 2006),  
468 especially under various hazards including drought (Westoby 1998).

469 The range of trait variation is the result of the plants trial to reach equilibrium between  
470 costs and benefits as the strategy of each plant individual is the best possible compromise  
471 within a given environment (Reich et al. 2003). In other words, the advantage provided by  
472 phenotypic plasticity per se, allows for adaptation to environmental conditions.

473 In all four species, the individuals of the south-facing habitat are better equipped to  
474 cope with environmental stress. In fact they are characterized by lower plant height, slower  
475 growing rate, thicker laminae, higher tissue density, lower photosynthetic efficiency, longer  
476 leaf life span (and lower leaf turnover), more investment in structural strength, and higher  
477 investment in seeds (stored energy) which ensure future successful performance of seedlings  
478 under stressful conditions.

479

#### 480 Data limitation

481

482 Both investigated habitats, i.e. the south- and north-facing slope, very likely have differences  
483 in their disturbance history. Due to local topography, grazing animals had more easily access  
484 to the south-facing slope before the closure of the reserve in 1970. This might explain in  
485 concert with the stressful environmental conditions that some of the late-successional plant  
486 species as *A. sempervirens* in the vegetation of the south-facing slope might have lower age.  
487 Plant age might influence the performances of some of the examined traits, mainly when it  
488 affects capacity of nutrient and water uptake by plant size and rooting depth. This might be  
489 the case for *A. sempervirens*, which is younger in the south- than in the north-facing slope.  
490 One representative individual of the south-facing slope was analyzed by dwarf shrub  
491 chronology and appeared to be 20 years old. One of the largest individuals on the north-facing  
492 slope was estimated according to annual increment of horizontal stem length and is around 60  
493 years old. In case of *S. nitida* no differences appear with age since the species does not  
494 develop huge individuals because clonal offspring disintegrate after some years. In case of *L.*  
495 *corniculatus* and *T. longicaulis*, both of which are larger and therefore probably older on the  
496 south-facing slope, we were able to adjust the testing for trait differences between habitats for

497 plant size (biomass) in all traits (see McCarthy and Enquist 2007) except seed traits.  
498 However, results were largely the same when testing for apparent plasticity, i.e. without  
499 adjusting for plant size which in turn relativizes the above stressed argumentation on the  
500 potential impact of plant size on phenotypic trait adaptation.

501 Another data limitation refers to the fact that we studied only two grassland sites (1 ha  
502 extension each) and could not include further spatial replicates, i.e. further valleys with north-  
503 and south-facing grasslands in this highly labour-intensive work. This limits the strength of  
504 conclusions drawn to the global change level and we suggest further studies in order to shed  
505 more light on the evidence indicated in our study.

506

#### 507 Implications for global change and nature conservation

508

509 Our findings demonstrate phenotypic differentiation of species at medium spatial scales, i.e.  
510 200 m air distance between the opposing slopes of a valley. Some species even vary their  
511 traits according to the fine scale (i.e. centimeters to meters) heterogeneity in temperature. The  
512 existing medium-scale climatic differences between the contrasting slopes, manifesting e.g. in  
513 a difference of 8 °C in the soil temperature, are larger than the overall climatic shifts predicted  
514 by coarse-scale scenarios (IPCC 2012) and partly cover the magnitude of European extreme  
515 events such as mega-heatwaves (Barriopedro et al. 2011). Therefore, we expect that these  
516 species can be pre-adapted (to some degree) to the overall expected environmental changes.  
517 Studying medium- and fine-scale intraspecific trait variability helps to quantify the magnitude  
518 of plasticity that can serve as adaptive potential of plant species.

519 Switching the focus on land-use change, the abandonment of traditional grazing  
520 regimes in the study area resulted in succession and subsequent increase in competition in the  
521 climatically and edaphically favorable north-facing slope. The related differences in nutrient  
522 availability at medium and fine spatial scales resulted in an adjustment of functional traits,  
523 e.g. SLA and LDMC.

524 As phenotypic specialization might enable a differentiated response to land-use and  
525 climate change it ultimately might increase plant fitness and survival. Nature conservation  
526 should therefore protect environmental heterogeneity between and within habitats in order to  
527 maintain larger intraspecific variability and thereby a variety of phenotypic specialization that  
528 finally can buffer future environmental extremities due to climate and land-use changes.  
529 These findings might support conservation planning with information on how and where to  
530 prioritize conservation objects and how to work in situ (Beier and Brost 2010; Groves et al.  
531 2012). This meets the purpose of moderating impacts of climate change and capitalizing on  
532 emerging opportunities, i.e. climate change adaptation (Groves et al.2012; IPCC 2012).

533

534

#### 535 Conclusions

536

537 Using ca. three times larger sample size than required by the standard protocol for measuring  
538 plant traits, we show the intermediate to high degree of intraspecific variability of whole  
539 plant, clonal, leaf and seed traits. We stress the adaptive significance of the key plant traits  
540 leading to intraspecific adaptation of strategies. We argue that protecting habitats with  
541 considerable medium- and small-scale environmental heterogeneity is important to maintain  
542 large intraspecific variability within local populations that finally can buffer against  
543 uncertainty of future climate and land use scenarios.

544

545

546 Acknowledgments

547 We thank the Analytical Chemistry of the Bayreuth Centre of Ecology and Environmental Research (BayCEER,  
548 Germany) for performing the analyses of soil chemical parameters. This research was partially supported by  
549 Montagna di Torricchio Nature Reserve. SB was supported by the Hungarian National Science Foundation  
550 (OTKA K 72561). CW was supported by the Bavarian State Ministry of Sciences, Research and the Arts within  
551 the FORKAST project. We thank Martin Hallinger (University of Greifswald, Germany) for help with the  
552 determination of age of *A. sempervirens*.

553

554

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**Table 1** Geo-physical and plant-sociological characteristics of the sampling sites (i.e. habitats)

	Site N	Site S
Coordinates	42°57'21.8"N–13°01'10.1"E	42°57'35.1"N–13°01'07.4"E
Elevation (m)	1200	1165
Exposition	320°N-NW	140°S-SE
Slope (°)	35	17
Soil	Rendzina on limestone	Lithosols and regosols, highly eroded soil
Vegetation	<i>Sesleria nitidae</i> – <i>Brometum erecti</i>	<i>Asperulo purpureae</i> – <i>Brometum erecti</i>
Series	Montane calcicolous <i>Fagus sylvatica</i> series	Montane calcicolous <i>Ostrya carpinifolia</i> series on steep rocky slopes

Both associations belong to the order Brometalia erecti W. Koch 1926, class Festuco-Brometea Br.-Bl. et Tx. in Br.-Bl. 1949

**Table 2** Traits definitions, respective life-history processes, and functional information from literature

	Life-history processes	Trait-environment links	Definition	Functional significance	Literature
<b>Leaf traits</b>					
SLA	Establishment; competition	Light; moisture, nutrients; temperature	The ratio of fresh leaf area to leaf dry mass	Positively correlated with potential relative growth rate and mass-based maximum photosynthetic rate; the relationships between SLA and light condition, leaf defence structures and environmental stresses are well known. Plasticity of SLA is well documented	Kleyer et al. (2008); Poorter et al. (2006); Cornelissen et al. (2003); Wilson et al. (1999); Westoby (1998)
LDMC	Establishment; competition	Nutrients; temperature	The ratio of dry leaf mass to fresh leaf mass	Used as a measure of leaf tissues density, which plays a central role in the use of nutrients by the species, determining the rate of turnover of biomass. At high values of LDMC, the rate of potential growth is low and the leaves tend to have a low turn-over to conserve nutrients, a big advantage in unproductive environments	Cornelissen et al. (2003); Kleyer et al. (2008); Ryser and Urbas (2000); Gross et al. (2007)
<b>Whole plant traits</b>					
Plant height	Competition	Nutrients; moisture; temperature	The distance between the highest photosynthetic tissues and the base of the plant	Associated with competitive vigour, whole plant fecundity and generation time after disturbance. There are also important trade-offs between plant height and tolerance or avoidance of environmental stress	Kleyer et al. (2008); Cornelissen et al. (2003); Westoby (1998)
Horizontal stem length	Competition	Light; nutrients; moisture; temperature	Maximum length of the horizontal stems of the plant	A measure of the maximum range of space occupancy. Lateral spread of horizontal stems enables the plant to place photosynthetic and reproductive organs at the microscale to new environments. This might help to minimize competition. Additionally, maximal lateral spread of sexual and vegetative reproduction is associated with increasing distribution of new individuals	Silvertown (2004); Klimešová and Klimeš (2006)



**Table 2** continued

	Life-history processes	Trait-environment links	Definition	Functional significance	Literature
<b>Clonal traits</b>					
No. of bifurcations/ nodes per cm	Competition; regeneration	Nutrients; moisture; temperature	No. of bifurcations or nodes per cm of a plants stem	A measure of the multiplication capacity which is associated with the abundance of space occupancy, i.e. density/packing of individuals/ramets	Knevel et al. (2005); Maillette (1992)
Distance between bifurcations/ nodes	Competition; regeneration	Light; nutrients; moisture; temperature	Distance between the bifurcations or nodes of a plants stem	A measure of the capacity of space occupancy by clonal growth. Lateral spread enables the plant to colonize a new substrate and avoid intraspecific competition. Limited lateral spread could be expected in situations where facilitation is important	Klimešová and Klimeš (2006)
<b>Seed traits</b>					
Seed mass	Establishment; dispersal	Moisture; nutrients; temperature	The air dried weight of seeds	Resources stored in large seeds help the young seedling to survive and establish in the face of environmental hazards. Connection with dispersion and persistence in the soil	Kleyer et al. (2008); Cornelissen et al. (2003); Westoby (1998); Westoby et al. (2002).
Seed germination	Establishment	Moisture; temperature	The percentage of seeds germinated under defined environmental conditions	Strongly associated with species ecology and competition. The relative performance of individual plants during the early stages of life, i.e. germination and seedlings establishment, can have important effects on subsequent adult growth and fitness	Baskin and Baskin (1998); Stanton (1984); Walck et al. (2011); Wellstein (2012)

**Table 3** Differences of environmental variables at the place of growth of individuals between species and sites (i.e. habitats)

Place of growth of individuals of respective species Environmental variables and resources	Site N + S	Main effect of site		Main effect of species' place of growth				
		Site N	Site S	Site N + S				
	All species	All species	All species	<i>Sesleria nitida</i>	<i>Lotus corniculatus</i>	<i>Astragalus sempervirens</i>	<i>Thymus longicaulis</i>	
	Mean and SD	Mean and SD	Mean and SD	Mean and SD	Mean and SD	Mean and SD	Mean and SD	
Light-PAR (%)	74.69 ± 25.72	65.85 ± 30.38 <b>a</b>	83.52 ± 15.78 <b>b</b>	48.10 ± 20.33 <b>b</b>	86.59 ± 21.73 <b>a</b>	74.17 ± 24.78 <b>c</b>	90.08 ± 8.65 <b>a</b>	
Soil temperature (°C)	18.59 ± 4.94	14.62 ± 1.74 <b>a</b>	22.66 ± 3.68 <b>b</b>	15.08 ± 2.58 <b>c</b>	18.58 ± 4.70 <b>a</b>	18.74 ± 4.53 <b>a</b>	21.97 ± 5.05 <b>b</b>	
Soil moisture (%)	10.98 ± 6.46	12.55 ± 6.84 <b>a</b>	9.37 ± 5.63 <b>b</b>	14.66 ± 4.81 <b>a</b>	13.68 ± 5.79 <b>a</b>	13.13 ± 3.22 <b>a</b>	2.41 ± 2.26 <b>b</b>	
pH	6.67 ± 0.41	6.60 ± 0.50 <b>a</b>	6.95 ± 0.14 <b>b</b>	6.90 ± 0.31 <b>b</b>	6.74 ± 0.60 <b>ab</b>	6.72 ± 0.32 <b>a</b>	6.73 ± 0.31 <b>a</b>	
Conductivity (µS/cm)	195.69 ± 61.72	220.84 ± 64.62 <b>a</b>	169.69 ± 46.05 <b>b</b>	203.20 ± 60.94 <b>ns</b>	182.06 ± 54.37 <b>ns</b>	197.14 ± 54.12 <b>ns</b>	201.22 ± 74.52 <b>ns</b>	
K (mg/100 g)	7.42 ± 3.65	9.11 ± 3.67 <b>a</b>	5.68 ± 2.70 <b>b</b>	7.83 ± 3.54 <b>ns</b>	7.10 ± 3.18 <b>ns</b>	7.60 ± 4.29 <b>ns</b>	7.20 ± 3.60 <b>ns</b>	
Mg (mg/100 g)	7.56 ± 2.30	7.92 ± 2.35 <b>a</b>	6.54 ± 1.73 <b>b</b>	6.83 ± 1.29 <b>b</b>	7.49 ± 2.20 <b>ab</b>	7.60 ± 2.51 <b>ab</b>	8.31 ± 2.72 <b>a</b>	
P (mg/100 g)	0.12 ± 0.14	0.20 ± 0.13 <b>a</b>	0.03 ± 0.11 <b>b</b>	0.10 ± 0.11 <b>a</b>	0.09 ± 0.11 <b>a</b>	0.18 ± 0.19 <b>b</b>	0.11 ± 0.14 <b>a</b>	
NH <sub>4</sub> (mg/100 g)	0.81 ± 0.53	1.09 ± 0.59 <b>a</b>	0.52 ± 0.23 <b>b</b>	0.90 ± 0.49 <b>a</b>	0.87 ± 0.56 <b>a</b>	0.96 ± 0.64 <b>a</b>	0.52 ± 0.25 <b>b</b>	
NO <sub>3</sub> (mg/100 g)	3.30 ± 4.14	4.81 ± 4.79 <b>a</b>	1.74 ± 2.54 <b>b</b>	6.01 ± 4.33 <b>c</b>	4.25 ± 5.41 <b>b</b>	2.19 ± 1.94 <b>b</b>	0.72 ± 0.48 <b>a</b>	
C/N (%)	10.81 ± 0.78	11.27 ± 0.57 <b>a</b>	10.33 ± 0.67 <b>b</b>	10.96 ± 0.57 <b>a</b>	10.78 ± 0.59 <b>ab</b>	10.54 ± 0.77 <b>b</b>	10.95 ± 1.04 <b>a</b>	
Interaction of site and species' place of growth								
	Site N	Site S	Site N	Site S	Site N	Site S	Site N	Site S
	<i>Sesleria nitida</i>		<i>Lotus corniculatus</i>		<i>Astragalus sempervirens</i>		<i>Thymus longicaulis</i>	
	Mean and SD	Mean and SD	Mean and SD	Mean and SD	Mean and SD	Mean and SD	Mean and SD	Mean and SD
	34.40 ± 18.63 <b>a</b>	61.80 ± 10.27 <b>b</b>	80.38 ± 27.61 <b>a</b>	93.01 ± 10.24 <b>b</b>	52.35 ± 15.70 <b>a</b>	95.26 ± 7.35 <b>b</b>	95.84 ± 7.26 <b>a</b>	84.33 ± 5.56 <b>b</b>
	12.60 ± 0.28 <b>a</b>	17.56 ± 0.87 <b>b</b>	14.36 ± 0.64 <b>a</b>	23.32 ± 1.58 <b>b</b>	14.46 ± 0.66 <b>a</b>	23.03 ± 1.83 <b>b</b>	17.19 ± 0.72 <b>a</b>	26.75 ± 2.01 <b>b</b>
	16.58 ± 5.44 <b>a</b>	12.67 ± 3.05 <b>b</b>	17.56 ± 5.05 <b>a</b>	9.63 ± 2.66 <b>b</b>	12.42 ± 1.70 <b>ns</b>	13.85 ± 4.14 <b>ns</b>	3.27 ± 1.23 <b>a</b>	1.54 ± 0.41 <b>b</b>

**Table 3** continued

Interaction of site and species' place of growth

Site N <i>Sesleria nitida</i>	Site S	Site N <i>Lotus corniculatus</i>	Site S	Site N <i>Astragalus sempervirens</i>	Site S	Site N <i>Thymus longicaulis</i>	Site S
Mean and SD	Mean and SD	Mean and SD	Mean and SD	Mean and SD	Mean and SD	Mean and SD	Mean and SD
6.75 ± 0.36 <b>ns</b>	7.06 ± 0.14 <b>ns</b>	6.55 ± 0.77 <b>ns</b>	6.94 ± 0.13 <b>ns</b>	6.55 ± 0.36 <b>ns</b>	6.88 ± 0.14 <b>ns</b>	6.55 ± 0.34 <b>ns</b>	6.92 ± 0.09 <b>ns</b>
219.07 ± 63.81 <b>ns</b>	186.79 ± 54.11 <b>ns</b>	194.18 ± 60.98 <b>ns</b>	168.73 ± 43.20 <b>ns</b>	218.52 ± 50.04 <b>a</b>	175.76 ± 50.13 <b>b</b>	254.17 ± 69.76 <b>a</b>	148.27 ± 25.13 <b>b</b>
9.74 ± 3.28 <b>a</b>	5.86 ± 2.61 <b>b</b>	7.91 ± 3.19 <b>ns</b>	6.21 ± 2.96 <b>ns</b>	10.39 ± 4.22 <b>a</b>	4.80 ± 1.86 <b>b</b>	8.57 ± 3.58 <b>a</b>	5.84 ± 3.12 <b>b</b>
7.46 ± 0.93 <b>ns</b>	6.18 ± 1.29 <b>ns</b>	7.87 ± 2.56 <b>ns</b>	7.07 ± 1.67 <b>ns</b>	9.17 ± 1.97 <b>a</b>	6.03 ± 1.98 <b>b</b>	9.77 ± 2.76 <b>a</b>	6.86 ± 1.76 <b>b</b>
0.17 ± 0.09 <b>a</b>	0.02 ± 0.05 <b>b</b>	0.14 ± 0.12 <b>a</b>	0.03 ± 0.06 <b>b</b>	0.27 ± 0.14 <b>a</b>	0.08 ± 0.19 <b>b</b>	0.22 ± 0.12 <b>a</b>	0.01 ± 0.04 <b>b</b>
1.17 ± 0.54 <b>ns</b>	0.64 ± 0.19 <b>ns</b>	1.18 ± 0.62 <b>ns</b>	0.53 ± 0.15 <b>ns</b>	1.35 ± 0.64 <b>ns</b>	0.56 ± 0.31 <b>ns</b>	0.68 ± 0.25 <b>ns</b>	0.36 ± 0.12 <b>ns</b>
7.11 ± 4.76 <b>ns</b>	4.87 ± 3.57 <b>ns</b>	7.60 ± 5.69 <b>a</b>	0.57 ± 0.28 <b>b</b>	3.35 ± 2.10 <b>a</b>	1.03 ± 0.66 <b>b</b>	0.85 ± 0.59 <b>ns</b>	0.58 ± 0.31 <b>ns</b>
11.23 ± 0.38 <b>a</b>	10.67 ± 0.60 <b>b</b>	10.99 ± 0.46 <b>a</b>	10.55 ± 0.63 <b>b</b>	11.11 ± 0.49 <b>a</b>	9.97 ± 0.54 <b>b</b>	11.65 ± 0.64 <b>a</b>	10.14 ± 0.67 <b>b</b>

Significant differences between species and sites were evaluated by two-way ANOVA followed by post hoc test (Tukey HSD). Main effect of site, species' place of growth, and their interaction are given subsequently. For the interaction effects, significant differences between sites are noted within species (not between species as this was not of interest). Significant differences are indicated by different lower case letters in bold; *ns* not significant

**Table 4** Mean trait values (untransformed data) per species in each slope (N,S), and their difference ( $\Delta$ )

		Leaf traits		Whole plant traits		Clonal traits		Seed traits	
		SLA (mm <sup>2</sup> mg <sup>-1</sup> )	LDMC (mg g <sup>-1</sup> )	Height (cm)	Horizontal stem length (cm)	No. of bifurcations/ nodes per cm ( <i>n</i> )	Distance between bifurcations/nodes (cm)	Seed mass (mg)	Seed germination (%)
<i>Sesleria nitida</i>	N	18.04 <b>a</b>	375.03 <b>a</b>	60.33 <b>a</b>	23.35 <b>a</b>	0.09 <b>a</b>	7.93 <b>a</b>	1.80 <b>a</b>	2.00 <b>a</b>
	S	15.77 <b>b</b>	408.61 <b>b</b>	51.00 <b>b</b>	8.37 <b>b</b>	0.46 <b>b</b>	2.88 <b>b</b>	2.30 <b>b</b>	8.00 <b>b</b>
	$\Delta$	2.27	33.58	9.33	14.98	0.37	5.05	0.50	6.00
<i>Lotus corniculatus</i>	N	38.43 <b>a</b>	227.47 <b>a</b>	20.33 <b>a</b>	18.69 <b>a</b>	0.10 <b>a</b>	1.99 <b>a</b>		
	S	24.32 <b>b</b>	254.98 <b>b</b>	13.10 <b>b</b>	16.90 <b>b</b>	0.13 <b>a</b>	3.25 <b>a</b>	No data	No data
	$\Delta$	14.11	27.51	7.23	1.79	0.03	1.26		
<i>Astragalus sempervirens</i>	N	17.31 <b>a</b>	343.48 <b>a</b>	12.80 <b>a</b>				2.30 <b>a</b>	2.00 <b>a</b>
	S	15.26 <b>b</b>	371.56 <b>b</b>	9.17 <b>b</b>	No data	Non clonal	Non clonal	2.80 <b>b</b>	11.00 <b>b</b>
	$\Delta$	2.05	28.08	3.63				0.30	9.00
<i>Thymus longicaulis</i>	N	14.44 <b>a</b>	361.06 <b>a</b>	6.97 <b>a</b>	21.10 <b>a</b>	0.30 <b>a</b>	2.64 <b>a</b>	0.053 <b>a</b>	29.00 <b>a</b>
	S	10.80 <b>b</b>	367.81 <b>a</b>	8.40 <b>b</b>	13.25 <b>b</b>	0.28 <b>a</b>	3.04 <b>a</b>	0.070 <b>b</b>	33.00 <b>a</b>
	$\Delta$	3.64	6.75	1.43	7.85	0.02	0.40	0.017	4.00

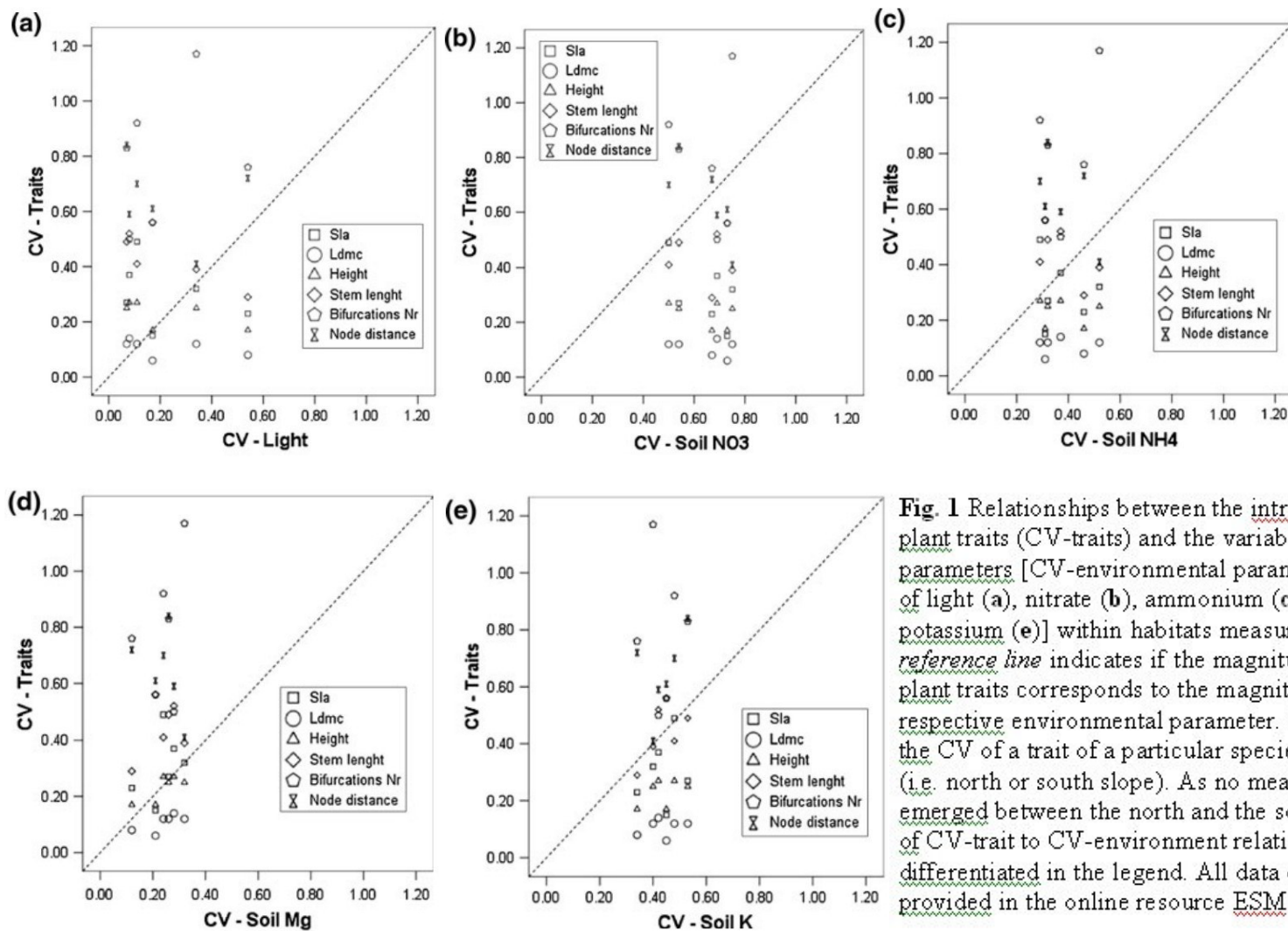
Significant differences between means ( $p < 0.05$ ) were evaluated by ANOVA (all traits of *S. nitida* and *A. sempervirens*; seed traits of all species) and ANCOVA using biomass as covariate (leaf, whole plant, and clonal traits of *L. corniculatus* and *T. longicaulis*). Significant differences are indicated by different lower case letters in bold. Identical lower case letters indicate homogeneous groups, i.e. no significant difference

**Table 5** Intraspecific trait-environment relationships of the study species within habitats (i.e. N: north-facing slope, S: south-facing slope)

Habitat	<i>Lotus comiculatus</i>		<i>Astragalus sempervirens</i>		<i>Thymus longicaulis</i>		<i>Sesleria nitida</i>	
	S	N	S	N	S	N	S	N
SLA	n.s.	n.s.	NO3(+)*	n.s.	n.s.	n.s.	n.s.	n.s.
LDMC	n.s.	n.s.	n.s.	n.s.	n.s.	K(-)* Mg(+)*	n.s.	n.s.
Height	light(+)** temp (-)* CN(+)*	temp (-)**	temp (-)**	n.s.	n.s.	n.s.	n.s.	n.s.
Horizontal stem length	n.s.	n.s.	no data	no data	n.s.	NH4(-)*	n.s.	Mg(+)** K(-)* P(-)* cond(-)*
N. of bifurcations / nodes per cm	n.s.	n.s.	non clonal	non clonal	n.s.	light(+)* NO3(-)* moist(-)* K(-)*	n.s.	n.s.
Distance between bifurcations / nodes	pH(-)** temp(+)*	n.s.	non clonal	non clonal	n.s.	K(+)* Mg(+)* P(+)*	n.s.	n.s.

significance: \*\* - p<0.01; \* - p<0.05; . - p<0.1

relative importance: >20% dark grey; 20%>x>10% grey



**Fig. 1** Relationships between the intraspecific variation of plant traits (CV-traits) and the variability of environmental parameters [CV-environmental parameter, i.e. availability of light (a), nitrate (b), ammonium (c), magnesium (d) and potassium (e)] within habitats measured as the CV. The reference line indicates if the magnitude of variation of plant traits corresponds to the magnitude of variation of the respective environmental parameter. Each point represents the CV of a trait of a particular species within one habitat (i.e. north or south slope). As no meaningful differences emerged between the north and the south slope in terms of CV-trait to CV-environment relation they were not differentiated in the legend. All data on CV values are provided in the online resource ESM 2

