

THE IMPACTS OF ENVIRONMENTAL CHANGE ON PLANT AND MICROBIAL
COMMUNITIES: A TURF TRANSPLANT EXPERIMENT IN THE COLORADO
ROCKY MOUNTAINS

by

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by: Lorah Seltzer, titled: The Impacts of Environmental Change on Plant and Microbial Communities: A Turf Transplant Experiment in the Colorado Rocky Mountains and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.



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PREVIEW

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ABSTRACT

Climate change is rapidly altering temperature and moisture regimes, reshaping species distributions, interactions, and ecosystem processes worldwide. High elevation mountain ecosystems are especially vulnerable due to accelerated climate change and limited opportunities for upslope range expansion, particularly as low elevation competitors move in. Responses to climate change are driven by a complex interplay of direct climatic effects and indirect species interactions. As a result, predicting how communities and ecosystems will change remains challenging, especially across biological scales.

To address this, I established a reciprocal whole-community turf transplant experiment in the Colorado Rocky Mountains, relocating intact plant and soil communities across an elevational gradient characterized predominantly by shifts in temperature and moisture. This design allowed me to assess how community composition, flowering phenology, functional traits, and ecosystem functioning respond to environmental change. By transplanting both up and down elevation I tested for asymmetries in ecological sensitivity to climate shifts.

Turfs transplanted downhill and exposed to a warmer environment had rapid, directional shifts in community composition, which were stronger in soil bacteria/archaea and weaker in plants and soil fungi. In contrast, turfs transplanted uphill and exposed to a cooler environment had slower, weaker, and more variable changes in community composition, with limited turnover. These asymmetrical responses suggest that upslope colonization by low-elevation species is more likely than downslope persistence, increasing the vulnerability of high-elevation communities to novel competitors and altered interactions.

Flowering phenology shifted rapidly with both uphill and downhill transplantation, suggestive of strong plastic responses to both warming and cooling that mirrored natural elevational patterns. Community-level flowering and production converged with the destination sites within five years, despite differences in species composition. However, phenological shifts were only weakly associated with reproductive output and unrelated to species dominance, suggesting that timing differences are consistent responses to climate, but are poor predictors of broader ecological outcomes.

Plant functional traits also shifted rapidly under warmer conditions, changing from traits associated with fast growth and resource use to traits linked to resource conservation and survival, similar to those found in the lower elevation, warm-adapted communities. In contrast,

cooling led to slower and more inconsistent changes. These shifts were driven primarily by plasticity and, to a lesser extent, changes in species dominance. Notably, carbon and water fluxes increased under warming despite more conservative trait profiles, which suggests context-dependent links between local conditions, traits, and function.

In summary, transplantation to warmer sites drove rapid reassembly of high elevation communities and altered ecosystem functioning, while transplantation to cooler sites overall led to slower, less consistent responses. These asymmetries indicate a heightened vulnerability of high elevation ecosystems to climate change, and the potential for upslope establishment of lower elevation species. While some community responses shifted predictably, others were context-dependent, indicating the complexity of ecosystem responses to environmental change. This dissertation reveals that complexity by empirically demonstrating the range of ecological responses to environmental change and exploring the mechanisms driving divergence. Although many ecological models and management plans depend on generalizable, predictable patterns, this work shows that in subalpine ecosystems, responses are often nonlinear, variable, and shaped by local context. In a time of urgent environmental decision-making, understanding this ecological complexity is essential for building informed strategies for a changing climate.

INTRODUCTION

Climate change is an urgent, global crisis of enormous complexity. The challenge climate change presents is multilayered, spanning governance, equity, infrastructure, and ecological resilience. According to the latest assessment by the Intergovernmental Panel on Climate Change (IPCC, Lee et al. 2023), global surface temperatures have risen by approximately 1.1 degree Celsius since pre-industrial times, already causing widespread damage to ecosystems and human societies. Extreme climate events, such as heatwaves, heavy rainfall, and droughts, are now more frequent and intense, affecting water availability, food production, and human health worldwide. Climate change is rapidly reshaping ecosystems by driving shifts in species distributions, altering the seasonal timing of key events like flowering, breeding, and migration, accelerating biodiversity loss, and disrupting ecosystem services (Parmesan 2006; Gottfried et al. 2012; Parmesan & Hanley 2015, Lee et al. 2023). Yet, despite international recognition of climate change and policy commitments, the scope and pace of mitigation efforts are insufficient to limit future warming.

Given this global urgency and the fact that many adaptation strategies rely on managing and restoring ecosystems, understanding how ecosystems respond to climate change is increasingly vital (Turner et al. 2020; Seddon et al. 2020; Cavicchioli et al. 2019). While climate change is a global phenomenon, broader environmental outcomes are shaped by local impacts (Wilbanks & Kates 1999; Chapin et al. 2000). This dissertation contributes to understanding of local responses by examining an ecosystem in depth and exploring how environmental changes impact its structure, function, and resilience. This research explores the mechanisms behind population, community, and ecosystem-level responses to better understand complex and often unpredictable impacts of climate change (Felton & Smith, 2017; Mori et al. 2013; Svenning & Sandel 2013).

To investigate these dynamics, I established a whole-community transplant experiment in the subalpine meadows of the Colorado Rocky Mountains (Vandvik et al. 2020). Fellow students and I relocated large, intact blocks of plant and soil communities across a 400-meter elevation gradient characterized by differences in temperature and moisture. From 2017 to 2023, we monitored changes in plant communities, soil fungal, bacterial, and archaeal assemblages, and carbon and water fluxes.

By integrating measurements across multiple biological levels, this research reveals how environmental change influences systems from the scale of individual organisms to ecosystem-level processes. It provides new insight into how plant and soil microbial communities respond to shared environmental changes, the role of phenological shifts as indicators of plant performance, and the strengths and limitations of using plant functional traits to predict ecosystem function. Together, these findings contribute to a broader effort to understand and anticipate the impacts of climate change.

The present study is divided into three Appendices, each including figures and tables, references, and supplementary materials:

Appendix A, *Experimental warming restructures soil microbial and plant community composition in subalpine meadows*, addresses the gap in our understanding of how climate change is reshaping both above-ground plant and below-ground soil microbial communities. These communities are often studied in isolation, which limits our ability to compare the timing and magnitude of community responses, as well as how those responses vary under warmer versus cooler conditions (Bektaş et al. 2024; Broadbent et al. 2021; Fierer 2017; Bardgett & Van Der Putten 2014; Ladau et al. 2018). I found that turf transplantation downhill to warmer sites led to rapid and directional shifts in species composition, with both plant and microbial communities becoming more similar to those at lower, warmer elevations. Response magnitude varied, with soil bacterial and archaeal composition changing the most, followed by fungi and then plants. In contrast, transplantation uphill to cooler sites led to slower, weaker, and more variable responses, with most communities retaining their original composition. This difference in response magnitude suggests a risk of forming novel communities under future climate change. Species-level analyses revealed that the most responsive taxa were those that already varied in abundance across the elevation gradient, indicating that these species may possess traits important in destination site conditions. Together, these findings suggest that low elevation species may expand upslope if dispersal and establishment barriers are overcome, increasing the vulnerability of high elevation species to novel competitors.

Appendix B, *Plasticity drives phenological shifts with limited links to performance in a subalpine whole-community transplant experiment*, investigates how the timing of biological events, or phenology, responds to environmental change. Although phenological shifts are one of the most widespread global responses to climate change (Piao et al. 2019; Chambers et al. 2013;

Parmesan et al. 2003; Menzel et al. 2020), species-level responses are highly variable and are less understood at local scales (CaraDonna et al. 2014; Diez et al. 2012; Iler et al. 2013; Cleland et al. 2012). Using the transplant experiment, I tracked the flowering phenology of 50 plant species to assess the variability of these responses and test whether this variability is linked to plant performance, which remains a critical but unresolved question for predicting species persistence with climate change (McLean et al. 2016; Iler et al. 2019, 2021; Cleland et al. 2012; Collins et al. 2025). I found that community-level flowering phenology shifted rapidly toward that of the destination site despite differences in species composition, suggesting that phenotypic plasticity may buffer communities against short-term environmental change. However, species-level responses varied in magnitude and direction, indicating that phenological shifts are not consistent or easily generalized. Additionally, while the species-level flowering shifts were correlated with flowering output, shifts were not correlated with changes in plant cover, a proxy for growth and survival. These results suggest that phenology alone may be an unreliable indicator of long-term ecological change, particularly in subalpine systems dominated by long-lived perennial plants.

Lastly, **Appendix C, *Climate warming outpaces cooling in driving plant functional trait and ecosystem change***, investigates how plant communities respond to transplantation through shifts in functional traits, or the morphological, physiological, or phenological characteristics that affect fitness through performance (Violle et al. 2007). This chapter addresses key uncertainties in global change biology, including whether trait shifts arise from phenotypic plasticity (Henn et al. 2018; Jónsdóttir et al. 2023; Nicotra et al. 2010; Violle et al. 2012) or species turnover (Schuchardt et al. 2023; Thompson & Gonzalez 2017), and how these shifts scale up to affect ecosystem-level carbon and water fluxes (Adler et al. 2014; Díaz et al. 2016; Norberg et al. 2001; Savage et al. 2007; Reich et al. 2014). Transplantation to warmer sites led to rapid, directional shifts in physical leaf traits towards those of the lower elevation destination community. In contrast, transplantation to cooler sites led to slower and more variable trait shifts. Intraspecific trait plasticity was the dominant mechanism of the trait shifts, suggesting that communities may exhibit short-term resilience to environmental change. Community turnover also drove trait shifts with warming, but not cooling. Despite a shift toward more resource-conservative trait profiles, which are typically associated with lower productivity, communities at warmer sites showed increased carbon and water fluxes, indicating

context-dependencies between local conditions, traits, and ecosystem functioning (Blouin et al. 2024).

Together, the findings presented across the three appendices underscore the complex, scale-dependent, and often contrasting ways that ecosystems respond to climate change. By integrating responses from soil microbial and plant communities to functional traits and fluxes, this research demonstrates that rapid ecological shifts can occur with environmental change. As climate change continues, understanding the interacting processes that govern community and ecosystem resilience will be essential for anticipating and managing ecological outcomes. The transplant experiment remains in place and, at the time of writing, has been renewed through 2028 as a resource for ongoing and future studies.

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L.S., P.S., V.V., and B.E. conceived the study; L.S., P.S., J.N., A.V., and B.E. conducted fieldwork; L.S. and P.S. conducted lab work; L.S., A.H., and R.T. developed R code used in the analysis; L.S. analyzed the data; L.S., P.S., and J.N. curated the data; L.S. wrote the manuscript; V.V., B.E., and P.S. provided feedback on the manuscript.

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APPENDIX A. Experimental warming restructures soil microbial and plant community composition in subalpine meadows

Abstract

Climate change is altering both above-ground plant communities and below-ground microbial assemblages, yet their simultaneous responses to altered climate remain poorly understood. To address this, we conducted a whole-community transplant experiment across a 400-meter elevation gradient in the Colorado Rocky Mountains, examining how plant and soil fungal and bacterial/archaeal communities respond to experimental warming and cooling. Transplantation to warmer sites led to rapid, directional shifts of all groups, with compositions changing to resemble those at lower, warmer sites, and diverging from higher origin sites. Soil bacteria and archaea responded most strongly, followed by fungi and then plants, indicating differing sensitivities. Variation in responses may lead to novel communities, potentially disrupting relationships that affect ecosystem resilience. In contrast, transplantation to cooler sites led to weaker, slower, and more variable responses, with limited evidence of community turnover. This indicates that low elevation species may successfully establish upslope if dispersal barriers are overcome, potentially increasing the vulnerability of high elevation communities to novel competitors.

Introduction

Plant and soil microbial communities are interconnected. Plants influence microbial communities through litter inputs and root exudates, while microbes affect plants through impacts on nutrient availability and soil structure (Bever 1994; Bever et al. 1997; Ehrenfeld et al. 2005; Kulmatiski et al. 2008; van der Putten et al. 2013; Bennet et al. 2019; Hagedorn et al. 2019; Sayer et al. 2017). Together, the communities play critical roles in regulating resource flow, influencing soil properties, and generating feedbacks that connect ecosystems to climate (Li et al. 2025; Whitman et al. 1998; Bardgett & Van Der Putten 2014; Zhang et al. 2017; Luo et al. 2014). Despite these tight links, plant and microbial responses to climate change are often examined in isolation or through the lens of specific functions (e.g., carbon cycling), limiting our understanding of how entire ecosystems respond (Bahram and Netherway 2022; Liang et al. 2015; Schattler et al. 2015; van der Putten et al. 2016).

Both plants and microbes can respond to environmental change through shifts in physiology and community composition. These shifts in community structure can offer early signals of broader ecosystem change (Sayer et al. 2017). However, shifts may differ in rate or direction, potentially disrupting the feedbacks between them that regulate ecosystem functioning (Schlatter et al. 2015; Classen et al. 2015; Broadbent et al. 2021). Understanding how both communities respond to shared environmental changes is critical for predicting future impacts to ecosystem functioning and resilience.

Plant responses to climate change are relatively well understood, particularly along elevation gradients. Some species are shifting ranges upward to track suitable climates (Feeley et al. 2020; Rumpf et al. 2018; Steinbauer et al. 2018), while others persist temporarily in unsuitable conditions, leading to a phenomenon known as “extinction debt” (Nomoto and Alexander 2021; Alexander et al. 2018; Dullinger et al. 2012). Plasticity allows some species to remain in place, while others decline or go locally extinct. Climate-driven changes in plant-plant interactions can also restructure communities (Lyu & Alexander 2022; Alexander et al. 2015).

In contrast, soil microbial responses remain less understood. Although microbes can respond quickly to environmental change due to short generation times and high dispersal capacity (Allison & Martiny 2008; Liang et al. 2015; DeVries & Shade 2013; Foley et al. 2024; Castro et al. 2010), delayed responses also occur, especially when mediated by slow-changing soil properties or novel environmental conditions (Ladau et al. 2018; Hawkes & Keitt 2015). The nature and speed of these responses is due to variation in traits that affect growth rates, resource use, and acclimation capacity (Schimel et al. 2007).

When plant and microbial communities respond to environmental change in different ways, the feedback loops that maintain ecosystem stability may be disrupted (Hagedorn et al. 2019). However, few studies examine how plant and microbial communities respond simultaneously to shared environmental change (Bektaş et al. 2024; Broadbent et al. 2021; Fierer 2017; Bardgett & Van Der Putten 2014; Ladau et al. 2018; Bryant et al. 2008; Buzzard et al. 2019). To address this, we established a whole-community turf transplant experiment that relocates entire ecosystems, with plant and soil microbial communities, to novel climate conditions across an elevation gradient (Vandvik et al. 2020a,b). Transplantation creates immediate shifts in temperature, growing season length, and water availability, which are key climate variables that are changing in the study area due to climate change. This approach

enables us to compare the rate, magnitude, and direction of community responses across multiple biological groups and treatments.

Our central question is: Do above-ground plant and below-ground microbial communities respond to transplantation with shifts in composition, and, if so, do shifts vary between communities, treatments, and populations? To answer this, we test five hypotheses:

Hypotheses 1 & 2: The composition of the communities will shift in response to transplantation, and shifts will be in the direction of the new “destination” environment due to exposure to the change in environmental conditions.

Hypothesis 3: The magnitude of community response to transplantation will be stronger for microbes than plants.

Hypothesis 4: The magnitude of community response to warming will be stronger than the response to cooling.

Hypothesis 5: The plant and microbial taxa that have the strongest change in abundance in response to transplantation will be those that also change most in abundance across the elevation gradient.

Methods

Turf transplant experiment

In 2017, we established study sites at High (3300 m, 38.97274 N, -107.04908 W), Mid (3150 m, 38.96046 N, -107.03262 W), and Low (2900 m, 38.93248 N, -107.01018 W) elevation in the subalpine meadows of the Elk Mountains near Crested Butte, Colorado, USA. This system is sensitive to climate change due to rapid warming, reduced snowpack, and earlier snowmelt (IPCC 2021; Pepin et al. 2022), which has altered critical seasonal cues (Sorensen et al. 2020) and driven changes in plant communities (Zorio et al. 2016; Anderson & Gezon 2014).

Each site location had a southern aspect and approximate 10% slope. From the High to Low site, the mean growing season daytime air temperature increased by 2.6 degrees Celsius and the gravimetric water content decreased by a mean of 0.17 g H₂O per g dry soil (Table 1, Wild et al. 2019). Snowmelt, which initiates the start of the growing season (Cook et al. 2012; Inouye 2022; Diez et al. 2012), occurred an average of 21 and 10 days earlier at the Low site than at the High and Mid sites, respectively. Earlier snowmelt is correlated with a longer growing season, less snowfall, and lower water availability during the growing season (Iler et al. 2019).

Each site was divided into five blocks of five 0.5 m x 0.5 m turfs arranged perpendicularly to the hillslope (Fig 1). We focused on grassland (grass/forb) vegetation and excluded shrubs when selecting turfs. Treatments were assigned randomly within each block and included: two ‘untouched controls’ (UT), a disturbance control that was locally transplanted (LT), and two warmed or cooled transplants, relocated either one or two sites higher or lower (W1, W2, C1, C2). Each transplanted turf was moved intact and contained the entire plant and soil community and an average depth of 23 cm (SD = 4.8 cm) of intact soil. Immediately after the transplant, turfs were watered from local streams and covered by a shade-cloth canopy for one month. Fences were built around the sites to exclude cattle grazing. In anticipation of natural disturbance from gophers, a sixth block of transplanted turfs (5 at the High site, 4 at the Mid site, and 6 at the Low site) was added in 2018, bringing the total to 90 turfs.

Nets were installed around each turf (1 m x 1 m) in the growing season months in order to prevent gene flow from the transplanted plants to the surrounding plant communities. One untouched control was left un-netted in each block to test for the effect of nets, which none was found (Table S1), as was the case in similar experiments (Guittar et al. 2016; Henn et al. 2018; Vandvik et al. 2020a). Throughout the paper, turfs are referenced by origin site and treatment (e.g., “High W2” refers to a turf warmed two sites down from the High site).

The goal of this experiment was to expose entire ecosystems to the net effect of a changing climate. This included both the abiotic changes expected for this ecosystem (increased temperatures, longer growing seasons, and reduced water availability at lower elevations), and biotic changes, since whole plant and soil communities were transplanted into new, destination communities (Vandvik et al. 2020; Yang et al. 2018). We refer to turfs transplanted downslope as “warmed” and those transplanted upslope as “cooled” to represent, in simplified terms, the complex environmental changes associated with warming and cooling. The reciprocal design also allows us to test whether ecological responses are symmetric or asymmetric depending on the direction and magnitude of environmental change— a key question for forecasting ecological change under directional climate forcing.

Sampling - Plant community

In 2017, baseline plant species abundance was recorded by counting individuals in one turf (out of five) per block at each site during peak plant biomass. From 2018-2023 (excluding

2020), species percent cover data was estimated during peak summer biomass on all turfs using a 50 x 50 cm frame with 10 x 10 cm subplots to the nearest 1%. Abundance and percent cover data were scaled by the largest count or cover value in each turf to create a value of proportional cover between 0-1 for analyses. In 2019, dominant plant species percent cover was also recorded weekly from early season to peak biomass using the same gridded frame. In each subplot, the dominant species or ground cover type (i.e., soil, litter) was recorded, and percent cover was calculated based on presence in the 25 subgrids.

The plant communities at all sites are dominated by perennial forbs and graminoids, many clonal. The growing season is short with snow cover from October through May. From 2017-2023, there were a total of 72 species in the control turfs (50 at the Low site, 38 at the Mid site, and 40 at the High site). On average, each turf contained 12.5 species (sd = 3.1). In the controls, Low and Mid site turfs shared 31 species out of 57 (54%), Mid and High site turfs shared 26 species out of 52 (50%), and Low and High site turfs shared 24 species out of 66 (36%).

Sampling - Soil microbial community

Soil sampling was conducted in three control turfs and three transplanted turfs at each site. A total of 81 soil cores (diameter = 2 cm, depth = 5 cm) were collected at three seasonal time points: late in the growing season in 2018, immediately after spring snowmelt in 2019, and at peak summer plant biomass in 2019 (n = 9 samples per site and time point).

Total soil DNA was extracted from all soil samples in duplicate using the Qiagen PowerSoil DNA extraction kit. Replicate extractions were pooled prior to quantifying the yield of the DNA extract and two methods verified the purity of the extracted DNA. First, a small aliquot of extracted DNA was run on an electrophoresis gel to check for excessive, low-molecular weight DNA (i.e., DNA shearing). Second, purity was verified by measuring the absorbance ratio at 230, 260, and 280 nm on a UV-spectrophotometer. The concentration of the pooled-DNA extractions was quantified using a QUBIT fluorometer. During this process, 10 samples were mislabeled and treatments could not be identified; thus, 71 samples remained.

In order to assess responses of microbial taxa to the experiment, PCR amplification of the bacterial/archaeal 16S Small subunit ribosomal RNA (16S rRNA, V-4 region) and fungal internal transcribed spacer region 1-2 (ITS1-2) biomarkers was used following previously described PCR

methods (Sorensen et al. 2020). The PCR amplicons were pooled and sequenced using MiSeq V3 300-bp paired-end sequencing. Microbial sequences were given taxonomic assignments by comparing sequence identity to sequence databases for bacteria, archaea, and fungi.

The data were then quality-filtered to include only amplicon sequence variants (ASVs) with at least 30 read counts and in at least three samples from the same origin site. This resulted in 1663 unique bacterial/archaeal ASVs (1305 in Low site controls, 1313 in Mid site controls, and 1248 in High site controls), and 693 unique fungal ASVs (527 in Low site controls, 432 in Mid site controls, and 463 in High site controls) for the analysis. There were an average of 567 bacterial/archaeal ASVs per sample ($sd = 201$), and an average of 217 fungal ASVs per sample ($sd = 64$). In the controls, for bacteria/archaea, the Low and Mid sites shared 1065 ASVs out of 1553 (69%), Mid and High sites shared 1087 ASVs out of 1474 (74%), and Low and High site turfs shared 996 ASVs out of 1557 (64%). In the controls, for fungi, the Low and Mid sites shared 341 ASVs out of 618 (55%), Mid and High sites shared 335 ASVs out of 560 (60%), and Low and High site turfs shared 344 ASVs out of 646 (53%).

Total copy number of bacterial and archaeal 16S rRNA genes were quantified to measure microbial abundance and to correct ASV relative abundance to ASV absolute abundance in each sample. Quantitative PCR was performed following methods that have been described previously (Wang et al. 2021). Briefly, 20 μ L qPCR reactions contained 10 μ L SYBR Green Supermix (Bio-Rad, Hercules, CA, United States), 1 μ L of forward and reverse primer (338f-806r; described in Mori et al. 2014) and were amplified using the Bio-Rad iCycler single-color real-time detection system (Bio-Rad, Hercules, CA, United States) using the following thermocycler settings: 95 °C for 3 min, 35 cycles of 95 °C for 30 s, 52 °C for 40 s, and 72°C for 90 s with an extension of 10 min at 72 °C. Standard curves were generated using linearized plasmids with a target gene inserted using a tenfold dilution series ranging from 10⁻¹ to 10⁻⁷ of the transformed plasmid that contained the gene target. The absolute abundance of all bacterial and archaeal taxa was calculated from the relative abundance of each ASV multiplied by the total absolute abundance in the sample (from qPCR), then divided by the 16S copy number. 16S copy numbers were obtained from the ribosomal RNA operon copy number database (rrnDB version 5.7, Stoddard et al. 2015). When no copy number was available, the uncorrected absolute abundance was calculated.

Statistical analysis

In order to examine patterns in community response to treatments in comparison to controls over time, and to test all hypotheses, Principal Response Curve analysis (PRC), a type of Redundancy Analysis (RDA), was conducted (Van den Brink & Braak 1999). Turfs transplanted in 2018 were excluded. ASV abundance and scaled cover data were used. The plant and microbial data were filtered to the treatment comparisons of interest and the *prc* function in the *vegan* R package was run. To test for PRC significance, ANOVA was run on the PRC results for each treatment pair using the *anova.cca* function. From this, we tested Hypotheses 1 and 2 to determine if transplanted turfs became different from the origin controls (transplant vs origin $p < 0.05$), converged with the destination controls (transplant vs destination $p > 0.05$), or if there was change in the direction of the destination but no convergence (transplants vs origin and transplant vs destination $p > 0.05$). If the p values were both significant, this could mean change in the direction of the destination site or divergence from both the origin or the destination, and ordination plots were used to verify direction. To test Hypothesis 3 and 4, we examined PRC treatment weights between groups and treatments.

To test Hypothesis 5, we examined PRC species/taxa weights, which are used to build the principal response curves. A high positive weight indicates that the species/taxa is more likely to follow the overall pattern of the curve, whereas a high negative weight indicates that the species/taxa is more likely to follow the opposite pattern (Van den Brink & Braak 1999). Weights near zero indicate that the species/taxa either did not respond or have a response unrelated to the principal curve. In the PRCs containing transplants and both origin and destination controls, the PRC axis and species weights are defined by shifts in species across the natural gradient. Thus, separate PRCs were run with the transplants and the origin control only, and a Pearson's correlation coefficient was calculated for 1) species weights vs. those defined by the natural gradient to test if gradient patterns were re-established following transplantation, and 2) with each other to test if species responded similarly to warming and cooling.

In addition, for bacteria/archaea and fungi, the taxa with the greatest change in abundance with warming and cooling relative to the origin control were determined with differential abundance analysis using the *DESeq2* R package (Love et al. 2014). The analysis tested for the effect of warming/cooling on abundance in comparison to the origin control, controlling for the effect of seasonal time point. It calculated the logarithmic fold change for each taxa, which is a

ratio between the abundance in the contrast condition (i.e., warmed or cooled turf) and the abundance in the reference condition (i.e., origin control turf). A negative logarithmic fold change indicates that the taxa decreased in abundance in the warmed or cooled turf relative to its origin control, whereas a positive logarithmic fold change indicates an increase in abundance. The differential abundance analysis performed a Wald test, correcting p values with the Benjamini-Hochberg method.

Results

Community response

With transplantation to warmer sites, we found evidence in all groups consistent with Hypotheses 1 and 2 that community composition shifted, and shifts were toward the destination site. Plants composition differed in the controls at each site across the elevation gradient (Table 2, $p = 0.001$ for each control pair), setting a baseline expectation for directional change toward the destination site. With warming, plant composition became different from both the origin and destination controls beginning 1-2 years post-transplant. At six years post-transplant, this shift toward the destination site was maintained, but communities never converged with the destination (Fig 2). Bacterial/archaeal composition differed between the controls at the Low site in comparison to both the High and Mid site (Table 2, $p < 0.05$ for each control pair), but not between the High and Mid sites ($p > 0.05$). With warming, at early and peak season time points, bacterial/archaeal composition of High W2 turfs converged with the destination, and the Mid W1 turfs shifted towards the destination (Table 2). High W1 turfs did not differ from the origin or destination, as expected based on the lack of change in the controls. Fungal composition differed in the controls at all sites across the elevation gradient (Table 2, $p < 0.05$ for each control pair). With warming, fungal composition did not differ from that of the origin or destination sites, and there were shifts in the direction of the destination (Table 2).

With transplantation to cooler sites, evidence was less consistent with Hypotheses 1 and 2, and there were fewer compositional shifts overall and toward the destination. Plant composition remained similar to the origin in the Low C2 and Low C1 turfs, but became different from both the origin and destination in the Mid C1 turfs (Table 2). Bacterial/archaeal composition remained similar to the origin in the Low C2 turfs, but shifted towards the destination at early and peak growing season time points in the Low C1 turfs (Table 2). Mid C1

turfs did not differ from the origin or destination, which was as expected based on the lack of change in the controls. Fungal composition remained similar to the origin in turfs transplanted from the Low site (Low C2, Low C1), but shifted toward the destination in the Mid C1 turfs (Table 2). There were no consistent seasonal patterns in fungal composition shifts between treatments.

Mean PRC treatment weights indicate that community responses over time aligned with Hypothesis 3, with stronger responses in bacteria/archaea and fungi than plants (Table 3). The mean absolute abundance of bacteria/archaea also increased to destination site levels (Fig S3). Treatment weights were in line with Hypothesis 4, and communities overall had greater changes in composition with warming than cooling (Table 3). There were a greater number of plant and microbial taxa that responded strongly to warming than cooling (Table 4).

Species-level response

The evidence was consistent with Hypothesis 5, that the species/taxa with the greatest change in abundance in response to treatment were those that also changed the most across the gradient. The plant and microbial species that were expected to have the greatest increase in abundance relative to the origin were either more common or only present at the destination sites (Table 4). Likewise, species that were expected to have the greatest decrease in cover relative to the origin were either more common or only present at the origin site.

The correlations between PRC species weights of warmed/cooled and gradient communities also indicate that the plant, fungal, and bacterial/archaeal species that respond strongest to temperature change along the gradient also respond strongest to experimental warming and cooling (Warmed vs gradient: Plants: Pearson's $r(63) = 0.75$, Fungi: $r(657) = 0.87$, Bacteria/Archaea: $r(1606) = 0.91$. Cooled vs gradient: Plants: Pearson's $r(56) = 0.77$, Fungi: $r(672) = 0.83$, Bacteria/Archaea: $r(1595) = -0.31$ (moderate correlation). All p values < 0.001).

There was no correlation between the PRC species weights of warmed and cooled plants, indicating that species sensitive to warming are different from those sensitive to cooling. But there were moderate to strong correlations for soil microbes, indicating that some microbial species are disproportionately more temperature sensitive than others (Warmed vs Cooled: Plants: Pearson's $r(52) = 0.22$, $p = 0.11$, Fungi: $r(652) = 0.51$, $p < 0.001$, Bacteria/Archaea: $r(1558) = -0.40$, $p < 0.001$).

Out of the 78 bacterial/archaeal taxa (ASVs) with a significant log₂ fold change in abundance with warming (52 enriched, 19 depleted, and 7 either enriched or depleted depending on treatment), a total of 65 (83%) also changed naturally from high to low elevations (Fig 4A). Out of the 67 ASVs that had a significant log₂ fold change in abundance with cooling (26 enriched, 33 depleted, and 8 either enriched or depleted depending on treatment), a total of 48 (71%) also changed naturally from low to high elevations (Fig 4A). Out of the 173 fungal taxa (ASVs) with a significant log₂ fold change in abundance with warming (117 enriched, 40 depleted, and 16 either enriched or depleted depending on treatment), 148 (85%) also changed naturally from high to low elevations (Fig 4B). Out of the 146 fungal taxa with a significant log₂ fold change in abundance with cooling (94 enriched, 42 depleted, and 10 either enriched or depleted depending on treatment), 123(84%) also changed naturally from low to high elevations (Fig 4B).

Discussion

In order to understand how plants, bacteria/archaea, and fungi simultaneously respond to shared environmental change, we compared the compositional changes of transplanted communities. Consistent with Hypothesis 1 and 2, transplantation to warmer sites led to rapid, directional shifts in all communities, with compositions becoming more similar to those at warmer, lower elevation sites. These shifts were likely driven by both direct climate effects and indirect influences from altered species interactions and demography (i.e., immigration, growth, reproduction, and/or mortality) (Blois et al., 2013; Pickett, 1989). Directional compositional shifts in response to warming are likely to reflect a broader trend where high elevation species, adapted to cooler and wetter conditions, are outcompeted by upward dispersing lower elevation species, more suited to the warmer and drier environments. The 2018 drought also showed community vulnerability to extreme climate events, which are becoming more intense and frequent (Cinto Mejía & Wetzl 2023). In contrast to the effects of transplantation to warmer sites, transplantation to cooler sites led to weaker and more variable responses, with community composition remaining more like the origin site (see discussion of Hypothesis 4 below).

In line with Hypothesis 3, the magnitude of the compositional shifts varied by community, with bacteria/archaea showing the strongest shifts, followed by fungi, then plants. Bacterial/archaeal communities transplanted to warmer sites converged with destination

communities within two years, while plant and fungal communities did not fully converge during the duration of the experiment. This may lead to the formation of novel communities, potentially disrupting the feedbacks between plants and microbes that regulate ecosystem functioning (Schlatter et al. 2015; Classen et al. 2015; Broadbent et al. 2021; Hagedorn et al. 2019).

Microbial communities may respond more quickly than plants due to short generation times, high dispersal, and sensitivity to resource availability (Classen et al. 2015; Liang et al. 2015; Waring et al. 2013). Responses may involve immigration from surrounding destination soils, shifts in resident taxa, or the resuscitation of dormant microbes (Hawkes & Keitt 2015). Fungi, which generally grow and turn over more slowly, may be more resistant to change (Waring et al. 2013; deVries & Griffiths 2018). Increased nutrient inputs from more productive plants under warming could also accelerate microbial changes (Appendix C, DeAngelis et al. 2015). However, over longer timescales, microbial responses may be shaped by slower-changing conditions (e.g., organic matter accumulation or substrate depletion) (Zhang et al. 2017; Walker et al. 2018).

Plant communities changed more slowly, likely due to long generation times, perennial life histories, and the ability of some species to persist via plasticity (Alexander et al. 2018; Dullinger et al. 2012). Major compositional turnover is likely to take much longer than the duration of the experiment (Sayer et al. 2017). Indeed, there was limited immigration from ingrowth of the more warm-adapted clonal species at the edges of the turfs and limited mortality (Appendix C; Berauer et al. 2019; Debouk et al. 2015). The slower plant and fungal responses suggest that the communities may have been more dependent on each other to drive change (Kilvin et al. 2013; Sayer et al. 2016). These patterns point to a potential extinction lag in high elevation species, where some persist temporarily under unfavorable conditions (Bektaş et al. 2024; Hagedorn et al. 2019; Alexander et al. 2018).

As predicted in Hypothesis 4, responses to cooling were consistently weaker than to warming. Plant community composition was 3 years slower to change with cooling than with warming, and soil bacteria/archaea and fungi composition overall remained like that of the origin site. This stability under cooling suggests that low elevation taxa are more resistant to environmental change, with the potential to persist under the abiotic and biotic conditions at the high elevation sites.