Methods copied from: Lynn, J.S., D.A. Duarte, & J.A. Rudgers. Accepted. Soil microbes that may accompany climate warming increase alpine plant production. Oecologia.

Greenhouse experimental design

The experiment used a 2x2x3x3 factorial design, including an inoculum sterilization treatment (sterile vs. live soil inocula), soil provenance (inocula collected from resident, high elevation vs. novel, low elevation), three independent mountains to replicate elevation geographically, and three grass species. This design resulted in 36 treatment combinations with 10 replicates per treatment (N=360 pots). Plants were grown in ~150 mL plastic root trainer pots (Stuewe and Sons, Inc., Tangent, Oregon, USA). Each pot was filled with 80% of sterilized sand (80 mL, QUIKRETE Play Sand, Atlanta, Georgia, USA) topped with 10% of treatment inoculum (10 mL), and then topped with 10% of sterilized sand (10 mL) to reduce splash contamination. Sand was autoclave-sterilized for three cycles (each cycle: 1 h at 121C and 20 psi) separated by a 24-hour cooling and rest period. Sand enabled easy cleaning of roots. Each pot was initially sown with 10 seeds of one focal species, to ensure at least one germinant. On 11-Oct and 30-Nov-2017, pots were thinned to one individual. All tools used for thinning were sterilized with 8.5% sodium hypochlorite bleach between pots. There were no treatment effects on rate of germination (93% of pots had germination, electronic supplementary material Table S4) or final plant survival (all pots with germination survived to harvest). Seedling survival was not monitored prior to thinning. Samples were fully randomized across eight blocks (trays) of pots. Plants were watered four times daily for 5 min using an automatic overhead spraying system (~50 mm per pot). The experiment began 15-Sep-2017. On 22-Nov and 21-Dec-2017, each pot was fertilized with FloraGro 2:1:6 % N:P:K solution (General Hydroponics, Sebastopol, CA, USA) to reduce possible nutrient stress during long-term growth.

Study area

We collected soil inocula and seed from the Colorado Rocky Mountains within the Upper Gunnison Basin, Colorado, USA. The Colorado Rockies are expected to experience more pronounced warming trends over the coming decades compared to lower lying regions (McGuire et al. 2012, Rangwala and Miller 2012). All plant material and soil inocula were collected from three independent mountain peaks and associated valleys (Avery, Cinnamon, and Treasury), with a high (resident) and low (novel) site on each gradient (site coordinates in electronic supplementary material, Table S1), all within the Gunnison National Forest, CO, USA. The two elevation sites per mountain were selected approximately 430m apart in elevation (range ~3200-3600m a.s.l.) to represent ~3 C difference in mean annual temperature (Pepin and Losleben 2002). The low sites were selected to be lower than the low elevation range boundaries of the three focal plant species.

Soil inoculum

At each of the six sites, live soil was collected to serve as inoculum in our greenhouse experiment. Within a site, soil was collected at three points along a 40m long transect (0, 20, and 40 m). We collected approximately 1 L of soil at each collection point using a 8.5% sodium hypochlorite bleach-sterilized trowel, resulting in 3 L of soil per site. All soils were collected 14-25 Aug-2017. Soils were immediately stored at 4 C after collection until used for inoculation 15-Sep-2017. In preparation for the experiment, soils were sieved through an ethanol-sterilized 2 mm grade sieve. Soil from each site was divided in half, with one half used for the live inoculum treatment and the other sterilized to better isolate the influence of living microbes from other edaphic factors. Sterilized soils were autoclave-sterilized for three cycles (each cycle: 1 h at 121 C and 20 psi) separated by a 24-hour cooling and rest period. Bulking soil may not accurately represent the spatial scale at which plants and microbes interact in the field (Rinella and Reinhart 2018). Our soils were combined over a 40 m area which may inflate the soil microbial diversity experienced by an individual plant as well as spread pathogens or mutualists among microsites; however, these potential effects may be consistent across all inocula. We focused replication at a larger spatial scale (six independent soil communities) rather than individual microsites, so that we could replicate high and low elevation sites on multiple mountains to more accurately capture elevation relative to comparing one low/high pair.

Focal plant species

We used three alpine-restricted grass species to test how soil communities affect plant growth and traits (all in family Poaceae, subfamily Pooideae; Poa alpina- tribe: Poeae, subtribe: Poinae; Festuca brachyphylla- tribe: Poeae, subtribe: Loliinae; Elymus scribneri- tribe: Triticeae). The focal species occupy the highest elevations within the region (~4000m a.s.l.); locally, they are rarely observed below ~3500m a.s.l. (Lynn et al. 2019). In the region, grasses have become more abundant in upland meadows and have often shifted their mean range to higher elevations (Zorio et al. 2016). Seeds for the focal plant species were collected in 2015 and 2016 from the same three high-elevation sites from which we collected resident soil inocula (Table S1). Seeds were bulked across years. Seeds were only grown in inocula from the same mountain gradient. We did not sterilize seeds. Therefore, microbes present in seeds occurred evenly across treatments and we only test the effects of novel soil microbes interacting with current seed microbes.

Response variables

Plant biomass harvest. We harvested plants on 16-18 Jan-2018. We washed roots through a 1 mm sieve. All biomass was dried at 60 C for 48 h then weighed to the nearest 0.001 g. Prior to drying roots, we placed ~ 0.2 g of wet root tissue in a plastic tissue cassette (Slimsette, Simport, Beloeil, QC, Canada) in preparation for microscopy. Root dry mass was corrected for removal of this sample by weighing the remaining roots both wet and dry. Cassettes were stored in 70% ethanol until processing for fungal colonization.

Plant traits. We measured the height of each individual in cm prior to harvest. We measured specific leaf area (SLA) on 3-5 fully expanded healthy leaves per plant. We scanned each leaf with a CanoScan LiDE 210 (Canon USA, Inc., Melville, NY, USA) flatbed scanner and measured area (in cm2) using ImageJ software (Schneider et al. 2012). Each leaf was weighed on a mass balance to the nearest 0.0001 g. SLA was calculated as cm2/g, and individual leaves were averaged to obtain a single SLA value per plant. Root to shoot biomass ratio (hereafter root:shoot) is the biomass of roots divided shoot biomass.

Fungal colonization. Roots were soaked in 10% KOH, rinsed in water, soaked in 0.1N HCl, and then soaked in preheated 5% Parker blue ink (Parker Pen Company, New Haven, East Sussex, UK) and vinegar stain, and rinsed in water again (Vierheilig et al. 1998). We scored root colonization via light microscopy with the magnified intersection method at 200x magnification (McGonigle et al. 1990) with 100 views per sample. Aseptate and septate hyphae were counted separately as indicators of AM fungi and non-AM fungi, respectively. Septate fungi were relatively rare (only ~34% of samples) and lacked sufficient data for analysis. A few individuals of each species lacked sufficient root material to assess colonization (7 of 333).

QA/QC Procedures

We performed QA/QC checks with data entry checking (entry and rechecking the entries), outlier analysis, scatterplots, and internal consistency checks.