What role do plant–soil interactions play in the habitat suitability and potential range expansion of the alpine dwarf shrub *Salix herbacea*?

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Abstract

Mountain plants may respond to warming climates by migrating along altitudinal gradients or, because climatic conditions on mountain slopes can be locally very heterogeneous, by migrating to different microhabitats at the same altitude. However, in new environments, plants may also encounter novel soil microbial communities, which might affect their establishment success. Thus, biotic interactions could be a key factor in plant responses to climate change. Here, we investigated the role of plant–soil feedback for the establishment success of the alpine dwarf shrub *Salix herbacea* L. across altitudes and late- and early snowmelt microhabitats. We collected *S. herbacea* seeds and soil from nine plots on three mountain-slope transects near Davos, Switzerland, and we transplanted seeds and seedlings to substrate inoculated with soil from the same plot or with soils from different microhabitats, altitudes and mountains under greenhouse conditions. We found that, on average, seeds from higher altitudes (2400–2700 m) and late-exposed snowbeds germinated better than seeds from lower altitudes (2200–2300 m) and early-exposed ridges. However, despite these differences in germination, growth was generally higher for plants from low altitudes, and there were no indications for a home-soil advantage within the current range of *S. herbacea*. Interestingly, seedlings growing on soil from above the current altitudinal distribution of *S. herbacea* grew on average less well than on their own soil. Thus, although the lack of a home-soil advantage in the current habitat might be beneficial for *S. herbacea* in a changing environment, migration to habitats beyond the current altitudinal range might be limited, probably due to missing positive soil-feedback.

Zusammenfassung

Pflanzen in Gebirgen können sich an ein wärmeres Klima anpassen, indem sie entweder in andere Höhenlagen, oder, da die klimatischen Bedingungen an Gebirgshängen lokal sehr heterogen sein können, in unterschiedliche Mikrohabitate auf gleicher Höhe wandern. An neuen Standorten treffen Pflanzen auch neuartige Gesellschaften von Bodenorganismen an, welche deren

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Etablierung beeinflussen können. Biotische Interaktionen stellen deshalb einen möglicher Schlüsselfaktor bei der Anpassung von Pflanzen an den Klimawandel dar. In unserem Experiment untersuchen wir, welche Rolle das Pflanze-Boden-Feedback für die Etablierung des Alpenen Zwergstrauchs *Salix herbacea* L. entlang eines Höhengradienten und zwischen Mikrohabitaten mit früher und später Schneeschmelze spielt. Wir haben Samen von *S. herbacea* und Bodenmaterial an neun Standorten gesammelt, die auf Transekte an drei Bergen in der Nähe von Davos, Schweiz, verteilt waren. Unter Gewächshausbedingungen haben wir die Samen und Keimlinge in Substrat verpflanzt, das mit dem Boden desselben Standorts oder mit dem Boden von unterschiedlichen Mikrohabitaten, Höhenstufen und Bergen inokuliert wurde. Unsere Ergebnisse zeigen, dass Samen von hohen Standorten (2400–2700 m) und von spät ausapernden Schneetälchen durchschnittlich besser keimten als Samen von niedrigen Standorten (2200–2300 m) und früh ausapernden Kämmen. Trotz dieser Unterschiede in der Keimung, war das Wachstum von Pflanzen von niedrigen Standorten im Allgemeinen stärker. Innerhalb des heutigen Verbreitungsgebiets von *S. herbacea* konnten wir keine Hinweise darauf finden, dass Pflanzen einen Vorteil hatten, wenn sie auf Substrat vom eigenen Standort wuchsen. Interessanterweise wuchsen Keimlinge auf Substrat von überhalb des heutigen Verbreitungsgebietes, schlechter als auf Substrat vom eigenen Standort. Obwohl das Fehlen eines Heim-Boden-Vorteils für *S. herbacea* unter sich ändernden Umweltbedingungen möglicherweise von Vorteil ist, könnte die Migration zu Standorten überhalb des heutigen Verbreitungsgebietes, aufgrund von fehlendem positivem Pflanze-Boden-Feedback, eingeschränkt sein.

Keywords: Biotic interaction; Range limit; Elevation; Genetic differentiation; Microhabitat; Microtopography; Migration; Snowmelt gradient; Soil feedback

Introduction

Over the last 100 years, the global surface temperature has increased by 0.7 °C (Stocker, Qin, Plattner, Tignor, & Allen, 2013) with the most pronounced temperature increases in the arctic and alpine regions by 2 °C (EEA, 2009). For these regions, climate models predict further increases in temperatures and precipitation, in the form of rain rather than snow, resulting in fewer days with snow cover, particularly in spring (Beniston, Keller, Koffi, & Goyette, 2003). As a result, 36–55% of European alpine plant species have been predicted to lose more than 80% of their suitable habitat by 2070–2100 (Engler, Randin, Thuiller, Dullinger, & Zimmermann, 2011). However there are many uncertainties surrounding these projections, since most of them are based on observed correlations between abiotic environmental factors and species occurrences, and ignore potentially important mechanisms of plant response to climate change (Thuiller, Albert, Araújo, Berry, & Cabeza, 2008).

There are many studies showing clear evidence that species are already migrating along altitudinal and/or latitudinal gradients as a consequence of climate change (reviewed in Chen, Hill, Ohlemüller, Roy, & Thomas, 2011). For example, a recent study by Pauli, Gottfried, and Dullinger (2012) showed that, because of upward migration, species richness of European mountain summits has increased by 3.9 species on average between 2001 and 2008. Climatic and other environmental conditions, however, do not only change across latitude and altitude, but also across microhabitats. This is especially true in mountain ecosystems, where microtopography is extremely heterogeneous, even over distances of a few metres, resulting in small-scale variation in temperatures and snowmelt timing (Körner, 2003; Scherrer & Körner, 2011).

This small-scale variation could explain why observed elevational shifts in contrast to latitudinal shifts lag behind the expected shifts of plant species (Chen et al., 2011). Therefore, not only altitudinal but also microhabitat shifts could provide opportunities for alpine plant species to escape warming climates (Scherrer & Körner, 2011).

The ability of a plant to successfully establish in a new habitat depends on how it deals with both climatic conditions and biotic factors. A home-site advantage of plants has been reported for various abiotic factors, e.g. along altitudinal gradients with different climatic conditions (Byars, Papst, & Hoffmann, 2007; Gonzalo-Turpin & Hazard, 2009) or habitats with different soil conditions (Sambatti & Rice, 2006). However, home-site advantages with respect to biotic interactions have rarely been studied (but see: Macel, Lawson, Mortimer, Smilauerova, Bischoff, et al., 2007; Grassein, Lavorel, & Till-Bottraud, 2014). Biotic factors that influence colonization success in new habitats include interactions with pollinators, herbivores, inter- and intraspecific competitors, pathogens and soil microbes (Tylianakis, Didham, Bascompte, & Wardle, 2008; Van der Putten, Macel, & Visser, 2010). Specifically, many soil microbes have negative effects on plant performance and act as pathogens, but others have positive effects on plants, e.g. by improving nutrient accessibility and uptake for the plant. A home-site advantage could emerge either because plants adapt to the local microbes or because plants select the most beneficial microbes. A home-site advantage may be disrupted when plant species shift their altitudinal range, migrate to other microhabitats or when soil microbial communities are altered due to climate change (Van Grunsven, van Der Putten, Bezemer, Tamis, & Berendse, 2007; Van der Putten, Bardgett, Bever, Bezemer, & Casper, 2013). Therefore, in order to be able to predict

how plants may respond to climate change, experiments testing for the effects of soil–microbial interactions are needed (Gellesch, Hein, Jaeschke, Beierkuhnlein, & Jentsch, 2013; Tylanakis, Didham, Bascompte, & Wardle, 2008; Van der Putten, Bardgett, Bever, Bezemer, & Casper, 2013).

The arctic-alpine dwarf shrub *Salix herbacea* typically occurs in late-snowmelt microhabitats but also on wind-exposed mountain ridges across an altitudinal range, which in the Alps ranges from 1800 to 2800 m a.s.l. (Beerling, 1998). Across such altitudes and microhabitats, soil microbial communities in mountains can differ considerably (Väre, Vestberg, & Ohtonen, 1997; Yao, Vik, Brysting, Carlsen, & Halvorsen, 2013). *Salix herbacea* is strongly associated with soil microorganisms such as ectomycorrhizal fungi (Graf & Brunner, 1996). For example, Mühlmann and Peintner (2008) found 93% mycorrhization of *S. herbacea* plants with high spatial heterogeneity across their study plots on a glacier forefield in the Austrian Alps. Both positive and negative plant–soil microbe associations may be altered by climate change. In order to make predictions about the potential of *S. herbacea* to migrate under climate change, we must first examine to what degree different microhabitats or altitudes affect population differentiation. We must also understand the importance of feedback with soil microbial communities at different altitudes and microhabitats within the species current range and beyond its current range limit, and determine the effects of changing microbial communities on plant establishment and growth.

In this study, we investigated the effect of soil biota on the establishment success of the alpine dwarf shrub *S. herbacea* across altitudes and microhabitats. We assessed the effect of local and non-local soil inoculates on germination and growth of *S. herbacea* in greenhouse experiments and asked the following questions:

- 1) Are there differences in seed germination and growth of *S. herbacea* among different microhabitats and altitudes of origin?
- 2) Does *S. herbacea* exhibit a home-soil advantage due to feedback with soil microbes at the scale of microhabitats, altitudes or mountains within its current range?
- 3) Can *S. herbacea* potentially establish in soils from higher altitudes, where it does not currently occur?

Materials and methods

Study species

Salix herbacea is a clonal, dioecious, long-lived dwarf shrub with an alpine-arctic distribution. It occurs in the northern and alpine regions of Europe and North America, and in western Siberia and the Arctic region (Beerling, 1998). Despite being a typical snowbed species, *S. herbacea* is also common on wind-exposed mountain ridges and scree with

little protection by snow cover (Beerling, 1998). It is adapted to harsh climatic conditions with winter minimum temperatures down to -20°C and a short growing period of two to three months (Beerling, 1998). *Salix herbacea* produces an extensive ramifying system with branched rhizomes forming flat mats (Beerling, 1998). The aerial branches are woody and usually reach 2–5 cm above the ground surface. Catkins appear with the leaves in June/July. Seed production may amount to >4000 wind-dispersed seeds/ m^2 (Nylén, Elven, & Nordal, 2000). Average clone size is estimated to be 1 m^2 (Reisch, Schurm, & Poschlod, 2007). *Salix herbacea* usually occurs on nutrient-poor, predominantly acidic, but also calcareous soils (Beerling, 1998). It is associated with at least 260 species of arctic-alpine Basidiomycete fungi, including pathogenic rust fungi (Graf, 1994; Pei, Royle, & Hunter, 1996). Graf and Brunner (1996) found up to 33 ectomycorrhiza species associated with *S. herbacea* within a 50 m^2 area in the Swiss Alps, close to one of our study sites.

Seed and soil sampling

We collected *S. herbacea* seeds and soil from a total of 12 study plots on three mountain-slope transects (Jakobshorn, Wannengrat and Schwarzhorn) near Davos, Switzerland. The plots were c. 100 m^2 , and resembled a typical late-exposed snowbed microhabitat type or a typical early-exposed ridge microhabitat type. Snowmelt in ridge microhabitats was on average ($\pm\text{SE}$) $39 (\pm 8)$ days earlier than in snowbed microhabitats in 2011 ($t_5 = -4.18$, $p < 0.008$) and $11 \pm (11)$ days earlier in 2012 ($t_5 = -0.90$, $p = 0.409$; see Appendix: Table A.1). Each transect consisted of one pair of contrasting microhabitat types in the upper part of the altitudinal distribution (2400–2700 m) and one microhabitat pair in the lower part of the altitudinal distribution (2200–2300 m) of *S. herbacea*. The mean altitudinal difference between lower and upper plots was $299 \pm (29)\text{ m}$ (for study plot characteristics see Appendix: Table A.1).

Within each plot, we collected a bulk sample of seeds from 100 catkins, which were, if possible, $>50\text{ cm}$ apart from each other. Closed capsules were collected between 17 July and 28 August 2012, when infructescences of nearby *S. herbacea* individuals started to open. We first kept the infructescences in paper bags for five days at room temperature until capsules dehisced and seeds were released, and then stored them at -20°C . Within two low snowbed plots (on the Jakobshorn and Wannengrat transect) and one high ridge plot (on the Schwarzhorn transect), very few seeds were released from the capsules. We excluded these three plots from the experiment, because we assumed the seeds to be immature.

Between September 3rd and 9th 2012, we excavated 1 L of soil to a depth of 20 cm from each of five different locations within each plot (5 L per plot). The soil from within the same plot was mixed and stored at 5°C until the start of the experiment.

Germination

In a growth room, we sowed *S. herbacea* seeds from the nine different origins with mature seeds in Petri dishes filled with agar and inoculated with different soil extracts. The inocula were prepared by stirring 100 ml of soil in 100 ml of deionized water, and filtering it using a 200 μ m sieve to obtain a filtrate of bacteria, fungi and other small soil organisms (Parepa, Schaffner, & Bossdorf, 2013). We used the following soil inoculum treatments: soil microbial inocula from (1) the same plot (home treatment), (2) the plot in the other microhabitat type at the same altitude in the same transect (microhabitat treatment), (3) the plot in the same microhabitat type but at the other altitude in the same transect (altitude treatment), and (4) a plot in the same microhabitat type and altitude, but in a different transect (transect treatment). In addition, (5) seeds from all five high altitude plots were also sown with inocula from soil collected at Schwarzhorn at an altitude of c. 3100 m, where *S. herbacea* does not occur (beyond-range treatment; Appendix: Table A.2).

For each of the soil treatments (four for the low- and five for the high-altitude plots) and seed origins, we sowed 10 seeds into each of five Petri dishes (diameter: 5 cm), filled with agar (1% agar, ½ Murashige & Skoog medium, pH 6). After watering each seed with 10 μ l of inoculum, Petri dishes were sealed with parafilm, and transferred to a climate chamber with a 20 °C day (14 h) and a 10 °C night (10 h) cycle. Every two or three days during three weeks, all seeds were checked for germination (appearance of the cotyledons).

Growth

After three weeks, we transplanted three seedlings per Petri dish into separate 88 ml pots filled with a 1:1:1 mixture of collected soil, sterile vermiculite and sterile sand. The soil treatments included the home, the altitude, the microhabitat and the beyond-range treatments. We excluded the transect treatment, because we had no seedlings available from the low snowbed plots of all three transects. Analysis of inorganic soil N concentrations using 2 M potassium chloride showed that soil N concentrations of the collected soils ranged from 4 to 78 μ g/g, with no clear differences between altitudes, microhabitats or transects. To avoid nutrient limitation in any of the treatments (Van Grunsven, van Der Putten, Bezemer, Tamis, & Berendse, 2007), we added 1.7 g Osmocote Exact Standard 3-4 M slow-release fertilizer to 1 l of soil mixture. For a sterile control soil mixture, this resulted in a soil N concentration of 826 μ g/g, so soil N concentrations in our pots were about 10 times higher than the most nutrient-rich collected soil. The pots were placed in a greenhouse with 20 °C day (15 h) and 18 °C night (9 h) temperatures.

Relative leaf-area growth rate was calculated as the rate of leaf area increase between day 35 and day 95 of the experiment. We estimated leaf area by multiplying averaged length and width of the two largest leaves and multiplied it with the

total number of leaves to gain an estimate of total leaf area. After 95 days, we harvested all the plants. We washed the roots free of soil, and determined above- and belowground biomass after drying for 72 h at 70 °C.

Data analysis

All statistical analyses were done using R version 2.15.2 (R Core Team, 2013). To analyze the proportion of germinated seeds, we used a generalized linear mixed model (GLMM) with a binomial error structure as implemented in the *glmer* function of the *lme4* package (Bates, Maechler, Bolker, & Walker, 2013). Total leaf area, total biomass and growth rates of seedlings were analyzed with linear mixed models as implemented in the *lmer* function of the *lme4* package.

For each response variable, the models included altitude of seed origin (high versus low), microhabitat of seed origin (ridge and snowbed), soil treatments (home, microhabitat, altitude, transect and beyond-range or a subset thereof) and their interactions as fixed effects. Plot of seed origin nested within transect, and plot of soil origin nested within transect were included as random effects to account for non-independence of replicates from the same plot and the same transect. We corrected for overdispersion in the GLMM by adding an observation-level random effect (Zuur, Hilbe, & Elena, 2013). We used maximum-likelihood-ratio tests to determine the overall significance of main effects and interactions (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). We also calculated marginal R^2 (proportion of variance explained by the fixed effect only) and conditional R^2 (proportion of variance explained by both the fixed and random factors) as a measure for a goodness-of-fit for each model (Nakagawa & Schielzeth, 2013). Since the “beyond-range” treatment (soil inoculum from 3100 m) was only used with seeds from high-altitude plots, we analyzed its effect in a separate model without seeds from low-altitude plots.

In addition to the analyses described above, we did analyses to test more specifically for a home-soil advantage by using the “home vs. away” and “local vs. foreign” criteria (Kawecki & Ebert, 2004). A significant “home vs. away” contrast indicates that seeds or seedlings have a higher performance on their home soils than on other soils, and a significant “local vs. foreign” contrast indicates that on each soil origin the local seeds or seedlings outperform the foreign seeds or seedlings. If in the main analysis described above, the soil-treatment effect was significant, we created contrasts for the “home vs. away” test by comparisons of the “home” treatment against each of the “away” treatments (i.e. altitude, microhabitat, transect). If the interaction of soil treatment with microhabitat of origin, altitude of origin or microhabitat of origin by altitude of origin was significant, we tested these contrasts separately for each of the seed origins. For the “local vs. foreign” test, we used a different fixed model, which included altitude of soil origin, microhabitat of soil origin and seed-origin treatment and their interactions. We

Fig. 1. Germination rates (means and standard errors) of *Salix herbacea* seeds from different altitudes, microhabitats and mountain transects when sown on inoculates of their own soil (Home), on inoculate of the other microhabitat at the same altitude (Microhab), on inoculate of the same microhabitat at the other altitude (Altitude) or the same altitude at a different mountain transect (Transect). For easier interpretation of significant differences due to seed origin, we indicated the group means with horizontal lines: dotted for high vs. low altitude of seed origin, dashed for high ridge, high snowbed vs. low ridge, low snowbed seed origin, respectively.

included the same random effects as described for the main analysis. If seed-origin treatment was significant, we created contrasts of the “local” treatment against the “foreign” treatments (i.e. altitude of seed origin, microhabitat of seed origin, transect of seed origin). If the interaction of seed-origin treatment with soil origin (i.e. microhabitat of soil origin, altitude of soil origin or microhabitat of soil origin by altitude of soil origin) was significant, we did these contrasts separately for each of the soil origins. All contrasts were made using post hoc tests with adjusted *p*-values as implemented in the *glht* function of the *multcomp* package (Hothorn, Bretz, & Westfall, 2008).

Results

Differences in germination and growth between seed origins

Across all transects and soil treatments, *S. herbacea* seeds germinated better when they originated from high altitudes ($60.0\% \pm \text{SE } 2.7\%$) than when they originated from low altitudes ($39.2\% \pm 3.3\%$; Fig. 1). There was a significant interaction between altitude and microhabitat of origin: the germination rate of seeds from high-altitude ridges ($53.1\% \pm 4.0\%$) was lower compared to seeds from high-altitude snowbeds ($65.0\% \pm 3.5\%$), whereas germination rate

of seeds from low-altitude ridges ($43.4\% \pm 3.8\%$) was higher compared to low-altitude snowbeds ($30.3\% \pm 6.2\%$; Fig. 1). Note, however, that the comparisons with the low altitude snowbed are based on the Schwarzhorn transect only.

Growth rates of seedlings did not differ significantly among the seed origins (Table 1). However, at the end of the greenhouse experiment, total leaf area and biomass were greater for seedlings originating from low altitudes than for those originating from high altitudes ($3457 \pm 317 \text{ mm}^2$ versus $2380 \pm 245 \text{ mm}^2$ for total leaf area; $0.109 \pm 0.011 \text{ g}$ versus $0.077 \pm 0.008 \text{ g}$ for total biomass; Fig. 2 and Table 1).

Effects of soil inocula on germination and growth within the current range

The soil treatments significantly affected seed germination (Table 1). On average, germination was lowest in the altitude treatment ($45.2\% \pm 4.5\%$), followed by the transect treatment ($53.3\% \pm 4.5\%$) and the home treatment ($56.3\% \pm 3.7\%$), and was highest in the microhabitat ($60.5\% \pm 4.3\%$) treatment. There was no evidence of a general home vs. away effect of soil inocula. Plants germinated equally well on their home soil inoculum as on inocula from other microhabitats ($z = 1.091$, $p = 0.602$), altitudes ($z = -1.939$, $p = 0.144$) or transects ($z = -0.008$, $p = 1.000$). This was consistent across seed origins, as there were also no significant seed

Table 1. The effects of altitude and habitat of seed origin, different soil treatments, and their interactions, on the germination and growth of *Salix herbacea*. The factor “soil treatment” included soil inoculates from the same origin as the seeds, from different microhabitats at the same altitude, from the same microhabitat at different altitudes, and (only for germination) from the same microhabitat at a different mountain transect (see Materials and methods for details).

Source of variation	Germination rate			Growth rate			Total leaf area			Total biomass		
	df	Chi ²	p-value	df	Chi ²	p-value	df	Chi ²	p-value	df	Chi ²	p-value
Seed origin altitude	1	7.80	0.005	1	0.24	0.625	1	6.30	0.012	1	5.68	0.017
Seed origin microhabitat	1	0.01	0.999	1	0.77	0.379	1	1.18	0.278	1	1.89	0.168
Soil treatment	3	8.26	0.040	2	2.02	0.364	2	0.15	0.927	2	0.83	0.660
Seed origin altitude: Seed origin microhabitat	1	6.26	0.012	1	2.93	0.087	1	2.45	0.118	1	1.17	0.277
Seed origin altitude: Treatment	3	4.08	0.252	2	4.06	0.131	2	3.13	0.209	2	2.24	0.325
Seed origin microhabitat: Treatment	3	6.02	0.110	2	0.80	0.671	2	6.78	0.034	2	6.60	0.036
Seed orig. altitude: Seed orig. microhabitat: Treat	3	5.13	0.162	2	2.92	0.233	2	3.00	0.223	2	4.58	0.101
Model fit: marginal R^2 ; conditional R^2	0.697; 0.779			0.052; 0.057			0.171; 0.342			0.188; 0.341		

origin by soil treatment interactions. The model used for testing the local vs. a significant interaction between seed origin treatment and soil altitude (df = 3, LRT = 10.327, $p = 0.015$; Appendix: Table A.3). On inocula from high altitudes, local seeds germinated better than foreign seeds (local: $61.3\% \pm 5.0\%$, foreign: $41.2 \pm 6.6\%$; $z = -5.343$, $p < 0.001$), and on inocula from low altitudes, local seeds germinated equally well as foreign seeds (local: $50.0\% \pm 5.6\%$, foreign:

$48.4 \pm 6.3\%$). This effect was additionally dependent on the microhabitat, as indicated by a significant three-way interaction between the seed origin treatment, soil altitude and soil microhabitat (df = 3, LRT = 10.504, $p = 0.014$).

There were no consistent overall effects of soil treatment on growth rate, total leaf area or total biomass of plants, but soil treatment effects depended on the microhabitat of seed origin for total leaf area and biomass

Fig. 2. Total biomass (means and standard errors) of *Salix herbacea* seedlings grown from seeds from different altitudes, microhabitats or mountain transects in substrate inoculated with soil from their origin (Home), with soil from the other microhabitat at the same altitude (Microhab), or with soil from the same microhabitat at the other altitude (Altitude). For easier interpretation of significant differences due to the altitude of seed origin, we indicated the group means of high vs. low altitude with dotted horizontal lines.

Fig. 3. Germination (a), growth rate (b), leaf area (c) and biomass (d) of *Salix herbacea* seeds and seedlings, respectively, on substrate inoculated with soil from beyond their current altitudinal range (3100 m) compared to substrate inoculated with their home soil. Dashed and dotted lines indicate group means of seedling growth rates on home and beyond range soil, respectively.

(significant treatment $\times$ seed origin microhabitat interactions in Table 1). However, there were no significant home vs. away contrasts for this interaction. The model used for testing the local vs. foreign contrast revealed that the effects of seed origin treatment depended on the altitude of soil origin for total leaf area and biomass (total leaf area: $df=2$, $LRT=7.931$, $p=0.018$; total biomass: $df=2$, $LRT=7.289$, $p=0.026$). On soil from low altitudes, local plants had more leaf area and biomass compared to foreign (i.e. high-altitude) plants (total leaf area: local: 2580 ± 354 mm², foreign: 1263 ± 239 mm², $z=2.751$, $p=0.022$; total biomass: local: 75.77 ± 11.12 mg, foreign: 43.24 ± 8.15 mg, $z=-2.701$, $p=0.027$), whereas on soil from high altitudes the biomass of local plants was smaller than that of foreign (i.e. low-altitude) plants (total biomass: local: 99.87 ± 13.66 mg, foreign: 132.59 ± 23.40 mg, $z=2.374$, $p=0.066$).

Effects of soil inocula from above the current altitudinal range limit

There was no evidence that soil biota from above the current altitudinal limit of *S. herbacea* influenced seed germination (soil treatment: $df=1$, $LRT=2.397$, $p=0.121$, marginal $R^2=0.073$, conditional $R^2=0.127$; Fig. 3a), or the total leaf area ($df=1$, $LRT=2.178$, $p=0.139$) and biomass ($df=1$, $LRT=0.623$, $p=0.429$) of *S. herbacea* plants. However, plants had a higher growth rate when growing on their own soils compared to soils collected at 3100 m ($df=1$,

$LRT=4.432$, $p=0.035$; Fig. 3b). However, this higher growth rate did not result in a higher final biomass at the end of the experiment (Fig. 3c and d).

Discussion

We found differences in germination rate and growth among *S. herbacea* from different altitudes and microhabitats. Although the soil inocula from different sites sometimes had significant effects on germination and growth, there was overall no consistent evidence for a home-soil advantage within the current range of *S. herbacea*. However, plant growth rate was significantly reduced on soils from beyond the current altitudinal range, which indicates that plant-soil feedbacks may limit the establishment of *S. herbacea* beyond its current range.

Altitude and microhabitat of seed origin influence germination and growth

We found that seeds from high altitudes had higher germination rates than seeds from low altitudes, regardless of the soil inoculum. A possible explanation for this apparent difference in seed quality in our study is that plants from low and high altitudes may differ in dormancy breaking requirements. Several other studies on alpine species found similar

results, and suggested that plants at high altitudes delay germination to late spring with high temperatures and lower probabilities of late season frosts (Cavieres & Arroyo, 2000). Therefore, it is possible that the relatively high temperatures in our climate chamber conferred an advantage to the seeds from high altitudes. Quality of seeds from low altitudes could also be reduced due to increased exposure to spring frosts. However, this is probably not the case in our experiment, because seed production happens later in the year when frost events in our study area are very unlikely (Wheeler et al., 2014).

An altitudinal effect of seed source was also apparent in plant growth traits, as total leaf area and biomass of plants originating from high altitudes were reduced compared to those from low altitudes. These results are consistent with other studies that found genetic differentiation in size of alpine plants along altitudinal gradients (Clausen, & Hiesey, 1958; Byars et al., 2007). Plants from high altitudes are often smaller, presumably because stressful climatic conditions (i.e. increased transpirative forces and frequent frost events) select for smaller plant sizes, whereas at lower altitudes, milder conditions and biotic pressures such as competition might favor larger and more vigorous plants (Körner, 2003).

Interestingly, we found that the microhabitat type of the seed origin influenced seed germination, and that this effect depended on the altitude of seed origin. Very few studies have investigated differences in germination success across alpine microhabitats. Shimono (2003), for example, found that *Potentilla matsumurae* seeds from snowbeds needed a warmer temperature regime for germination than seeds from ridges. On the other hand, Shimono and Kudo (2005) did not find a clear pattern of differences in germination success between seeds from snowbeds and ridges of four other alpine plants. In another study, we found that at high altitudes plants produce more (26.3%) seeds per fruit in snowbeds than in ridge habitats (Sedlacek et al. unpublished data), which, together with this study, indicates that resource allocation to seed quantity and quality might be generally higher in high-altitude snowbeds.

Our finding of differences in germination and growth among different origins of *S. herbacea* under common environmental conditions suggests possible genetic control for those traits. A genetic study along the same transects found no genome-wide genetic differentiation among altitudes and microhabitats, despite strong phenological separation (Cortés et al., 2014). This does not necessarily preclude the existence of trait differentiation or local adaptation (Gonzalo-Turpin & Hazard, 2009). However, the differences in germination and growth could also be caused by maternal carry-over effects due to differences in seed provisioning (Roach & Wulff, 1987) or by epigenetic differences (Bossdorf, Richards, & Pigliucci, 2008). Seedling germination and growth have been demonstrated to be influenced by maternal effects via seed size (Fenner, 2000). However we did not attempt to measure seed size in *S. herbacea*, because

its minute and hairy seeds, are very difficult to measure accurately.

Lack of home-soil advantage within the current range

We found that the germination rate of *S. herbacea* seeds depended on the soil treatment, indicating that soil microbial communities differed among our study sites. The site differences in soil microbial community composition are likely driven by the strong environmental differences between the sites, such as the snowmelt-time difference of up to 40 days between ridge and snowbed plots and other environmental factors such as soil moisture, temperature and plant community composition (Nussbaumer, Rixen, & Schmidt, 2012).

In spite of trait differences between plants of different origins and the observed soil-origin effects, we found no evidence for a home-soil advantage with respect to soil microbes within the current range of *S. herbacea*. Although we found that in inocula from high altitudes germination of local seeds was higher than that of foreign seeds, this was not true in inocula from low altitudes. Similarly, local plants grew better than foreign plants in soil from low altitudes, but the opposite was true in soil from high altitudes. Overall, we found no consistent home vs. away or local vs. foreign contrasts. Similarly, a transplant experiment of *M. effusum* found no evidence of a home-soil advantage, but increased seedling emergence and growth of seedlings growing in colder soil relative to their home soil, mainly driven by below-ground biotic interactions (De Frenne et al., 2014). Despite the lack of a home-soil advantage within its current range, *S. herbacea* might exhibit adaptive differentiation to other important factors, which we did not test in this study.

There are several possible explanations why we found no home-soil advantage with regard to soil microbiota. In a genetic study along the same transects, Cortés et al. (2014) found high gene-flow in *S. herbacea* populations among altitudes and microhabitats. High levels of gene flow between our study populations may have counteracted local adaptation to soil microbes (Sambatti & Rice, 2006). Local adaptation could also be constrained by genetic drift (Blanquart, Gandon, & Nuismer, 2012) and low genetic variation (Kawecki & Ebert, 2004). Furthermore, it could be that soil-microbial communities pose only very small or no selection pressures on the measured traits, or that temporal changes in these communities involve opposing selective pressures, and thus constrain local adaptation (Galloway & Fenster, 2000). Finally, we cannot exclude the possibility that the nitrogen addition, which we applied to avoid nutrient limitation, has masked potentially small effects of soil biota. In any case, the lack of a discernible home-soil advantage may be beneficial for *S. herbacea*, if climate change forces the dwarf shrub to migrate to new microhabitats or altitudes within the current range, where it might face new soil microbial communities.

Limited establishment above the current altitudinal range

There were no differences in the germination rates of seeds on soil inocula from their own habitat compared to soil inocula from beyond the current altitudinal range limit. Likewise, final biomass and leaf area were not significantly different for plants grown with their home-soil biota versus soil biota from beyond the current range. However, growth rates of seedlings were on average lower when they were grown with soil inocula from beyond the current altitudinal range, which suggests that in the long term biomass production may be lower for these plants. These results are consistent with several other experiments demonstrating reduction in fitness and growth of plants transplanted beyond their altitudinal range (reviewed in Hargreaves, Samis, & Eckert, 2014). This effect can be explained by missing positive plant-soil feedback above their range limit, as it was found for the mutualistic symbiosis of legumes and rhizobia (Stanton-Geddes & Anderson, 2011) or invasive plants and mycorrhiza (Nunez, Horton, & Simberloff, 2009). Väre et al. (1997) did not find a decrease in mycorrhization rates of *S. herbacea* up to an altitude of 900 m in Northern Scandinavia, which is comparable to an altitude of ca. 2200 m in the Alps, and is therefore not reflective of the altitudinal gradient of our study. Other studies support a decrease of mycorrhization rates with altitude (Read & Haselwandter 1981; but see: Ruotsalainen & Väre, 2004). Our findings demonstrate that interactions with soil biota may constrain migration of *S. herbacea* to higher altitudes.

Conclusions

Soil inocula from different origins affected seed germination rates and growth of *S. herbacea*, indicating that soil microbial communities likely differed among our study sites. However, we did not find any consistent evidence of a home-soil advantage, i.e. plants did not generally germinate and grow better in the presence of their local soil biota. When we tested the effects of soil biota from above the species' current range limit, we found seedling growth rate to be on average reduced with soil inocula from above the range limit. Taken together, this suggests that climate-driven migration to new sites within the current range might not be limited by soil biota, but that the migration of *S. herbacea* to higher altitudes beyond its current range may be constrained by a lack of positive plant-soil feedbacks.

Conflict of interest

The authors declare that they have no conflict of interest, and that all experiments comply with the current laws of Switzerland and Germany.

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