The Effect of Climate Change and Site on the Above- and Belowground Bacterial Communities in Subalpine Conifer Seedlings

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INTRODUCTION

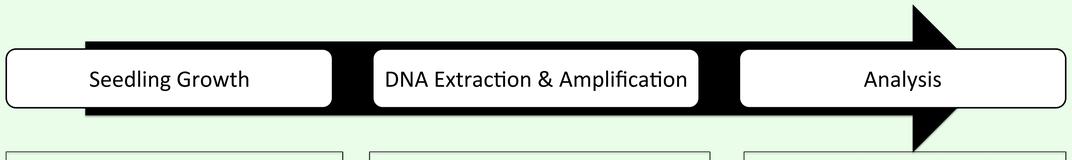
Climate change is predicted to shift the distributions of forest trees upward, moving the current forest boundary into the alpine zone. However, upward elevational shifts depend on the ability of seedlings to establish from seed. In the alpine-treeline ecotone, climate change could enhance establishment by reducing cold stress, while at lower elevations, climate change could exacerbate heat and drought stress impairing seedling recruitment. The ability of seedlings to survive depends on interactions between genetic and environmental controls on seedling physiology [1–3]. Additionally, though rarely considered, seedling interactions with microbes in the soil, as well as in and on seedling tissues, could influence seedling establishment. Increasingly recognized as a dynamic constituent of plants [4], the plant microbiome has the potential to buffer plants against climate stressors. Microbial symbioses can accelerate host adaptation to different climates and stressful environments [5,6], potentially promoting climate-induced expansion at host range limits. Alternatively, a lack of microbial taxa resident in newly suitable habitat or climate change in existing habitat could disrupt adapted host-microbe associations, thereby limiting expansion and persistence [7]. Subalpine conifer seedlings are exposed to extremes of temperature, humidity, radiation, and soil moisture [8–10], possibly assisted by the native seedling microbiome, including mycorrhizal fungi [11–16]. With climate change, new and existing seedling-microbe associations could contribute to establishment beyond the current range, as well as to seedling recruitment under warmer and dryer conditions within the current range. On the other hand, seedling-microbe associations could constrain uphill migration of forest trees if host and microbes differ enough in their dispersal capacity or response to climatic change [7,17–21].

The plant microbiome consists of bacteria and fungi in the rhizosphere, phyllosphere, and endosphere. Given their different roles, dispersal capacities, and degrees of host specialization, members of the microbiome will likely respond independently to climate change, and differ in their capacity to buffer their hosts against associated stressors. Recent work on grasses demonstrates that fungal endophytes can mediate plant response to climate change [22] and broaden the geographic niche of their host [23]. Additionally, rapid changes in belowground fungal and bacterial communities has been shown to influence plant response to drought stress [24], suggesting a potential role for soil microbes in mediating plant response to climate change. The role of the bacterial endophyte community in mediating plant response to climate change is less clear [25], but like their fungal counterparts bacterial endophytes can buffer plants under stress [26–31]. Endophytes of adult limber pine, a subalpine conifer that is widespread in the Western United States, are thought to fix atmospheric nitrogen (N), which they potentially share with the host [36]. Little is known about the endophytic bacteria throughout a tree's lifetime, and the function of the endophytic community in seedlings may differ from that in adults. To better understand the potential functional roles of endophytes at the earliest tree life stages, we characterized the above- and belowground bacterial endophyte communities in limber pine seedlings establishing in *in situ* common gardens within and beyond the elevation range of subalpine forest.

AIMS

1. **To gain a better understanding of the endophyte community in pine seedlings, including its potential functional role**
2. **To understand how the endophyte community varies across sites across an elevation and canopy gradient**
3. **To understand whether the endophyte community is sensitive to experimental climate change within the alpine-treeline ecotone**

METHODS



- 1 year old seedlings were sampled from the Alpine Tree Line Warming Experiment at Niwot Ridge, CO [2,3,37]
- Three sites:
  - Forest, 3060m
  - Treeline, 3430m
  - Alpine, 3540m
- Four Treatments (Only USA)
  - Control
  - Heated
  - Watered
  - Heated & watered

- Seedlings were surface sterilized with 30% H202 [38]
- Separated into above- and belowground tissue
- Ground tissue and extracted DNA using CTAB protocol [39]
- Amplified using nested PCR: [40-43]
  - PCR1: 799f-1492R
  - PCR2: 799f-1115R
- Sequenced on Illumina MiSeq platform

- Processed sequencing using UPARSE and QIIME [44-51]
- Generated OTUs based on 97% similarity
- Calculated differences in community composition using:
  - Unweighted and Weighted UniFrac
- Measured α diversity
  - Observed Species
  - Chao1
  - Shannon Index
  - Faith's Phylogenetic diversity

Conclusions

We found that the most important factor structuring seedling endophyte communities to be tissue type (above vs below) similar to studies in *Arabidopisis* [52]. The communities were dominated by Betaproteobacteria specifically the family *Oxalobacteriaceae* which have been shown to increase biomass, protect against stress, fix nitrogen and protect against fungal pathogens [31,53-56]. Relative to adult communities seedlings were significantly more diverse, which has been previously reported by fungal communities in *Pinus taeda* (loblolly pine)[57]. This could be a difference in acquisition routes.

We observed differences between shoot communities from seedlings in the forest and those from higher elevations. This was likely not do to provenance due to seedlings being from a common seed pool. Studies of soil bacterial communities in the high-alpine environment above Niwot Ridge show that these have significant spatial autocorrelation in community composition up to a distance of 240 m [58], and that there is high correlation between soil microbiota and plant abundance distribution [59]. Provided that these patterns hold true at lower elevations, we would expect the soil bacterial communities at treeline (3430 m) and alpine (3540 m) sites to be more similar to each other than to those in the forest site, with possible implications for endophyte community assembly.

The climate treatments did not significantly shift the aboveground community at treeline, suggesting that it is relatively robust to environmental change. We saw a significant difference with heating in root samples when weighted UniFrac was used to measure the community dissimilarity between samples (but not unweighted UniFrac). This suggests that heating altered relative abundance of root bacteria without turnover in the identity of taxa, with potential consequences for community function. However, the relative abundance of the major OTUs, which we hypothesize may play a role in biotic stress protection, did not shift significantly with climate treatments, indicating that these associations are relatively robust to environmental change, potentially consisting of taxa that well adapted to or selected by the host .

To conclude, our results do not exclude the possibility that bacterial endophytic microbiomes of forest tree seedlings can shift as a consequence of climate change, but suggest that local effects of climate change are small compared to site-level variation across an elevation gradient, at least in the first year of a seedling's life. Beneficial associations with bacteria such as those contributing pathogen defense may therefore be relatively robust to climate change, but seedling uphill migration could be constrained by lack of such associations in newly suitable habitat, in particular if beneficial endophytes are sourced from surrounding trees.

RESULTS

Fig. 1 Unweighted and weighted UniFrac distances visualized by PCoA colored by tissue type

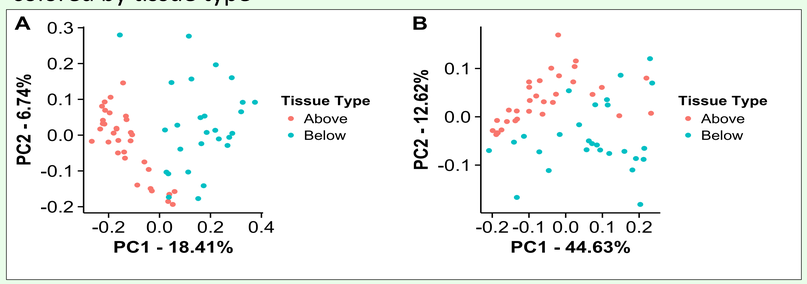


Fig. 3 Unweighted and Weighted UniFrac distances visualized by PCoA colored by site and treatment

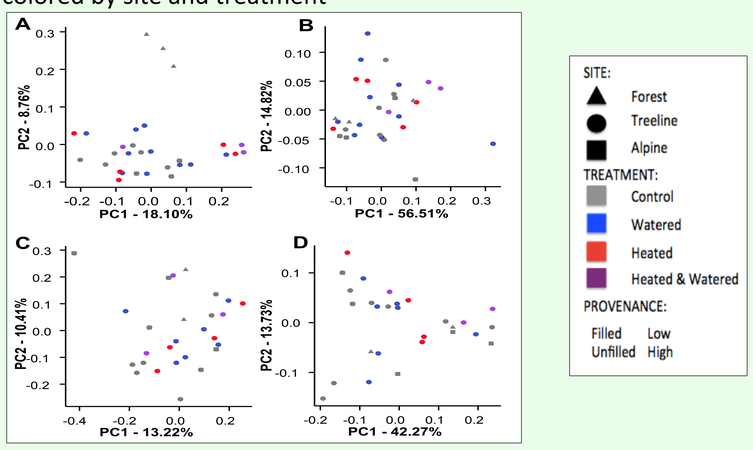


Fig. 2 Heatmap of the top 10 OTUs for each tissue type

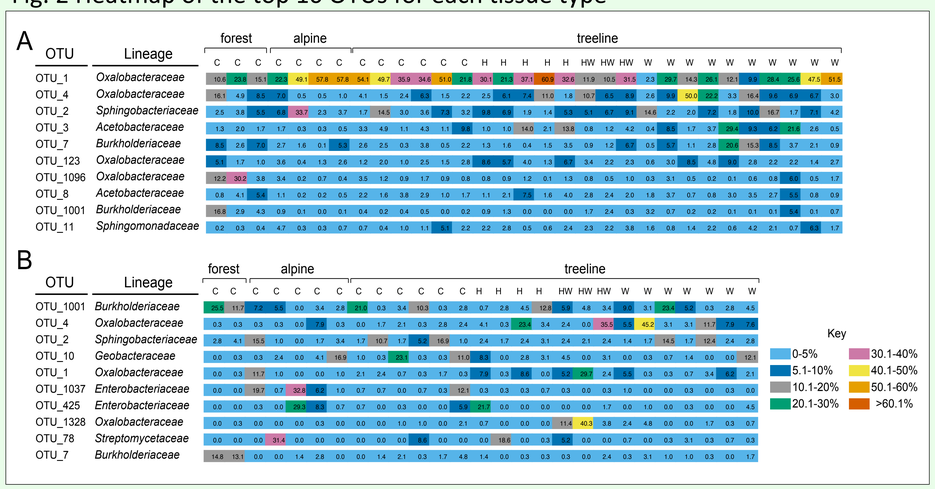


Figure 1 Unweighted and weighted UniFrac distances visualized by PCoA colored by tissue type (Top left). Samples clustered by tissue type with aboveground tissues forming one cluster and belowground tissues forming another cluster. This was significant when looking at both (A) unweighted distances which only takes into account presence/absence (ANOSIM: R-statistic=0.6503 P-value=0.001 and PERMANOVA: Pseudo-F=8.9379 P-value=0.001) and (B) weighted distances which takes into account relative abundances of the species (ANOSIM: R-statistic=0.3520 P-value=0.001 and PERMANOVA: Pseudo-F=11.8849 P-value=0.001) .

Figure 2 Heatmap showing the 10 most abundant OTUs and their relative abundances as percentages of all the 16S rRNA gene sequences in each sample. (Top right). (A) aboveground and (B) belowground tissues types. Both above- and belowground tissues were dominated by *Betaproteobacteria,* 58.7% and 32.7% respectively. Belowground tissues had a larger portion of the endophytic community comprised of *Deltaproteobacteria* (7.0% vs. 1.1%) and *Gammaproteobacteria* (12.3% vs. 2.7%). The top two OTUs (OTU_1 and OTU_4) together made up between 4.9% and 71.0% of all aboveground samples and 0.2% and 40.3% of all belowground samples. Color tones range from warm (orange) to cool (blue) to indicate the highest and lowest abundances. The value in each square is the percentage of the sample that is made up of that OTU. The lineage shows the taxonomic order for which each OTU has been classified. Each column is a single sample, the first letter in the sample name represents the treatments, control (C), heated (H), watered (W) and heated and watered (HW). Samples with at or above 16,912 and 290 sequences per samples were included in the heatmaps for shoot- and root samples respectively.

Figure 3 Unweighted and Weighted UniFrac distances visualized by PCoA colored by site and treatment (Bottom left). (A) unweighted and (B) weighted UniFrac distance matrix for shoot samples. (C) unweighted and (D) weighted UniFrac distance matrix for root samples Points that are closer together have more similar communities.

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