

Plant responses to simulated warming and drought: a comparative study of functional plasticity between congeneric mid and high elevation species

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Abstract

Aims

Effects of climate change, especially changes in temperatures and precipitation patterns, are particularly pronounced in alpine regions. In response, plants may exhibit phenotypic plasticity in key functional traits allowing short-term adjustment to novel conditions. However, little is known about the degree of phenotypic plasticity of high elevation species relative to mid elevation congeners.

Methods

We transplanted 14 herbaceous perennial species from high elevation into two common gardens (1050 and 2000 m.a.s.l.) in the Swiss Alps, and we examined plastic responses in key functional traits to changes in temperature and soil water availability. This design was replicated with 14 congeneric species from mid elevation to assess if the degree of phenotypic plasticity differs between mid and high elevation species. Survival was assessed across two growing seasons, while aboveground biomass and specific leaf area (SLA) were measured after the first growing season, and biomass allocation to belowground and reproductive structures after the second. Moreover, a phenotypic plasticity index was calculated for the functional traits to compare the degree of plasticity between mid and high elevation species.

Important Findings

Aboveground biomass was higher in mid elevation species relative to high elevation congeners in all treatments, yet decreased for both with elevation and drought. Similarly, SLA decreased with elevation and drought. Root mass fraction (RMF) was generally higher in high elevation species, and decreased with drought at the lower site. Drought increased the allocation to reproductive structures, especially when plants were grown at their elevation of origin. Interestingly, no difference was found in the degree of phenotypic plasticity averaged across mid and high elevation species for any of the studied functional traits. These results indicate that phenotypic plasticity in the focal traits did not depend on the elevation of origin of the species. Plasticity was not related to environmental heterogeneity, nor constrained by selective pressures at high elevation. However, both species groups showed a remarkable capacity for short-term acclimation to a prospective climate through rapid adjustments in key functional traits.

Keywords: biomass allocation, common garden, climate change, perennial herbaceous species, phenotypic plasticity, SLA, Swiss Alps, transplant experiment

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INTRODUCTION

In the late nineteenth century, pioneer experimental botanists began using transplantation experiments along elevational gradients to investigate the degree of transformation

of plants in novel environments (Bonnier 1890; Kerner 1869; reviewed in Briggs *et al.* 1997). Soon after, the genetic component of ecotypic differentiation of plants from different elevations was recognized by Clausen *et al.* (1941), along with the possibility that plants might change their phenotype

depending on a given environment. This particular finding, later termed phenotypic plasticity, has received growing attention in the past decades (Bradshaw 1965; Schlichting 1986; Sultan 1987; Thompson 1991), and the current interest results in part from an urgency to predict species responses to global change (Valladares et al. 2006).

In Europe, increasing temperatures and changes in precipitation patterns have been reported by the IPCC (Hartmann et al. 2013; Kovats et al. 2014), and it has been suggested that the effects of global change are proportionally more important at high elevation (Beniston et al. 1997). Indeed, in alpine regions, the amplitude of temperature changes during the past decades is greater than globally observed changes (Beniston et al. 1994), and summer droughts are predicted to become more frequent (Kovats et al. 2014), leaving mountain biota particularly vulnerable to climate change (Körner 2003; Theurillat et al. 2001). In this context, phenotypic plasticity may play a crucial role in the short-term adjustment to novel conditions and could promote long-term adaptive evolution by buffering against rapid change (Nicotra et al. 2010; Price et al. 2003; Richter et al. 2012).

A number of studies have investigated shifts in plant traits in response to climate change. While modifications in flowering phenology are probably the best documented worldwide (Parmesan et al. 2003), adjustments in other key plant functional traits have also been reported in response to changes in temperature and soil water availability. Leaf traits and particularly specific leaf area (SLA) are considered as most informative (Scheepens et al. 2010; Wright et al. 2004), as SLA is an indicator of relative growth rate, stress tolerance and leaf longevity (Atkin et al. 2006; Lavorel et al. 2002; Poorter et al. 2009). SLA has been shown to strongly correlate with temperature, irradiance and soil water availability (Poorter et al. 2009), and generally decreases with increasing elevation (Körner 2003; Ma et al. 2010; Scheepens et al. 2010), and with reduced soil water availability (Poorter et al. 2009). SLA is a highly plastic trait, which adjusts rapidly to changing environmental conditions (Scheepens et al. 2010).

Increasing elevation and decreasing soil water availability are important factors limiting plant productivity. Indeed, aboveground biomass generally decreases with increasing elevation and drought (Körner 2003; Lambers et al. 1998). More specifically, the allocation of biomass to different plant organs differs along elevational and soil moisture gradients. Plant growth theory predicts that plants from stress dominated and cold habitats allocate a high portion of dry matter to belowground organs thereby increasing survival (Bloom et al. 1985; Grime 2001). Indeed, Körner et al. (1987) showed in an extensive study on 49 perennial herbaceous species, that high elevation plants allocate more dry matter to roots, especially fine roots, than typical lowland plants, and these results were generally corroborated since (Ma et al. 2010; Prock et al. 1996; Poorter et al. 2012a). Similarly, in the context of drought stress, greater proportional root biomass presumably increases the uptake surface area and thus the water

acquisition potential (Heschel et al. 2004; Huang et al. 2013; Pang et al. 2011). However, increased allocation to belowground structures may come at the expense of allocation to reproductive structures and/or photosynthetic organs such as leaves, and a trade-off between these structures has been found in several studies (Körner et al. 1987; Ma et al. 2010; Prock et al. 1996). Furthermore, when comparing high and low elevation species, it was found that high elevation species allocated three times more of their aboveground biomass specifically to floral structures (Fabbro et al. 2004), indicating a clear prioritization of reproduction over growth. Similarly, under drought stress, trade-offs at the expense of reproductive structures have also been found (Huang et al. 2013). However, another study showed that two out of 11 alpine species had a higher reproductive biomass when grown under drought (Peterson et al. 1982), indicating that investment in sexual reproduction can be favored in some species under drought stress or competition (Rautiainen et al. 2004).

Although a number of studies have examined the effects of warming and drought on plant traits (Arft et al. 1999; Atkin et al. 2006; Gilgen et al. 2009; Heschel et al. 2004), only few have studied the effects of these factors in combination, and simultaneously on multiple herbaceous species (Bloor et al. 2010; Cleland et al. 2006). Furthermore, to this day, we know of only two studies, which have used a comparative approach to examine if species or populations growing at high elevation harbor the same potential for phenotypic plasticity as their counterparts growing at lower elevation. While a reciprocal transplant experiment with three grassland species revealed no difference in the plasticity of growth, phenology and leaf traits between low and high elevation populations (Frei et al. 2014a), Vitasse et al. (2013) found lower phenological plasticity in high elevation deciduous tree species. Theory predicts that phenotypic plasticity is advantageous in spatially and temporally heterogeneous environments (Alpert et al. 2002; van Kleunen et al. 2005; Via et al. 1985). One could hypothesize that high elevation species, adapted to habitats with great spatial and temporal heterogeneity (Scherrer et al. 2011) might display greater plasticity in response to environmental variation than plants from lower more homogeneous sites. On the other hand, high elevation species have evolved under strong selective pressures, imposing directional or stabilizing selection on plant traits, and thereby constraining their capacity to respond plastically to changes in external conditions (Vitasse et al. 2013). In a parallel study, we found lower plasticity in the flowering phenology of high elevation species, which are probably constrained by canalized selection for rapid flowering after snowmelt in regard of the short growing season at high elevation (Gugger et al. 2015). In the traits studied here, we expect the opposite because high plasticity in SLA and biomass allocation might be advantageous in a highly heterogeneous environment such as the alpine habitat.

Here, we examine the combined effects of warming and drought on specific plant traits, known to be particularly plastic

to these environmental factors (i.e. SLA, biomass allocation). We reciprocally transplanted 14 congeneric pairs of herbaceous perennial species originating from mid and high elevation sites in the Swiss Alps to common gardens differing c. 1000 m in elevation to mimic changes in temperature, and installed rain shelters to control soil water availability. Our factorial design allows to quantify the effects of simultaneous warming and drought on key plant functional traits and to test for differences in direction and magnitude of plastic responses between mid and high elevation species. Specifically, we expect: (1) plant productivity to decrease with elevation and drought (2) SLA to decrease with increasing elevation and drought (3) allocation to belowground and reproductive structures to increase with drought and elevation and to be proportionately greater in high elevation species (4) the degree of phenotypic plasticity to be higher in high elevation species relative to congeneric mid elevation species, resulting from adaptation to high environmental heterogeneity at high elevation.

MATERIALS AND METHODS

Common gardens and study species

Common gardens, with four plant beds each, were established at 1050 and 2000 m.a.s.l on the same mountain of the Bernese Highland in Switzerland. The difference in elevation between the common gardens entails for an annual mean air temperature difference of 5–6 °C (Körner 2003), which mimics extreme warming scenarios of the IPCC by 2100 (Kovats *et al.* 2014). Specific site location and abiotic conditions have previously been described in a related paper (Gugger *et al.* 2015).

Fourteen species pairs of congeneric perennial herbs naturally growing in the region and originating from mid and high elevations were selected for this study (Table 1), covering a broad taxonomic and growth form range. Mid elevation species were selected from elevations between c. 300 and 1000 m.a.s.l and high elevation species between c. 1600 and 2400 m.a.s.l (Aeschimann *et al.* 2004; Lauber *et al.* 2001), as to avoid an overlap in their altitudinal range of distribution (see details in Table 1 in Gugger *et al.* 2015). Seed mixes, originally collected from wild flower populations from the aforementioned distributional ranges and then proliferated in gardens for two years, were purchased from Swiss seed producers (Samen und Pflanzen AG Schutz, Filisur; UFA-Samen, fenaco Genossenschaft, Winterthur; Wildstaudengärtnerei, Eschenbach).

Experimental design

For a detailed experimental design refer to (Gugger *et al.* 2015). In short, seeds were germinated in spring 2012 and seedlings were later transferred into multitrays (4 cm Ø *6*9 = 54 pots) filled with low-nutrient soil (Anzuchterde Ökohum, Herrenhof, Switzerland). In early July, plants were transported to the common gardens and transplanted into bigger pots (11.5 × 11.5 × 21.5 cm) with identical soil. At each site, 12 individuals per species were placed in the control beds and 12 in the beds receiving rain shelters (i.e. drought treatment), leading to a full factorial design including 12 replicates × 28 species (14 mid and 14 high elevation species) × 2 sites (mid/high) × 2 treatments (control/dry) = 1344 individuals in total. Rain shelters were installed after 2 weeks of acclimation and consisted of triangular aluminium frames

Table 1: overview of the congeneric pairs of mid and high elevation species included in our study (or subspecies in the case of *Anthyllis*, *Silene* and *Trifolium*)

Family	Mid elevation species	High elevation species
Lamiaceae	<i>Acinos arvensis</i> (Lam.) Dandy	<i>Acinos alpinus</i> (L.) Moench
Poaceae	<i>Anthoxanthum odoratum</i> L.	<i>Anthoxanthum alpinum</i> Löve
Fabaceae	<i>Anthyllis vulneraria ssp. vulneraria</i> L. s.l.	<i>Anthyllis vulneraria ssp. alpestris</i> Schult
Brassicaceae	<i>Arabis hirsuta</i> L.	<i>Arabis alpina</i> L. s.l.
Campanulaceae	<i>Campanula rotundifolia</i> L.	<i>Campanula scheuchzeri</i> Vill.
Asteraceae	<i>Centaurea scabiosa</i> L. s.l. ^a	<i>Centaurea montana</i> L. ^a
Caryophyllaceae	<i>Dianthus deltooides</i> L.	<i>Dianthus sylvestris</i> Wulfen
Rosaceae	<i>Geum urbanum</i> L. ^a	<i>Geum montanum</i> L. ^a
Fabaceae	<i>Lotus corniculatus</i> L.	<i>Lotus alpinus</i> Ramond
Fabaceae	<i>Onobrychis vicicifolia</i> Scop. ^a	<i>Onobrychis montana</i> DC. ^a
Poaceae	<i>Phleum phleoides</i> (L.) Karsten	<i>Phleum alpinum</i> L.
Plantaginaceae	<i>Plantago lanceolata</i> L.	<i>Plantago alpina</i> L.
Caryophyllaceae	<i>Silene vulgaris ssp. vulgaris</i> ^a (Moench) Garcke s.l.	<i>Silene vulgaris ssp. glareosa</i> (Jord.) ^a Marsd.-Jon & Turill
Fabaceae	<i>Trifolium pratense ssp. pratense</i> L. ^a	<i>Trifolium pratense ssp. nivale</i> (Koch) ^a

Mid elevation species were collected from 300 to 1000 m a.s.l., and high elevation species from 1600 to 2400 m a.s.l. (Aeschimann *et al.* 2004; Lauber and Wagner 2001). For details on the natural range of distribution of each species refer to Gugger *et al.* (2015).

^aSpecies marked with asterisks were excluded from analysis for the 2013 data.

with a base area of 2.4×3.0 m and a height of 1.2 m, covered by a UV-B transmissible greenhouse film (Luminance AF Window, Folitec, Germany; Samuel Schmid and Michael Scherer-Lorenzen, personal communication). A minimal water input was provided every 2 weeks during the growth period by distributing 20 L of rainwater equally over the plants in both the control and the drought treatment. It follows that the difference in soil water availability between the two treatments equaled the amount of natural precipitation (blocked by the rain shelters in the drought treatment). Rain shelters were removed after the first growing season to allow plants to overwinter under snow cover. After snowmelt in spring 2013, rain shelters were re-installed (mid-May at the low common garden and mid-June at the high common garden).

At each site, data loggers (TidBit v.2 UTBI-001; Onset Computer Corporation, Bourne, MA, USA) recorded temperatures at 0.5 m above the ground in both treatments (control and drought) to assess possible warming effects of rain shelters. Similarly, light intensity loggers (Hobo pendant light data logger 64K-UA-002-64, Onset Computer Corporation, Bourne, MA, USA) were installed in each common garden and treatment to control for shading effects induced by the rain shelters. Volumetric soil moisture content (VSMC $\text{m}^3 \text{m}^{-3}$) was measured monthly on a subset of pots in each treatment with a HH2 Moisture Meter and a Theta Probe type ML2x (Delta-T Devices, Cambridge, UK).

Abiotic treatment effect

Over the growing season (from May to October 2013), the recorded temperature averaged 15.7°C at the lower site and 11.3°C at the higher site (Table 2), and differed on average by 4.4°C between both common gardens. Rain shelters only marginally increased the temperature of the plant beds by 0.3°C on average (Table 2). Light intensity (measured in klux at 1 PM, Table 2) was greater at the higher site but at both sites rain shelters intercepted c. 30% of light without having limiting effects on plant growth (see Fig. 11.11 in Körner 2003). Volumetric soil moisture content (VSMC in $\text{m}^3 \text{m}^{-3}$, Table 2) was significantly reduced (at least 6-fold) in the drought treatment relative to the control at both common gardens ($W = 900$, $P = 10^{-4}$; $W = 844.5$, $P = 10^{-4}$, respectively).

Table 2: mean temperature, light intensity and volumetric soil moisture content (VSMC) for each treatment averaged over the second growing season (May–October 2013)

	Temperature ($^\circ\text{C}$)	Light Intensity (klux)	VSMC ($\text{m}^3 \text{m}^{-3}$)
Low site/control	15.5	11.53	0.4
Low site/dry	15.9	8.45	0.06
High site/control	11.2	13.98	0.48
High site/dry	11.4	10.12	0.08

Assessment of plant traits and fitness proxies

At the end of the first growing season in 2012 (12 weeks after transplantation, from October 1 to 4), survival of individuals was recorded. Aboveground biomass was harvested at c. 2 cm above the ground, stored in individual parchment bags, dried for 72 h at 80°C and weighed to obtain dry mass. Specific leaf area was measured during harvest by taking circular corings from three newly grown, mature leaves per individual, while avoiding the central leaf vein (Scheepens et al. 2010). The diameter of the corings differed between species and ranged between 2.5 and 10 mm depending on leaf size. The three leaf corings from one individual were pooled in individual parchment bags, and dried for 48 h at 60°C . Leaf corings of one individual were weighed together to a precision of 0.0001 g. SLA was calculated for every individual by dividing the area of corings by their average dry mass (Perez-Harguindeguy et al. 2013).

At the beginning of the second growing season (2013), over-winter survival of individuals was recorded before re-installing the rain shelters. Final harvest was done from September 15 to 17 at the lower common garden and from October 15 to 17 at the higher common garden. The intentional difference between both harvests allowed plants to grow for 18 weeks at both sites. For every individual, aboveground biomass was harvested at ground level, separated into vegetative and reproductive biomass and stored in parchment bags. Reproductive biomass includes flower heads and flower stems. Individual root biomass, including all belowground organs, was sampled from pots and additional roots were dug up when they had grown out of the pots (rare occurrence). After roughly cleaning roots of soil, they were stored in parchment bags. All samples were kept refrigerated until transporting them back to the laboratory (max. 3 days), where vegetative and reproductive biomass was dried for 72 h at 80°C and weighed for dry mass. Root samples were carefully washed to remove all sediment particles above a 2-mm mesh sieve to minimize loss of fine roots. Clean roots were dried for 72 h at 80°C and weighed. Plant mass fractions (Poorter et al. 2012b) were calculated as the proportion of total plant biomass allocated to each structure (RMF: root mass fraction, FMF: flower mass fraction).

Degree of phenotypic plasticity

The degree of phenotypic plasticity in response to warming and drought was estimated as a phenotypic plasticity index (P_i) (Valladares et al. 2006). This index was calculated as the difference between the maximum and the minimum mean value of a given trait and species over all treatment combinations divided by the maximum mean, which serves to standardize the index ranging from zero (no plasticity) to one (maximum plasticity). The P_i was examined for the functional plant traits (i.e. SLA, RMF, FMF) of every species, in order to compare the degree of phenotypic plasticity between mid and high elevation species for traits related to but not directly indicative of plant fitness (i.e. biomass).

Statistical analysis

To test if the transplantation and drought treatment had an effect on plant functional traits and fitness proxies of mid and high elevation species, linear mixed-effect models were applied. 'Elevation' (mid elevation or high elevation site), 'drought' (control or drought treatment), 'origin' of species (mid elevation and high elevation species) were included as fixed effects, along with their respective two-way and three-way interactions. To account for variances between species, species nested within genus was included as random effect in the models. The environmental effects of 'elevation' and/or 'drought' indicate trait variation due to different environmental conditions (i.e. phenotypic plasticity), while the 'origin' of species effect indicates differences between mid and high elevation species. The interaction between 'origin' of species and 'elevation' and/or 'drought' indicates a difference in the responses to environmental conditions between mid and high elevation species. All proportions were arc sine transformed prior to analysis (Crawley 2007). Initially, the growth form, taxonomic and functional group of species were included in the models to check for patterns induced by these factors, but these terms were removed because they were never significant. All linear mixed-effect models were performed with the 'lmerTest' package for R software (Kuznetsova et al. 2013) and based on Type 3 errors and Satterthwaite approximation for denominator degrees of freedom. We report F -values and P values for fixed effects and χ^2 -values and P -values for random effects using the 'rand' function in lmerTest. Normality was verified for all variables to ensure accuracy of the estimated P -values (Pinheiro et al. 2000). *Post hoc* Tukey HSD tests for multiple comparisons were performed using the 'mult-comp' package (Hothorn et al. 2008) for R software.

The number of individuals that survived the first growing season and the following winter was counted at each site and

for each treatment and analyzed using Fisher's Exact Test for Count Data.

Finally, to test for differences in the degree of phenotypic plasticity of focal plant traits between mid and high elevation species, the calculated Phenotypic Plasticity Index (P_i) was analyzed with a paired Wilcoxon signed rank test (accounting for species genera).

All the analyses were performed on R version 3.0.2 software (R Development Core Team 2013).

RESULTS

Fitness proxies (survival and biomass)

96.7% of individuals survived transplantations to the common gardens and the first growing season. Not surprisingly, aboveground biomass differed between species nested within genus, because of inherent differences in productivity (Table 3; $\chi^2 = 768$, $P < 10^{-4}$). Although certain genera produced larger plants (i.e. *Anthyllis*, *Silene*) or smaller plants (i.e. *Campanula*, *Dianthus*), grouping of species in functional and taxonomic groups or growth forms did not yield further insight (factors were subsequently removed from final models). However, after the first growing season (c. 12 weeks in 2012) interesting overall patterns emerged between grouped mid and high elevation species in response to elevation and manipulated water availability (Fig. 1a). Specifically, aboveground biomass decreased significantly with elevation for both mid and high elevation species (Fig. 1a; See online supplementary Table S1). On average, mid and high elevation species differed in their response to transplantation, as indicated by a significant interaction between elevation and origin of species (Table 3; $F = 28.7$, $P < 10^{-4}$). High elevation species had a consistently lower biomass than mid elevation species at both sites, but this effect was significant only at the lower elevation

Table 3: linear mixed effect model for the responses of functional traits to the elevation and drought treatment, the origin of the species (mid vs. high elevation species) and their respective interactions

	Above-ground biomass (g)			Total biomass (g)			SLA (mm ² mg ⁻¹)			RMF			FMF		
	df	F/ χ^2	P	df	F/ χ^2	P	df	F/ χ^2	P	df	F/ χ^2	P	df	F/ χ^2	P
Elevation	1	254.53	<10 ⁻⁴	1	3.07	0.08	1	331.45	<10 ⁻⁴	1	0.002	0.95	1	0.41	0.53
Drought	1	20.95	<10 ⁻⁴	1	14.33	0.0002	1	265.84	<10 ⁻⁴	1	24.16	<10 ⁻⁴	1	27.36	<10 ⁻⁴
Origin	1	3.96	0.06	1	1.57	0.24	1	13.01	0.77	1	9.89	0.01	1	0.33	0.58
Elevation: drought	1	2.85	0.09	1	4.79	0.02	1	36.76	<10 ⁻⁴	1	38.82	<10 ⁻⁴	1	0.35	0.55
Elevation: origin	1	28.69	<10 ⁻⁴	1	0.36	0.54	1	0.51	0.47	1	3.58	0.05	1	10.45	0.001
Drought: origin	1	4.29	0.03	1	0.03	0.85	1	6.21	0.012	1	1.52	0.22	1	0.05	0.81
Elevation: drought: origin	1	2.46	0.11	1	2.31	0.12	1	0.002	0.96	1	0.45	0.51	1	1.26	0.26
Species/genus	1	768.8	<10 ⁻⁴	1	294	<10 ⁻⁴	1	830	<10 ⁻⁴	1	463	<10 ⁻⁴	1	188	<10 ⁻⁴

We report F -values and P -values for fixed effects and χ^2 -values and P -values for random effects. The significant P -values are shown in bold ($P < 0.05$).

^aAboveground biomass and SLA were measured in 2012 on a total of 1300 individuals, while the total biomass, the RMF and the FMF were measured in 2013 on the surviving individuals after removal of 5 genera ($n = 556$).

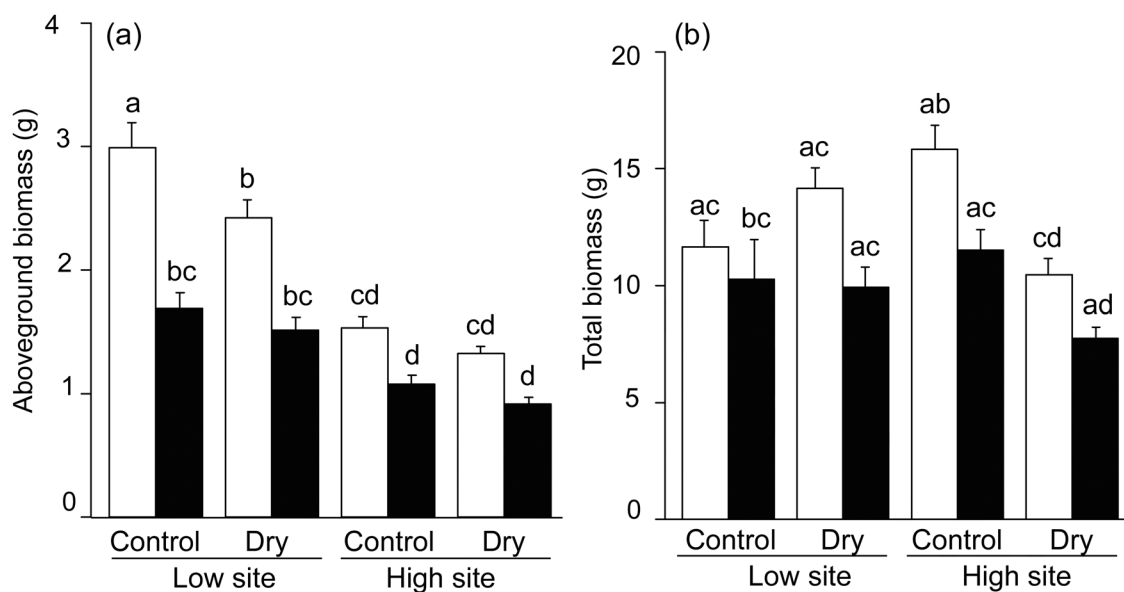


Figure 1: mean \pm 1 SE of aboveground biomass (a) measured in 2012, and total biomass (b) measured in 2013, for mid elevation species (white bars) and high elevation species (black bars) in each treatment combination (i.e. low and high elevation, control and drought). Results from *post hoc* Tukey tests can be seen in the letter contrasts. *Aboveground biomass was measured in 2012 on a total of 1300 individuals, while the total biomass was measured in 2013 on the surviving individuals after removal of five genera ($n = 556$).

site (Fig. 1a). Furthermore, a significant interaction between drought and origin of species was found (Table 3; $F = 4.29$, $P = 0.03$). While drought generally decreased aboveground biomass for both mid and high elevation species, the negative effect of drought was significant only for mid elevation species at the lower site (Fig. 1a).

Survival assessment in 2013 revealed that c. 25% of individuals had died over winter 2012/13. Mortality was however independent of site of transplantation, treatment and origin of species (Fisher's Exact Test for Count Data: $P = 0.33$). Additionally, another 13% of individuals were damaged by herbivores or were not reproductive during the following growing season. This resulted in highly unbalanced data across treatment combinations for species of five genera (*Centaurea*, *Geum*, *Onobrychis*, *Silene* and *Trifolium*), leading to the complete exclusion of these genera from analysis of data collected in 2013 to avoid any statistical biases.

After the second growing season (18 weeks), total biomass still differed between species nested within genus (Table 3; $\chi^2 = 294$, $P < 10^{-4}$). Differences between genera were larger than those between species pairs within genera, with some being inherently larger (i.e. *Anthyllis*, *Lotus*, *Silene*), compared to others (i.e. *Campanula*, *Dianthus*). More importantly, total biomass differed across treatment combinations, as revealed by a significant interaction between elevation and drought (Table 3; $F = 4.79$, $P = 0.02$). While drought marginally increased total biomass of mid elevation species at the lower site, total biomass of mid elevation species significantly decreased with drought at the high elevation site (Fig. 1b; See online supplementary Table S1). High elevation species were only marginally affected by drought at the lower site, yet total biomass of plants was

significantly reduced when grown under dry conditions at the higher site relative to the control treatment at the lower site (Fig. 1b; See online supplementary Table S1).

Specific leaf area

Across the sites and treatments, SLA ranged from $25.7 \pm 0.30 \text{ mm}^2 \text{ mg}^{-1}$ at the lower elevation site, under control conditions to $15.8 \pm 0.13 \text{ mm}^2 \text{ mg}^{-1}$ at the high elevation site under dry conditions (Fig. 2a; See online supplementary Table S1). SLA also differed between species nested within their genus (Table 3; $\chi^2 = 830$, $P < 10^{-4}$). On average, SLA decreased with elevation and drought, and the negative effect of drought was more pronounced at the lower site (Fig. 2a; See online supplementary Table S1), as indicated by the significant interaction between elevation and drought (Table 3; $F = 36.7$, $P < 10^{-4}$). Additionally, the negative effect of drought on SLA was also more pronounced for high elevation species, leading to a significant interaction between drought and origin (Table 3; $F = 6.2$, $P = 0.01$). SLA was however very similar between mid and high elevation species within each site and treatment (Table 3; $F = 13.01$, $P = 0.7$, and Tukey; Fig. 2a).

Biomass allocation (to roots and reproductive structures)

On average, plants allocated 26% of total biomass to belowground structures and 13% to reproductive structures (61% to vegetative structures). While the proportion of biomass allocated to roots or reproductive structures differed between species nested within their genus (Table 3; $\chi^2 = 463$, $P < 10^{-4}$, $\chi^2 = 188$, $P < 10^{-4}$, respectively), interesting patterns emerged when averaged across mid and high elevation species.

The proportion of total biomass allocated to belowground structures (RMF, Fig. 2b) differed significantly between mid and high elevation species, as indicated by a significant origin effect (Table 3; $F = 9.89$, $P = 0.01$). Indeed, RMF of high elevation species was significantly higher compared to mid elevation species when grown under control conditions at the lower site, and marginally higher compared to their mid elevation congeners in all other treatment combinations (Fig. 2b; See online supplementary Table S1). For both species' groups, RMF was surprisingly highest when grown at the lower site under control conditions, but drought had opposite effects at both sites, as revealed by the significant interaction between elevation and drought (Table 3; $F = 38.8$, $P < 10^{-4}$). For both mid and high elevation species, drought significantly decreased the allocation to roots at the lower site relative to the control treatment, while allocation to roots was only marginally increased at the higher site (Fig. 2b).

The investment in reproductive structures (FMF, Fig. 2c) differed between elevation of transplantation and species' origin, as indicated by the significant interaction between elevation and origin (Table 3; $F = 10.45$, $P = 0.001$). On average, species tended to have a higher FMF when growing at their elevation of origin relative to their foreign congeners (not revealed by individual *post hoc* test Fig. 2c). Moreover, drought had a significant effect on the FMF (Table 3;

$F = 27.4$, $P < 10^{-4}$). Specifically, both mid and high elevation species significantly increased the allocation to reproductive structures when growing under limited water conditions at their elevation of origin (Fig. 2c; See online supplementary Table S1).

Phenotypic plasticity index (Pi_v) of mid and high elevation species

The phenotypic plasticity index did not significantly differ between mid and high elevation species for the measured plant functional traits (Table 4). RMF was the only trait for which a marginally lower Pi_v was found for high elevation species (Table 4; $P < 0.10$). The phenotypic plasticity indices were however highly species and trait specific (see ranges Table 4). For example, plasticity in SLA ranged from 0.24 to 0.56 in mid elevation species and from 0.20 to 0.58 in high elevation species. Plasticity in RMF ranged from 0.09 to 0.54 in mid elevation species and from 0.10 to 0.35 in high elevation species. The highest ranges were found for plasticity in FMF, which ranged from 0.28 to 0.89 in mid elevation species and from 0.27 to 0.91 in high elevation species. Finally, from the average Pi_{vs} and their ranges, it also becomes apparent that the FMF was the most plastic trait, SLA had an intermediate degree of plasticity and the RMF was the least plastic trait (Table 4).

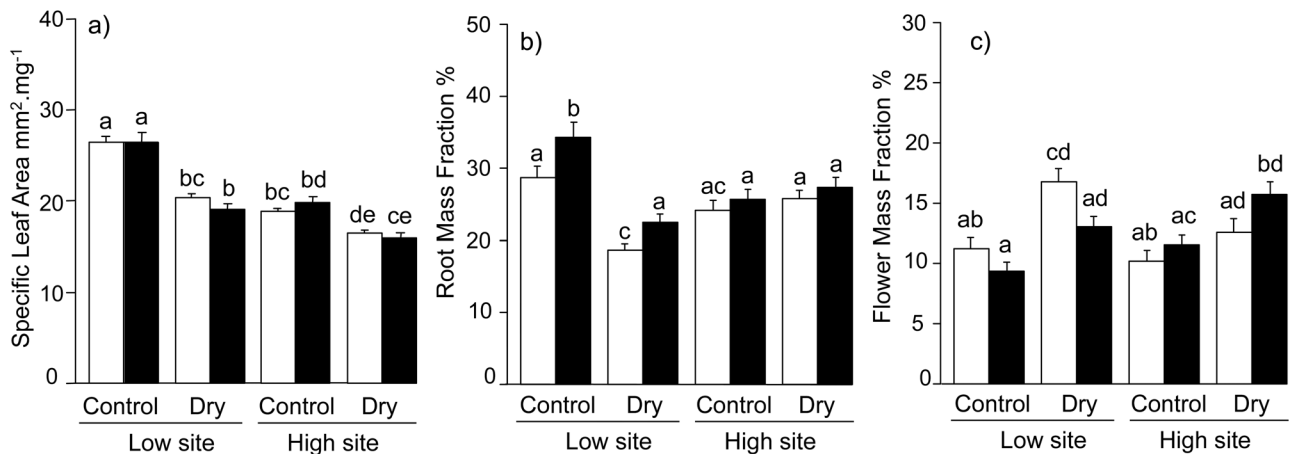


Figure 2: mean \pm 1 SE of key functional plant traits (a) SLA, (b) RMF and (c) FMF in response to treatment combinations (i.e. low or high elevation, control and drought). Mid elevation species are represented in white bars and high elevation species in black bars. Results from *post hoc* Tukey tests can be seen in the letter contrasts. *SLA was measured in 2012 on a total of 1300 individuals, while the RMF and the FMF were measured in 2013 on the surviving individuals after removal of five genera ($n = 556$).

Table 4 : mean \pm SD phenotypic plasticity indices (Pi_v) for key functional traits (SLA, RMF, FMF) compared between mid and high elevation species with a paired Wilcoxon test (V , P)

	Pi_v mean (range) of mid elevation species	Pi_v mean (range) of high elevation species	
SLA	0.41 \pm 0.10 (0.24–0.56)	0.45 \pm 0.11 (0.20–0.58)	$V = 23$, $P = 0.13$
RMF	0.29 \pm 0.15 (0.09–0.54)	0.24 \pm 0.08 (0.10–0.35)	$V = 37$, $P = 0.09$
FMF	0.56 \pm 0.19 (0.28–0.89)	0.56 \pm 0.24 (0.27–0.91)	$V = 24$, $P = 0.9$

We also report ranges of Pi_v (in parentheses) for mid and high elevation species to highlight species-specific responses.

DISCUSSION

In this study, we investigated the effects of changes in temperature (through transplantations to different elevations) and soil water availability on phenotypic variation in key functional plant traits (i.e. SLA, RMF, FMF) of 14 congeneric pairs of mid and high elevation species. We further examined if trait plasticity varied in direction and magnitude between species originating from mid and high elevation.

Plant productivity in response to elevation and drought

After the first growing season, plant productivity, measured as the aboveground biomass, was in accordance with expectations as it decreased with elevation and lower temperatures (Fig. 1a). Positive warming effects indicate that plant growth is constrained by low temperatures (Körner 2003). A moderate warming could thus have beneficial effects on high elevation plant performance and productivity, as suggested by a meta-analysis of *in situ* warming experiments with arctic and alpine tundra species (Arft et al. 1999) and by a climate chamber warming experiment on three grassland species (Frei et al. 2014b). Drought stress, however, had a negative effect on plant productivity, especially for mid elevation species and significantly reduced the production of aboveground biomass (Fig. 1a). This result confirms the efficiency of our drought treatment, which reduced the volumetric soil moisture content 6-fold, and that water availability is an important limiting factor for plant productivity (Lambers et al. 1998).

Variation in total biomass in response to the different treatment combinations during the second growing season was less consistent (Fig. 1b). Although, total biomass was significantly reduced by drought at the high site, at the lower site drought had no effect on the total biomass of high elevation species and seemed to marginally increase the productivity of mid elevation species. Increased biomass productivity of grasslands subjected to drought stress has also been reported by Gilgen et al. (2009) and was explained by improved soil oxygenation. Higher soil oxygen concentrations are expected to increase soil mineralisation rates and consequently nutrient availability, which could rapidly lead to higher plant productivity (Brilli et al. 2011; Gilgen et al. 2009).

Finally, while inherent differences in productivity were detected between genera, no significant effects of growth forms or taxonomic and functional groups were detected. However, across the species' origin we detected that on average high elevation species always had lower aboveground and total biomass than mid elevation species. This result highlights the fundamental differences in growth strategies between species from mid and high elevation, with high elevation species displaying smaller or even dwarfed morphologies (Billings et al. 1968; Körner et al. 1987; Körner 2003), allowing them to better withstand harsh alpine conditions (i.e. temperature extremes, snow, wind, irradiance etc.). Clearly, our results

confirm that these differences in growth form are genetically determined.

Plastic responses of key functional traits to elevation and drought treatment

Specific leaf area showed substantial phenotypic plasticity after 12 weeks, as indicated by a significant decrease in SLA with increasing elevation and drought (Fig. 2a), in accordance with literature (Prock et al. 1996; Pang et al. 2011; Poorter et al. 2012a; Scheepens et al. 2010). At both sites, drought stress reduced SLA and the highest SLA values were found for leaves of individuals grown under control conditions at the mid elevation site and lowest values were found under dry conditions at the high elevation site. However, SLA values did not vary between the dry treatment at mid elevation and the control treatment at high elevation, possibly implying that transplantations to the higher site and the drought treatment at low elevation exerted comparable pressures on plants, resulting in similar SLA values. Additionally, SLA values of mid and high elevation species did not differ within treatment combinations, suggesting similar responses to external conditions. The decrease in SLA with increasing elevation and drought stress can be achieved through increases in leaf density and/or leaf thickness (Körner 2003; Poorter et al. 2009). Though we did not measure these traits separately, Scheepens et al. (2010) found in *Campanula thyrsooides* that leaf thickness significantly decreased with elevation and thus explained the decrease in SLA through substantial increases in leaf density, leading to smaller cells and more cells per unit leaf volume. This might also be true in our case, especially in the event of drought stress, which restricts cell expansion by decreasing internal turgor pressure of the cells (Sharp et al. 1989; Tardieu et al. 2000). Overall, high plasticity in SLA is highly advantageous as it allows plants to adjust growth rate, leaf longevity and stress tolerance to prevailing environmental conditions (Scheepens et al. 2010; Wright et al. 2004).

The proportion of total biomass allocated to belowground structures, measured as the RMF, only decreased under drought stress at the mid elevation site, and was unaffected by site elevation in general. This result is counter to predictions, as investment in roots usually increases with elevation (Körner 2003) and under limited soil water availability (Bell et al. 1999; Heschel et al. 2004; Larcher et al. 2010). Similarly to our results, Kreyling et al. (2008), Gilgen et al. (2009), and Backhaus et al. (2014) found small increases or no alterations in plant belowground biomass in response to limited soil water availability. While we cannot exclude that some root material was lost during sampling or cleaning, leading to biases in our data, we rather hypothesize that the similar values in RMF are due to the fact that root morphology differed between treatment combinations. Körner et al. (1987) showed that with increasing elevation, investment in fine roots increases, and a similar result was found in response to low water potential (Fraser et al. 1990). Fine roots, which have a thinner diameter and are less lignified and suberised

than coarse roots (Lavelle *et al.* 2005), probably result in less dry weight than thicker roots and we argue that this morphological difference could potentially explain that changes in RMF between site elevations and soil water availability were relatively small. In accordance with literature, our results however showed that high elevation species generally invested more biomass in belowground structures relative to their mid elevation congeners (Billings *et al.* 1968; Körner 2003). Additionally, among the studied functional traits, RMF was the least plastic trait (P_i c. 0.265) and showed particularly little variation in high relative to mid elevation species, and lesser variation when mid elevation species were grown at the higher site, indicating the constraints acting on allocation patterns at high elevation.

The proportion of total biomass allocated to reproductive structures, measured as the FMF tended to be greater for mid elevation species when growing at the lower elevation site and for high elevation species when growing at the high elevation site. As the FMF is closely associated with seed production and plant fitness, these results seem to indicate a home-site advantage of species to the conditions at their habitat of origin (Blanquart *et al.* 2013; Joshi *et al.* 2001). Furthermore, drought also increased the allocation to reproductive structures for mid and high elevation species at their respective elevation of origin. These results suggest a prioritization of reproduction at the expense of growth under drought stress. Interestingly, FMF showed the highest plasticity among the studied plant traits (P_i of 0.56), probably indicating the importance of adjusting this trait to environmental conditions to maintain fitness homeostasis.

Degree of phenotypic plasticity compared between mid and high elevation species

We found very little evidence for differences in the degree of phenotypic plasticity in key plant traits between mid and high elevation species. Only the plasticity in RMF was marginally smaller for high elevation species (Table 4). This indicates that high elevation species were less capable of adjusting the allocation to belowground structures to changing external conditions, probably reflecting their genetically fixed higher allocation to below-ground structures. Similar results were found by Frei *et al.* (2014b), where plasticity was reduced in only a single trait (leaf length) in high elevation populations of *Trifolium montanum*. Consequently, the magnitude but also the direction of plasticity in key plant traits in response to transplantations and soil water availability in mid and high elevation species was similar, suggesting rather uniform responses to climate change between these two groups of species (Frei *et al.* 2014b).

More generally, and contrary to our hypothesis, phenotypic plasticity did not seem to depend on environmental heterogeneity more commonly observed at high elevation (Scherrer *et al.* 2011). In contrast to the general consensus that phenotypic plasticity should be selected for in heterogeneous environments (Via *et al.* 1985), other studies also

reported no differences in plant trait plasticity compared between populations from habitats with constant and more variable environmental conditions (Franks 2011; Heschel *et al.* 2004). These previous results, in combination with our study, indicate that increased environmental variation does not necessarily lead to a greater degree of functional plasticity. Two combined factors are predicted to favor selection for phenotypic plasticity: when the rates of environmental change are similar or slower than the response rate of an organism and when said change is highly but not completely predictable (Scheiner 1993). In alpine environments, change might be rather unpredictable and could thus explain why the advantages of being plastic in response to environmental heterogeneity do not necessarily outweigh the costs (i.e. maintenance, production and information acquisition cost; DeWitt *et al.* 1998).

Although no difference was found in functional plasticity between mid and high elevation species, some traits were more plastic than others. SLA and the allocation to reproductive structures (FMF) were highly plastic in response to treatment combinations, while the allocation to belowground structures (RMF) was comparatively less plastic, hence more strongly genetically controlled. This result indicates constrained phenotypic plasticity in this specific trait, which could be related to potential stabilizing selection acting on allocation patterns at high elevation. Constrained plasticity was also found in the reproductive phenology of high elevation species, which was monitored in a parallel study during the second year of this experiment (Gugger *et al.* 2015). Particularly, high elevation species were less plastic than their lower elevation congeners in the timing of peak flowering, suggesting that adaptation to short growing seasons in alpine environments limits the potential for plasticity of flowering phenology in high elevation species in response to environmental change (Gugger *et al.* 2015), and leads to a higher genetic canalization of the timing of peak flowering (Ghalambor *et al.* 2007; Price *et al.* 2003; Pigliucci *et al.* 2006). This however does not seem to apply to all functional traits of high elevation species, as we have shown here that SLA and FMF were highly and equally plastic in mid and high elevation species.

CONCLUSION

To conclude, both mid and high elevation species displayed great functional plasticity in key plant traits related to eco-physiological characteristics in response to changing temperatures and soil water availability. As the direction and magnitude of functional plasticity was similar between mid and high elevation species, our results suggest rather uniform responses of these species groups to climate change. While plasticity in functional traits was highly species and trait specific, the general capacity of species to respond plastically to environmental changes may offer a short-term strategy to face climate change.

SUPPLEMENTARY DATA

Supplementary material is available online at *Journal of Plant Ecology* online.

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