Ecology, 0(0), 2020, e03095

Growing-season length and soil microbes influence the performance of a generalist bunchgrass beyond its current range

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Citation: Bueno de Mesquita, C. P., S. A. Sartwell, S. K. Schmidt, and K. N. Suding. 2020. Growing season length and soil microbes influence the performance of a generalist bunchgrass beyond its current range. Ecology 00(00):e03095. 10.1002/ecy.3095

Abstract. As organisms shift their geographic distributions in response to climate change, biotic interactions have emerged as an important factor driving the rate and success of range expansions. Plant–microbe interactions are an understudied but potentially important factor governing plant range shifts. We studied the distribution and function of microbes present in high-elevation unvegetated soils, areas that plants are colonizing as climate warms, snow melts earlier, and the summer growing season lengthens. Using a manipulative snowpack and microbial inoculation transplant experiment, we tested the hypothesis that growing-season length and microbial community composition interact to control plant elevational range shifts. We predicted that a lengthening growing season combined with dispersal to patches of soils with more mutualistic microbes and fewer pathogenic microbes would facilitate plant survival and growth in previously unvegetated areas. We identified negative effects on survival of the common alpine bunchgrass *Deschampsia cespitosa* in both short and long growing seasons, suggesting an optimal growing-season length for plant survival in this system that balances time for growth with soil moisture levels. Importantly, growing-season length and microbes interacted to affect plant survival and growth, such that microbial community composition increased in importance in suboptimal growing-season lengths. Further, plants grown with microbes from unvegetated soils grew as well or better than plants grown with microbes from vegetated soils. These results suggest that the rate and spatial extent of plant colonization of unvegetated soils in mountainous areas experiencing climate change could depend on both growing-season length and soil microbial community composition, with microbes potentially playing more important roles as growing seasons lengthen.

Key words: *alpine plants; biotic interactions; climate change; plant–microbe interactions; range shifts.*

Introduction

Global environmental change has led to a redistribution of the earth's flora and fauna, with warmer temperatures typically allowing species to move to higher elevations and higher latitudes (Chen et al. 2011, Settele et al. 2014). Such responses to abiotic conditions do not occur in isolation; rather, they are mediated by complex biotic interactions that can either facilitate or inhibit, and either speed or slow, range shifts (van der Putten 2012). For example, recent research has demonstrated that both plant–pollinator and plant–plant interactions can influence plant range shifts (Meier et al. 2010, Pellissier et al. 2010, Meineri et al. 2012, Giannini et al. 2013, HilleRisLambers et al. 2013). Plant–microbe interactions are a ubiquitous and important biotic interaction that has largely been overlooked in the context of plant range shifts in response to climate change, with recent exceptions (Pellissier et al. 2013, Bueno de Mesquita et al. 2016, Van Nuland et al. 2017). Here we first surveyed soil microbial community composition across an unvegetated landscape and then used a manipulative inoculation and transplant experiment to test the importance of these microbial communities in facilitating or inhibiting plant establishment beyond their range as climate changes and growing season lengthens.

Soil microbes are drivers of vegetation productivity, diversity, community assembly, and community structure (Klironomos 2002, Wardle et al. 2004, van der Heijden et al. 2008). The majority of plants rely on mutualistic or beneficial microbes either to mobilize or help acquire nutrients, and cope with abiotic and biotic stressors (van der Heijden et al. 2008). On the other hand, soil-borne microbial pathogens can have devastating impacts on plant fitness (Jackson 2009). Plant interactions with this wide variety of fungal and bacterial enemies, mutualists, and decomposers can contribute to plant–soil feedbacks that have important implications for plant fitness (van der Putten et al. 2016).

Manuscript received 22 March 2019; revised 14 July 2019; accepted 3 April 2020; final version received 29 April 2020. Corresponding Editor: Nicholas J. Gotelli.
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Importantly, soil fungi and bacteria also have biogeographic patterns based on environmental variables (Fierer et al. 2009, Lauber et al. 2009, Tedersoo et al. 2014), and thus, the microbiomes necessary for plant establishment in new habitats may not necessarily be present.

In mountainous regions, species typically move up in elevation to track a suitable climate (Parmesan and Yohe 2003, Pauli et al. 2007, Parolo and Rossi 2008, Frei et al. 2010, Chen et al. 2011). In many cases, for alpine plants, this requires colonizing areas that are currently unvegetated, and not necessarily uphill if there are suitable microclimates because of topography. Unvegetated soils are characterized by poor development and nutrient limitation (Aide and Cwick 1998, Schmidt et al. 2008*a*). But these seemingly barren soils are teeming with microbial life long before plant arrival (King et al. 2008). Indeed, complex microbial communities can develop in newly exposed deglaciated soil within 4 yr (Schmidt et al. 2008*a*). Bacterial taxa that perform nitrogen fixation, mineralization, immobilization, and nitrification are important in governing plant nutrient availability and may be important for "priming" soil for plant colonization (Schmidt et al. 2008*a*). Bacteria are also capable of solubilizing phosphorus (Rodríguez and Fraga 1999), which was recently suggested to be more limiting than nitrogen in early successional, high-alpine environments (Darcy et al. 2018*b*). Furthermore, some plant-associated fungi can be present in unvegetated soils. Dark septate endophytes (DSE) are a group of facultative root endophytes that are capable of surviving on organic debris and in biological soil crusts (Caldwell et al. 2000, Menkis et al. 2004, Mandyam and Jumpponen 2005, Green et al. 2008, Mandyam et al. 2010, Day and Currah 2011, Knapp and Kovács 2016). Additionally, aerially deposited spores of arbuscular mycorrhizal fungi (AMF) and other biotrophic fungi can be present in unvegetated soils (Jumpponen 2003).

In this study, we tested the importance of microbial communities on the success of an alpine plant upward range shift into unvegetated soil as climate changes and snow melts earlier. We first surveyed the distribution of microbes in unvegetated soils and then manipulated the abundance of plant-associated taxa in the soil by collecting soil inocula from eight different locations to simulate the scenario of a plant dispersing into unvegetated soil and encountering different microbial communities. We hypothesized that (1) a longer growing season because of earlier snowmelt will facilitate plant survival and growth in previously unvegetated soils, (2) microbial community composition and differences in the abundance of plant-associated microbes will affect plant survival and growth, and (3) growing-season length and microbial community composition will interact such that microbes will most strongly affect plant performance as the growing season lengthens.

Methods

Study site

The experiment was conducted in a late-melting snowbed (3,900 m above sea level [a.s.l.]) on the southeast facing slope of Navajo Peak in the Green Lakes Valley, part of the Niwot Ridge Long Term Ecological Research Site in the Front Range of the Rocky Mountains, Colorado, USA (Appendix S1: Fig. S1). The snowbed typically melts out in mid-August and snow starts falling again in September. Average precipitation from 1952 to 2012 was 1,090 ± 230 mm/yr, with a 60-mm/yr increase over that time period, driven mostly by increases in winter precipitation (Kittel et al. 2015). Recent mean annual temperatures (2011–2014) range from −4°C to −7°C, and mean daily summer temperatures range from 4°C to 10°C (Losleben 2017). Both annual and summer temperatures have been increasing over the last several decades (McGuire et al. 2012, Bueno de Mesquita et al. 2018*b*), leading to increased positive degree days (Caine 2010), earlier lake ice-off dates (Preston et al. 2016), and earlier snowmelt (Bueno de Mesquita et al. 2018*b*). Over the last several decades, concurrent with this summer warming trend, there have been increases in cover by alpine plants in areas that were previously unvegetated (Bueno de Mesquita et al. 2018*b*) or dominated by moss (Bueno de Mesquita et al. 2017).

We combined a field survey to describe the spatial variation in soil microbial communities that a plant colonizing unvegetated areas might interact with, with an experimental manipulation of the interactive influence of climate change (early snowmelt) and microbial composition (different soil inocula). For the survey, we described soil microbial communities across 24 unvegetated soils. For the manipulative experiment, we established four experimental blocks of unvegetated soils across a 52-m transect (blocks 2, 3, and 4, were 6, 30, and 52 m away from block 1, respectively), each of which contained paired 1.5 × 1 m early snowmelt and control plots (Fig. 1). Three alpine plant species grown in soil with one of eight inocula with varying microbial community composition (Fig. 2A, B) were transplanted into the plots ((8 inocula × 4 blocks × 2 snowmelt treatments × 3 species = 192 pots) + (2 of the inocula ("U1" unvegetated inoculum and "V1" vegetated inoculum) had 1 additional pot in each block and snowmelt treatment × 3 species = 48 pots) = 240 total pots).

Plants

We chose three focal plant species that are abundant at the highest-elevation plant communities at our site and likely colonizers of unvegetated soils as climate changes—the generalist bunchgrass *Deschampsia cespitosa* ((L.) P. Beauv.), the arctic/alpine cushion plant *Silene acaulis* ((L.) Jacq.), and the talus specialist *Oxyria*

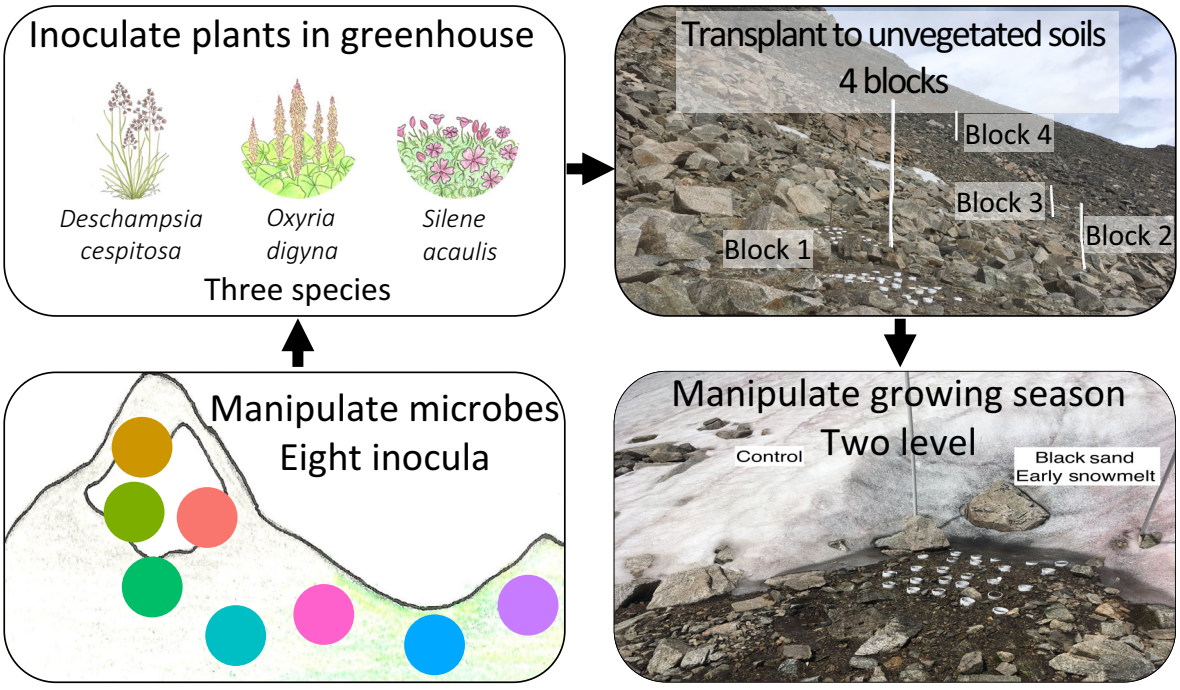

FIG. 1. Experimental design. We (1) manipulated microbial community composition by collecting eight different inocula across the landscape, (2) inoculated three abundant alpine plant species in the greenhouse, (3) transplanted them beyond their range into unvegetated soils distributed across four blocks, and (4) manipulated the growing-season length using black sand to speed snowmelt. At each of the four blocks there were paired control and early snowmelt plots. There were 8 inocula × 8 replicates × 3 species = 192 pots, plus 2 of those inocula (U1 and V1) with 8 additional replicates × 3 species = 48 pots, for a total of 240 total pots. Each species had 60 pots per block. Pots contained between one and five individual plants. Pots in which plants had died were not transplanted, such that a total of 210 pots and a total of 280 *Deschampsia*, 281 *Oxyria*, and 106 *Silene* individual plants were actually transplanted.

digyna ((L.) Hill). All of these species have been observed growing in poorly developed, early successional soils. *Silene* is often found in more windswept areas with shallower snowpacks and growing seasons ~4 months, and *Deschampsia* dominates moist meadow communities topographically below deep snowpacks with growing seasons ~3 months, and *Oxyria* can be found in rockier, more undeveloped areas with later melting snow and shorter growing seasons of ~2 months. We collected seed of each of these species from vegetated areas downslope of the transplant site (3,550–3,625 m elevation) in August and September 2015, which were stored at room temperature in paper bags until sowing in March 2016. Plump and likely viable seeds were selected from the seed collections. We sowed five seeds into open-bottom PVC cylinders 7 cm tall × 7.62 cm diameter, containing sterile soil and sand mixed with one of the eight inocula.

Inocula

In September 2015 we collected soils from four different unvegetated areas and four different vegetated areas, spanning elevations from 3,631 to 3,906 m, to use as soil inoculum (Appendix S1: Fig. S1). One vegetated inoculum (V1) was dominated by the plants *Geum rossii* and *Carex rupestris*, and the other three vegetated inocula (V2, V3, V4) were dominated by *G. rossii* and *Kobresia myosuroides*, but all were from habitats considered to be "dry meadow." There were no plant roots present in the unvegetated soils. Two quarts of soil at each collection location were collected from within a 2-m radius at a maximum of 10-cm depth and thoroughly homogenized by mixing for several minutes in a 2-gallon Ziploc bag. Soils were transported on ice to the lab where they were stored at −20°C until March 2016. We also collected bulk soil from the tundra to use in the greenhouse. This soil was collected at slightly lower elevations (~3,500 m) adjacent to the Niwot Ridge alpine dirt access road, also to 10-cm depth. Although this soil was near developed vegetation, it was mostly unvegetated from the disturbance. Soils were collected from a ~10-m^2 area and thoroughly homogenized. The bulk tundra soil was mixed with sand at a 1:1 ratio, similar to the sand content of the unvegetated soils at our site (76% sand; King et al. 2010). One gallon at a time, this mixture was sterilized by autoclaving for 1 h at 121°C, remixing, and autoclaving again. Postautoclaved bulk soil total inorganic nitrogen levels were 74.07 μg/g dry soil, much higher than average unvegetated soils (3.68 μg/g dry soil)

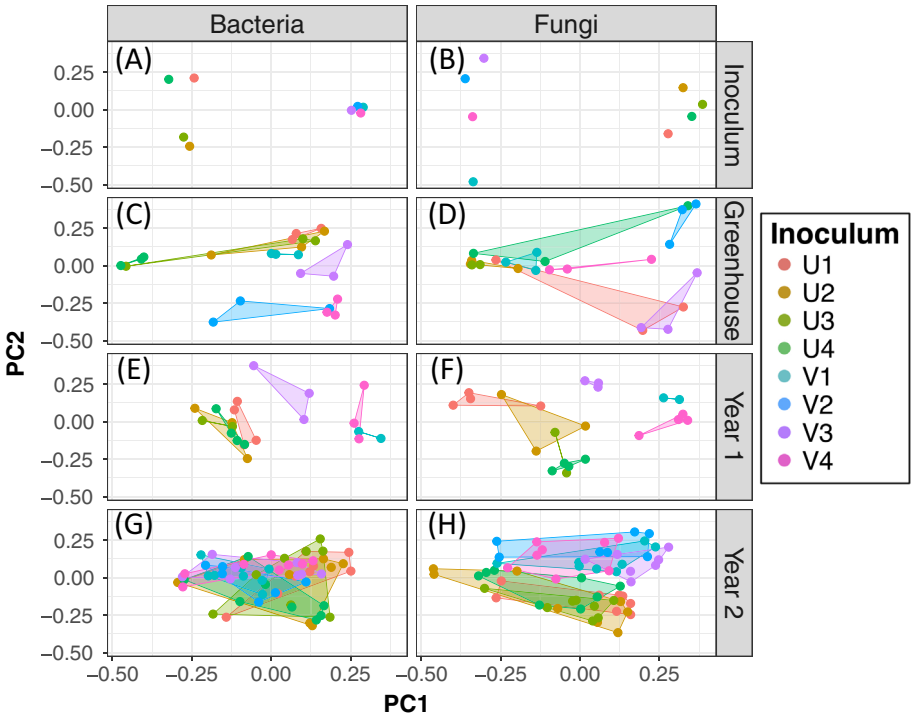

FIG. 2. Principle coordinates analysis of Bray–Curtis dissimilarities in bacterial and fungal community composition at the OTU level in the original eight soil inocula used in the experiment and in plant roots at three different time points in the experiment. $n = 3$ samples per treatment in the greenhouse and year 1 to minimize disturbance and sample loss; $n = 8$ samples per treatment in year 2 for the final destructive harvest. Four inocula were collected from vegetated areas (V1–V4), and four from unvegetated areas (U1–U4) (Appendix S1: Fig. S1). Inoculum was always a significant driver of community composition (PerMANOVA, $P < 0.05$), but variation declined over time. Percent variation explained by Axis 1 and Axis 2 in each panel: A = 54.23, 16.14; B = 34.73, 17.11; C = 14.61, 10.42; D = 18.68, 12.70; E = 12.90, 8.41; F = 12.22, 10.21; G = 7.44, 6.36; H = 8.93, 7.83).

and vegetated soils (1.95 μg/g dry soil) near our inocula collection locations (Porazinska et al. 2018), such that minor differences in nutrient concentrations among inocula were overwhelmed. To minimize differences in other soil properties, pots received a 30:1 ratio of sterile bulk soil to inoculum. Each pot was filled with sterile soil until 2 cm from the top, a layer of inoculum was spread, and then 1 cm of bulk sterile soil added on top of the inoculum. This layering of inoculum and sterile soils minimized cross-contamination among pots and ensured that roots passed through a layer of inoculum. Pots were watered every day until the first true leaves formed; then they were watered every other day. Plants grew from March to July in the alpine room of the University of Colorado greenhouse, with natural light conditions and diurnal temperature cycles ranging from 15°C during the day to 10°C at night.

Field manipulations

In July 2016, plants were transported to the University of Colorado Mountain Research Station (2,900 m a.s.l.) where they were exposed to ambient light and temperature conditions outside, but were kept in flats and watered every other day. The goal of this intermediate stage was to start acclimatizing the plants before final transplantation to higher elevations. In August 2016, a total of 212 pots containing 1–5 individual plants were transplanted to the field site where they were distributed among the four blocks. Pots were not thinned to maximize the sample size of individuals being transplanted. Each block was transplanted on a separate day, approximately 1–3 d after the snowbed melted at the block. Holes the size of the PVC cylinders were dug and the cylinders placed into the soil. Because the soils were saturated from snowmelt water, there was no need to water the transplants. To avoid contamination of unvegetated inocula by vegetated inocula, each plot was divided in half vertically such that all of the pots with unvegetated soil inocula were on the top half and all pots with vegetated soil inocula were on the bottom half. Within each half of the plots, plant species and inocula were randomly placed. Pots were 15 cm apart horizontally and 20 cm apart vertically. A HOBO air temperature and light sensor (Onset Corporation, Bourne, Massachusetts, USA) was placed in the center of each plot to help determine snowmelt date.

To manipulate snowmelt timing, a thin layer of inert black sand (composed of primarily silica dioxide, Mission Laboratories, Los Angeles, California, USA) was

applied to one plot in each block to speed snowmelt (Fig. 1; Blankinship et al. 2014), and then onto control plots after snowmelt. Sand was spread in a 3 × 3 m area with the plot in the middle (such that there was a 0.75-m buffer zone upslope and downslope, and 1-m buffer zone on each side of the plot), at a rate of 500 g sand/m as soon as 3-m-tall plot marker poles were visible. Survival and leaf number (from which biomass was calculated allometrically) were recorded for each individual in September 2017, and plants were harvested and aboveground biomass weighed in September 2018.

Staining and sequencing

Prior to transplanting, as well as at the end of summer 2017 and the end of 2018, soils and roots were collected for molecular analyses, and a subset of roots were collected for staining and microscopy. Note that for the first two harvests, we carefully extracted one isolated plant from near the wall of each pot such that other individuals in the pot were not disturbed, and the decrease in total individual sample size can be found in Appendix S1: Fig. S2. After rinsing and brushing off soil and any tangled loose roots, roots that could be traced back to the individual plant were selected for analysis. To assess the quantity of fine root endophytes, dark septate endophytes, and arbuscular mycorrhizal fungi, roots were cleared in 10% KOH for 1 h at 90°C, reacidified in 1% HCl for 20 min, and stained overnight in acidic glycerol trypan blue (modified from Koske and Gemma 1989, Schmidt et al. 2008*b*). In the morning, roots were destained in acidic glycerol and stored at 4°C until microscopy. Microscopy was done according to McGonigle et al. (1990). Briefly, ~20 cm of roots were placed horizontally on slides and passes were made up and down the slide at random intervals such that 100 intersections between the root and ocular crosshair were observed. The presence or absence of a fungal structure was recorded at each intersection, and percent colonization was calculated.

We used (Illumina, San Diego, California, USA) MiSeq sequencing of the 16S and ITS regions of the genome to describe bacterial and fungal communities, respectively, of plant roots, pot soils, the initial inoculum, as well as 20 additional unvegetated soils that we sampled for a previous study (Porazinska et al. 2018). For soils, DNA was extracted from 0.25 g of soil using the Qiagen PowerSoil Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocols. Roots were first surface sterilized by soaking for 1 min in 70% ethanol, then 1 min in 10% bleach, and then triple rinsing with sterile deionized water. Then, 0.1 g wet roots were frozen in liquid nitrogen and ground into a powder with a sterile mortar and pestle. DNA was extracted from this powder using the Qiagen DNeasy Plant Kit (QIAGEN) following the manufacturer's protocols. Extracted DNA was amplified via polymerase chain reaction, using the 515F/806R primers for 16S (Fierer et al. 2012) and ITS1F/ITS2 primers for ITS (White et al. 1990) following the methods of the Earth Microbiome Project (Amaral-Zettler et al. 2009, Bellemain et al. 2010, Caporaso et al. 2012). Amplified DNA was normalized with the SequalPrep Normalization Kit (Invitrogen Inc., Carlsbad, California, USA), and sequenced (paired-end 2 × 150 bp for 16S, 2 × 250 bp for ITS) at the BioFrontiers Next Generation Sequencing Facility (Boulder, Colorado, USA). Sequencing data were processed with the QIIME (Caporaso et al. 2010) and UPARSE pipelines to demultiplex, merge, quality filter, remove singletons, and select operational taxonomic units (OTUs) at 97% similarity, and remove chimeras. OTU tables were rarefied before analysis, so all samples within each sample type had the same sequencing depth (Appendix S1: Table S1). Taxonomy was assigned using the GreenGenes (DeSantis et al. 2006) and UNITE (Abarenkov et al. 2010) databases for bacteria and fungi, respectively. Fungal functional guilds were assigned using the program FUNGuild (Nguyen et al. 2016). Sequencing data are accessible on GenBank via the project accession number PRJNA525120.

Plant performance analyses

Plant growth data in the greenhouse were analyzed with Kruskal–Wallis tests to test for effects of inoculum (eight levels), and Wilcox tests to test for effects of inoculum type (two levels). Plant growth data in the field (average biomass per individual by pot per year) were analyzed with a linear mixed-effects regression (LMER) model (R package "lme4," Bates et al. 2015) with inoculum, growing-season length, and their interaction as fixed effects and block as a random effect. We tested for effects of inoculum (eight levels) as well as inoculum type (two levels, vegetated vs. unvegetated). We also added number of individuals per pot (could range from 1 to 5 based on germination and survival) as a random effect, such that the final model formula was specified as biomass ~ Inoculum × GSL + (1|Block) + (1|Individuals). We used growing-season length (calculated as the days from snowmelt until we measured the plants) as a continuous variable instead of categorical (control vs. black sand additions) because there was also considerable variation in growing season across blocks (Appendix S1: Fig. S3). Note that the field year 1 biomass data for inoculum V2 include some contaminants by *Festuca brachyphylla* that were not identified until field year 2. In field year 2, only confirmed *Deschampsia* individuals were included in the analysis. Plant survival data were analyzed with generalized linear models with a binomial distribution (GLM, where 1 is a plant that survived and 0 is a plant that died) with the same fixed effects but no random effects to avoid overfitting and model errors. When a significant inoculum effect was found, we conducted pairwise comparisons using the emmeans function from the R package "emmeans" (Lenth 2018). Relationships between fungal root

colonization and pathogen abundance and plant biomass were tested with linear regressions.

Microbial analyses

Microbial communities were visualized with principle coordinates analysis and differences between treatments were assessed with permutational multivariate analysis of variance (PerMANOVA; Anderson 2001, function adonis, R package "vegan", Oksanen et al. 2013) on Bray–Curtis dissimilarity matrices calculated from Hellinger-transformed relative abundances. Pathogen abundances and root colonization by potential mutualists were assessed with the same linear mixed effects regressions as plant growth. To analyze drivers of unvegetated microbial community composition further, we performed a targeted analysis of our four unvegetated inocula and a subset of data presented by Porazinska et al. (2018). We selected the 20 unvegetated soils from that study (Appendix S1: Fig. S1) and used the envfit function in "vegan" to assess relationships with total dissolved inorganic nitrogen, total dissolved inorganic phosphorus, dissolved organic carbon, pH, and soil moisture, which were measured as described in Porazinska et al. (2018). We partitioned variance of the Bray–Curtis dissimilarity matrix explained by either spatial distance or environmental variables using the varpart function in "vegan". To test correlations between space and community composition, we used Mantel tests (package "vegan", function mantel). All analyses were performed in R version 3.4.4 (R Development Core Team 2018).

Results

Deschampsia had the highest survival in both 2017 and 2018, whereas both *Oxyria* and *Silene* experienced high mortality in 2017 and additional mortality in 2018 (Appendix S1: Fig. S2). Ninety percent of *Oxyria* individuals were dug up and likely eaten by the American pika (*Ochotona princeps*), which has been observed near the transplant site, and herbivory rates were high across all blocks, snowmelt treatments, and inocula. Because of the low remaining sample sizes of *Oxyria* and *Silene*, we focused all analyses on *Deschampsia*.

Snowmelt timing was 2–3 d earlier in the black sand plots in 2017, and 2–10 d earlier in 2018 (Appendix S1: Fig. S3). The entire snowbed melted out earlier and earlier each year from 2015 to 2018 (Appendix S1: Fig. S3). There was a significant positive effect of growing-season length on *Deschampsia* survival in year 1 (GLM, $\beta = 0.59$, $P = 0.001$, Fig. 3A). In contrast, in year 2, there was significantly greater mortality with extended growing season (GLM, $\beta = -0.01$, $P = 0.002$, Fig. 3B). There was no significant main effect of growing-season length on growth in year 1 (LMER, df = 1,52, $\chi^2 = 0.32$, $P = 0.57$, Fig. 4A) or year 2 (LMER, df = 1,52, $\chi^2 = 2.35$, $P = 0.12$, Fig. 4B).

Soil inoculum had a significant effect on *Deschampsia* field survival in both years (GLM, $P < 0.05$, Figs. 3A, B). There were also significant differences in survival between vegetated vs. unvegetated inoculum types (GLM, $P < 0.05$, Fig. 3A, B), with greater survival in vegetated inocula. Soil inoculum also significantly affected *Deschampsia* growth in both years in the field (Fig. 4A, B; year 1 LMER df = 7,52, $\chi^2 = 49.99$, $P < 0.0001$; year 2 LMER df = 7,52, $\chi^2 = 23.97$, $P = 0.001$), although the effects changed over time (i.e., inocula with plants with the highest growth). Inoculum type (vegetated vs. unvegetated) had a significant effect on growth in year 1 (LMER, df = 1,64, $\chi^2 = 11.86$, $P = 0.0006$), with higher growth in vegetated inocula, but this effect disappeared in year 2 (LMER, df = 1,64, $\chi^2 = 3.50$, $P = 0.06$). In the greenhouse, *Oxyria* growth also differed significantly among soil inocula in the greenhouse (Kruskal–Wallis, df = 7,368, $\chi^2 = 71.24$, $P < 0.001$), whereas *Silene* growth did not (Kruskal–Wallis, df = 7,178, $P > 0.05$, Appendix S1: Fig. S4). Inoculum type did not significantly affect growth of any species in the greenhouse portion of the study (Wilcox test, $P > 0.05$).

Growing season and inoculum interacted to affect survival in both field year 1 and field year 2 (GLM, $P < 0.01$, Fig. 3). There was greater variability in survival among the inocula in plots with the shortest and longest growing seasons (Fig. 3). In year 1, inoculum and growing season interacted to affect growth (LMER, df = 7,52, $\chi^2 = 18.67$, $P = 0.009$, Fig. 4A), with growing season having either positive, negative, or neutral effects on growth depending on inoculum. There was no significant interactive effect of inoculum and growing season on growth in year 2 (LMER, df = 7,52, $\chi^2 = 8.54$, $P = 0.28$, Fig. 4B). Inoculum type did not interact with growing-season length.

Unvegetated soil microbial communities varied significantly across space, and plant-associated taxa showed patchy distributions (Appendix S1: Fig. S5). Obligate plant-associated fungi were patchily distributed in unvegetated soils, with arbuscular mycorrhizal fungi present in 5 of the 24 soils. Facultative plant-associated fungi were more widely distributed, with dark septate endophytes present in 22 of the 24, though in some cases in very low abundance. Fungi classified as plant pathogens were present in all 24 soils, though in some cases in very low abundance, and abundances varied by three orders of magnitude. We captured some of this variability in our inocula (Fig. 2A, B). Both fungal and bacterial community composition in roots varied by inoculum after growing in the greenhouse, after 1 yr in the field, and after 2 yr in the field (PerMANOVA, $P = 0.001$, Fig. 2C–H), though differences declined over time. Root colonization by arbuscular mycorrhizal fungi and fine root endophytes each varied significantly by inoculum (LMER, df = 7,48, $\chi^2 = 18.69$, $P = 0.009$; df = 7,48, $\chi^2 = 15.56$, $P = 0.03$, respectively), whereas dark septate endophytes did not (LMER, df = 7,48, $\chi^2 = 3.26$,

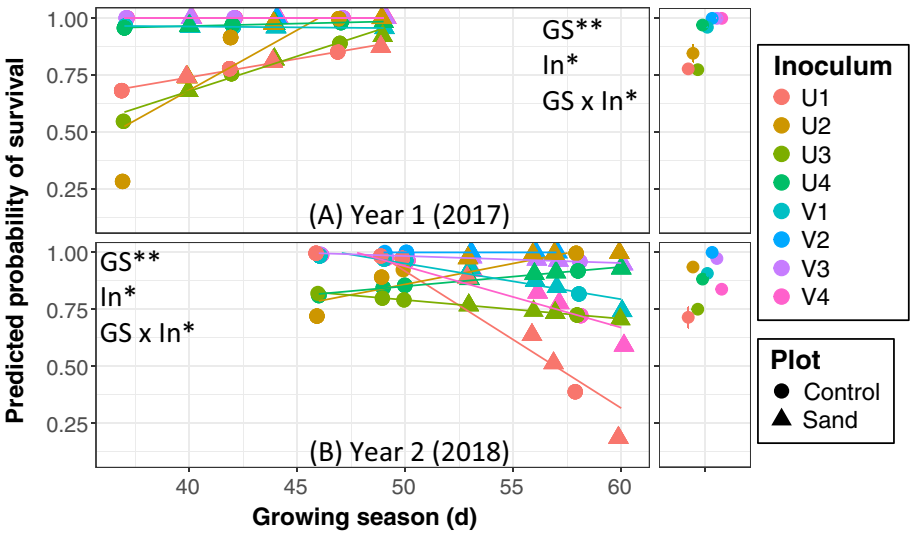

FIG. 3. Effects of growing-season length (days from snowmelt to plant measurement) and soil inocula on *Deschampsia cespitosa* survival in (A) field year 1 and (B) field year 2. Statistics are from logistic regression models (*p<0.05, **p<0.01). GS, growing season; In, inoculum. The right panels show the main effect of inocula, as mean (±SE) predicted probabilities across the growing-season lengths. Lines represent linear regressions between predicted probabilities and growing-season length and are included for each inoculum (even when fit not significant) to visualize the significant interactions in each panel. Growing season varied among the four blocks and with the control and black sand (for earlier snowmelt) plots at each block, such that there were six different growing-season lengths in year 1 (two pairs of plots had the same growing-season length), and eight different growing-season lengths in year 2. Points are slightly jittered for visualization.

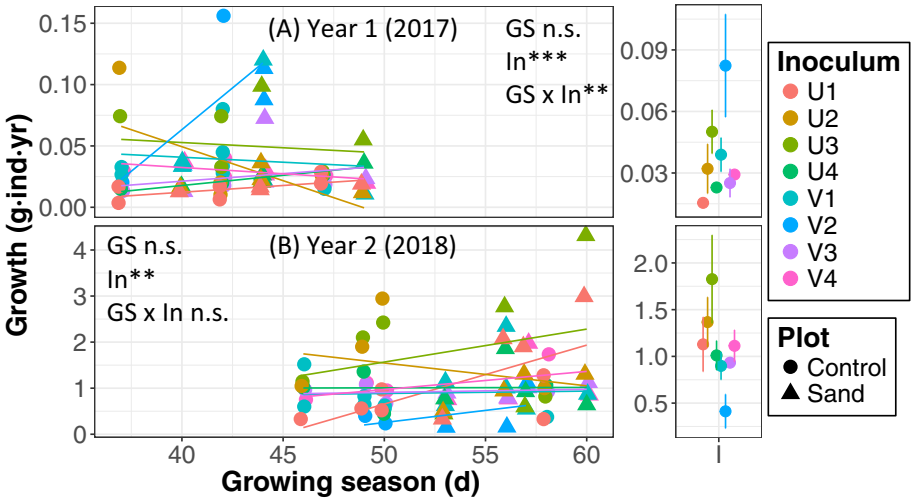

FIG. 4. Effects of growing-season length (days from snowmelt to plant measurement) and soil inocula on *Deschampsia cespitosa* growth in (A) field year 1 and (B) field year 2. Statistics are from linear mixed-effects models (**p<0.01, ***p<0.001, n.s.: not significant). GS, growing season, In, inoculum. The right panels show the main effect of inocula, as mean (±SE) predicted probabilities across the growing-season lengths. Year 1 growth was calculated allometrically from height and leaf number measurements. Lines represent linear regressions and are depicted for each inoculum (even if the fit is not significant) to demonstrate the significant interactions in each panel. Growing season varied among the four blocks and with the control and black sand (for earlier snowmelt) plots at each block, such that there were six different growing-season lengths in year 1 (two pairs of plots had the same growing-season length), and eight different growing-season lengths in year 2. Points are slightly jittered for visualization.

P = 0.86). However, only dark septate endophyte colonization was significantly positively correlated with plant growth, and the relationship was weak (linear regression, R^2 = 0.08, P = 0.02, Appendix S1: Fig. S6). Fungal pathogen abundance did not significantly differ among inocula (LMER, df = 7, 48, χ^2 = 11.93, P = 0.10), but tended to be higher in the inocula with the lowest plant growth (Appendix S1: Fig. S6). However, for the subset

of plants that were sequenced, there was no relationship between total fungal pathogen abundance and plant growth (linear regression, $R^2 = 0.01$, $P = 0.22$), or each individual pathogen's abundance and plant growth (linear regression, $P > 0.05$).

Discussion

Our results notably demonstrate that both snowmelt timing and soil microbial communities can influence plant survival and growth in areas beyond their current range. Although it was not a focus of our study, we also learned that herbivory can be an important limiting factor for some plant species, which has been the focus of other work (HilleRisLambers et al. 2013). Two of the three plants in the study experienced high levels of mortality, suggesting that not all alpine plants will be able to expand into new habitats that are currently unvegetated. Interestingly, it was the generalist species, rather than the two alpine specialists, that performed the best, which is in line with predictions based on species traits that promote range expansion (Angert et al. 2011). Angert et al. (2011) found that traits associated with more generalist strategies, such as greater diet breadths in birds, were associated with faster rates of range expansion.

Interestingly, the high mortality in *Oxyria* and *Silene* was driven by different factors. *Oxyria* experienced high herbivory by pika, which suggests that herbivory can be a limiting factor for some plants expanding their ranges. This contradicts other work that has found that range-expanding plants can be released from herbivory pressures, thus facilitating plant establishment (Engelkes et al. 2008). Herbivory, which can sometimes be higher outside of a plant's native range (Rotter and Holeski 2017), has been previously discussed as a factor limiting woody plant expansion into tundra (Cairns and Moen 2004), but further study is needed on tundra expansion into unvegetated areas. Pikas and *Oxyria* coexist in more developed vegetated areas below the transplant site, so the interaction between these two organisms is not novel. It is likely, however, that pikas preferentially eat other plant species if they are available, but at the transplant site they settle for *Oxyria*. Surveys of pika haypiles have demonstrated that the American pika is indeed selective in plants it eats, and selected species are typically some of the most abundant (Dearing 1996). At the same time, pikas also preferentially select plants high in secondary compounds, like *Oxyria*, for their haypiles, but other more abundant plants in the area, such as *G. rossii*, are also high in secondary compounds (Dearing 1996, 1997). It is unknown why so many *Silene* died, as our seedling survival rates are much lower than in a natural Alaskan population (Morris and Doak 1998). One likely explanation is the short growing season at the transplant site, which is well below the optimum growing-season length for *Silene* found at other sites (Doak and Morris 2010).

By combining survival data from 2017 and 2018, which had different growing-season lengths, we identified an optimum growing-season length at this site of 45–55 d for *Deschampsia*. As expected, too short of a growing season was detrimental for plant survival. However, the negative effect of extended growing season seen in year 2 of the experiment contradicts our hypothesis, but is likely due to moisture limitation. Much of the growing-season soil moisture in the alpine is supplied by snowpack, and earlier snowmelt can lead to earlier drying out of the soil, especially with warmer temperatures (Remke et al. 2015). Other work in dry and moist meadows on Niwot Ridge has shown that soil moisture is limiting for alpine tundra plants (Fisk et al. 1998, Knowles et al. 2015) and increased moisture limitation later in the growing season during long summers can decrease productivity of alpine plants (Berdanier and Klein 2011, Fan et al. 2016) and subalpine forests both at our site (Hu et al. 2010) and elsewhere (Buermann et al. 2013).

Unvegetated soil microbial communities were patchily distributed across space (Appendix S1: Fig. S5). However, more work is needed on community assembly in these ecosystems, as spatial and environmental variables did not explain substantial amounts of variation in both bacterial and fungal communities. It is possible that wind deposition and stochasticity play important roles in driving community composition (Jumpponen 2003), though spatial distance is also important. In subnival environments, patches of soil habitat are usually surrounded by an uninhabitable matrix of boulders and talus rocks (Fig. 1). In a recent paper applying island biogeography theory to microbial community assembly on glaciers, researchers found that island (cryoconite hole) size was positively correlated with diversity and that community similarity decayed with distance (Darcy et al. 2018*a*). Although we did not measure unvegetated soil patch size, we also found spatial autocorrelation of bacterial and fungal communities in unvegetated soils, similar to the results of King et al. (2010), who analyzed both unvegetated and vegetated communities at our site.

The hypothesis that the variability in microbial communities would lead to differences in plant performance was supported by both the survival and growth data. Although we cannot rule out the possibility that minor differences in other soil parameters led to some differences in plant growth, these were likely overwhelmed by homogeneous bulk soil that made up the majority of the pot soil. The differences in survival among the four unvegetated inocula is an important result that suggests current microbial distributions can mediate future plant distributions as well as the rate of distributional shifts. Growth in unvegetated inocula was equal to or exceeded that in vegetated inocula, which suggests that unvegetated soils can contain either enough beneficial plant-associated microbes, or fewer pathogens that are more abundant in vegetated soils, thus facilitating plant growth. This is surprising given that AMF are likely more dispersal limited than DSE and the most abundant fungal pathogens at our site, which are all ascomycetes. AMF produce spores belowground that range from 40

to 640 μm and can be either animal or wind dispersed typically <2 km (Kivlin et al. 2011), whereas ascomycete spores are more readily dispersed through air and water, and many taxa are capable of forcible discharge (Trail 2007). It is also possible that there were minor effects of location in the plot (all unvegetated inoculum pots slightly upslope of vegetated inoculum pots). These results contradict those found for trees and shrubs, whose range expansion can be limited by negative plant–soil feedbacks or a lack of mutualistic fungi in beyond-range soils (Nuñez et al. 2009, Sedlacek et al. 2014, Van Nuland et al. 2017). Our results are more in line with studies that have found plants escaping belowground enemies in beyond-range soils (Van Grunsven et al. 2007, Engelkes et al. 2008, McCarthy-Neumann and Ibáñez 2012).

The strong interactions between growing-season length and microbial communities is a striking result, especially for survival. The interaction suggests that in suitable growing seasons, microbial community composition is not as important for plant survival and growth, but if the growing season is too short or too long, there can be significant differences in plant performance based on microbial community composition. We hypothesized that the magnitude of the effects of microbial communities would be strongest with longer growing seasons, but we also found they are important in shorter growing seasons. This result is in line with theory on plant–soil feedbacks and resource allocation, whereby when resources are limiting (perhaps nutrient uptake in short growing seasons and moisture in long growing seasons), carbon allocation belowground increases and plant–soil feedbacks are important (i.e., lack of decrease in survival in suboptimal growing seasons in some inocula; Revillini et al. 2016, van der Putten et al. 2016, Lekberg et al. 2018). More work is needed to conclude whether this result is due to different microbial communities enabling plants to grow rapidly in short growing seasons, or helping them survive moisture limitation late in long growing seasons (Kim et al. 2012). Taxa such as DSE and AMF have been shown in previous studies to increase plant performance when moisture is limiting (Pagano 2014, Santos et al. 2017, Zhang et al. 2017). These interactive effects could be particularly important for plants shifting their ranges and for coping with interannual variability in climate. The strong effects of microbes on plant survival in the long growing season of year 2 is especially relevant, as climate continues to warm and growing seasons are predicted to lengthen in the future (Settele et al. 2014).

It remains difficult to identify which microbial taxa are affecting plant performance. Differences in plant survival and growth could be driven by either mutualistic/beneficial microbes, or pathogenic/detrimental microbes (van der Putten et al. 2010). Although some inocula had higher levels of pathogens than others, the lack of relationship with growth could be due to offsets from other taxa, or to limitations in pathogen assignment by FUNGuild. Taxa assigned as pathogens could either not be pathogenic to these plants, or not pathogenic in this environmental context. Our data suggest that the dark septate endophyte fungal group could be important, as greater colonization levels by this fungal group were weakly but positively correlated with plant growth. This is particularly interesting because these fungi are more frequently found in unvegetated soil than the other two root endophytic fungal groups. Although a metanalysis of dark septate endophyte effects on plant growth demonstrated typically beneficial effects (Newsham 2011), their function is still open to debate (Mandyam and Jumpponen 2015), as some studies have shown that they can negatively impact plant growth (Alberton et al. 2010, Mayerhofer et al. 2013), and have not benefited as wide a range of plants as arbuscular mycorrhizal fungi. However, DSE may play a relatively more important role in alpine ecosystems, where they are found in most plant species (Bueno de Mesquita et al. 2018*a*). Other important fungal genera identified in the sequencing data include the pathogen *Venturia*, and the saprotrophs *Fontanospora*, *Pseudogymnoascus*, and *Knufia* (Rikkerink et al. 2011, Crous et al. 2013, Tedersoo et al. 2014, Nguyen et al. 2016). Some of the important bacterial genera include the nitrifier *Nitrospira*, the denitrifier *Rhodanobacter*, the phosphorus-solubilizing *Agrobacterium*, and the antimicrofungus *Pseudonocardia* (Hameed et al. 2005, Kostka et al. 2012, Poulsen et al. 2012, Daims et al. 2015).

Our manipulative experiment provides the first evidence of the role of unvegetated soil microbes facilitating plant colonization as climate changes. We also provide evidence of potential detrimental effects of extended summers on the growth of an alpine plant. Although our experiment focused on earlier snowmelt and longer growing seasons, climate change is also causing warmer summers characterized by greater evapotranspiration rates and soil moisture deficits, which can interact with other factors such as nutrient deposition to affect vegetation (Farrer et al. 2015, Remke et al. 2015). Future work should seek to understand plant–microbe interactions in the context of range shifts (Bueno de Mesquita et al. 2019), and study the role of multiple climatic and resource variables. Another interesting avenue for future research is to investigate the role of seed-borne microbes in facilitating plant establishment in new ranges. Additional work using cultures and simplified microbial communities can further elucidate which microbial taxa are important for plants. Understanding plant–microbe interactions, and in general, biotic interactions, remains an exciting and crucial area of research for understanding geographic responses to climate change.

Acknowledgments

Funding was provided by National Science Foundation (NSF) grant DEB 1457827 to KNS and SKS. Additional funds were provided by the Indian Peaks Wilderness Association and the University of Colorado Graduate School. We thank Tom

Lemieux and Janice Harvey for help in the greenhouse. We thank the Niwot Ridge LTER program (NSF DEB 1637686) help in the greenhouse. We give special thanks to Jared Anderson-Huxley, Jon Flechsenhaar, Christian Prince, Adam Solon, and Kelsey Elwood for their hard work carrying the transplants to the field site, and Jane Smith, Lara Vimercati, Antoine Magre, Cormac Martinez del Río, Joshua Addison, Liza Hasan, and Elizabeth Buhr for additional field help. We thank Bill Bowman, Dan Doak, Noah Fierer, Jane Smith, Emily Farrer, and Dorota Porazinska for helpful feedback on this experiment. We thank Jessica Henley, Matt Gebert, and Jonathan Leff for assistance with DNA sequencing preparation and analysis. We thank the City of Boulder, Craig Skeie, and Eric "EJ" Johnson for permission to use the field site.

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3095/suppinfo

Data Availability

Sequencing data are accessible on GenBank via BioProject accession no. PRJNA525120. All other data sets are publicly available on the Niwot Ridge Long Term Ecological Research Site data portal on the Environmental Data Initiative website via Query URL https://portal.edirepository.org/nis/browseServlet?searchValue=NWT. R code can be found on Zenodo: https://doi.org/10.5281/zenodo.3776213