



Experimental warming decouples plant–fungal symbiont interactions and leads to a more conservative ecosystem

Lara Souza^{a,b,1} , Aimée T. Classen^{b,c} , Jennifer A. Rudgers^{b,d} , Cassandra M. L. Miller^d , Jessica A. M. Pyle^e, Ruth Simberloff^f, James A. Fordyce^f , John Harte^{b,g} , and Stephanie N. Kivlin^{b,f}

Affiliations are included on p. 7.

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Climate forecasts project rising temperatures for every land surface on Earth. Predicting the consequences of climate warming poses a challenge for science and society. Shifts in plant–microbial interactions can lead to significant changes in ecosystem-level functioning. In one of the world's longest climate change experiments, we studied the effects of 29 y of warming on a native subalpine grassland in the Rocky Mountains of North America. Warming caused a transition from herbaceous to woody-dominated vegetation, with shrubs increasing by 150% in warmed plots and a concurrent ~28% decrease in both forbs and grasses. This shift was accompanied by functional traits changing by 20% toward more conservative values relative to acquisitive ones. Consequently, plant–symbiont associations decreased with root-associated fungi—arbuscular mycorrhizal fungal and septate root colonization—both declining by 17 to 20% under warming. In turn, soil saprotrophic fungi increased by 10% with warming. Strikingly, warming decoupled above- and belowground communities and their functions. This decoupling was evident as plant–available phosphorus increased and both individual plants and plant communities reduced their reliance on mycorrhizal fungi, while plant connectedness to decomposer fungi tightened. Our results suggest that, as the planet warms, subalpine grasslands will become more conservative and desert shrubland-like in both above- and belowground properties.

warming | plant | fungal | coupling | montane meadow

Plant and soil communities are closely entwined. Plant–microbial mutualisms significantly contribute to global nutrient transfer and soil carbon sequestration (1, 2). Additionally, soil microbes degrade plant root and litter inputs to soils releasing nutrients that facilitate plant growth, directly linking carbon and nutrient exchanges (3). Competing hypotheses predict that climate warming will strengthen plant–microbial interactions due to heightened organismal physiological stress (4) or cause independent changes in soils and plants that decouple their roles in ecosystems and the functions they provide (5). However, it remains unclear whether warming strengthens or weakens plant–soil coupling and interactions over decadal timescales.

Mountain ecosystems are experiencing climate warming at a faster rate than other ecosystems, making them an ideal place to study how plants and soils will respond to future climates (6). Mountain ecosystems also provide essential services, including clean water and forage for grazers, to over 40% of the global population (7). Further, these heterogeneous mountain landscapes harbor abundant biodiversity (8) including diverse plants (9) and soil assemblages (10), creating valuable systems for investigating how plant and microbial communities may respond to warming.

In general, air temperature increases with decreasing elevation in mountains. Along such elevational temperature gradients, soil microbial biodiversity typically peaks at mid-elevation (11)—a “goldilocks effect” of not too hot or too cold—whereas plant diversity increases monotonically from mountain peaks to low elevation valleys. Thus, research on elevational patterns in mountain ecosystems suggests that plants and soils may become decoupled under climate change (*sensu* refs. 12 and 13). Yet, using such space-for-time substitutions for climate prediction can be flawed (14). Sustained, long-term warming experiments uniquely reveal how warming will disrupt above- and belowground community interactions and the ecosystem functions they support. While many experiments have examined how mountains and high latitude ecosystems respond to warming (15), few have extended beyond 10 y of active experimental warming (16, 17), fewer still beyond 20 y (but see ref. 18), and very few have assessed the coupled responses of plant and soil communities. Long-term studies are critical as the warming responses of

Significance

The decoupling of plant–microbial interactions resulting from warming-induced shifts in plant–microbial interactions can lead to significant changes in ecosystem-level structure and associated functioning. The reassortment of plants and associated fungi and alteration in above- and belowground strategies from acquisitive toward conservative resource uptake under warming scenarios can have strong implications for producer productivity and nutrient cycling in shrub-dominated meadows. Taken together, the shrubification of herbaceous will potentially alter key services provided by meadow ecosystems including forage for wildlife.

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¹To whom correspondence may be addressed. Email: lara.souza@ou.edu.

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communities and ecosystems can vary with the length of the study (18, 19), where responses that occur early during initial community reorganization may disappear or change over time (18).

In one of the world's longest-running active climate change experiments, ten experimental plots (30-m²) were established in a montane Rocky Mountain subalpine meadow grassland (hereafter, grassland) near Crested Butte, Colorado, United States of America (38.53°N, 107.02°W; elevation 2,920 m) in 1991. Overhead infrared radiators actively warmed five plots with a constant increment of 22 W/m² downward heat flux from 1991 to 2019 (20–22). Year-round warming advanced snowmelt by 10 to 14 d and warmed soils by ~2 °C (20). The site represents more than 12,000 km² of the subalpine meadow grassland biome in the western United States, supporting a diverse mixture of growth forms including 38 forb, graminoid, and shrub species, as well as several soil- and plant-associated fungal guilds (5, 11, 23). Nearby warmer, low-elevation biomes consist of semiarid dry shrublands that extend over ~110,000 km² of the western United States (24).

Here, we sampled plants and soil microbial communities under both ambient and warmed conditions to test whether aboveground transitions in plant community structure and function were linked with belowground changes in soil microbial communities. Previous research at the experimental site involved minimal sampling of plant and soil materials to facilitate long-term study. In July 2019, we harvested the “Warming Meadow” experiment, which was the longest running (29 y) in situ active field warming manipulation.

First, we measured plant species-specific cover and identified nine focal plant species that comprised at least 75% of the total aboveground cover across the experimental plots (SI Appendix, Table S1). We predicted that shrub biomass would increase and that plant traits would become more conservative with warming. We measured aboveground stem and leaf cover, aboveground plant biomass, and traits for three randomly selected individuals of each focal species within each plot. Next, we collected plant-specific rhizosphere soil cores (2.5 × 15 cm) centered over each focal plant stem to measure plant-specific root traits, root fungal communities, soil microbial biomass, soil edaphic properties, soil bacterial and fungal community composition, and soil functions (e.g., enzyme activity). Once soils were collected, we harvested the rest of the roots of each focal plant to estimate root biomass for each individual. In addition, we collected root and bulk soil samples in 10 cm increments to a soil depth of 1 m.

By excavating belowground structures and communities, we tested whether aboveground responses to warming were concordant with the belowground microbial responses. Here, above- and belowground components of ecosystems are considered *coupled* when they are coordinated with one another (e.g., positively correlated) (25–27) and *decoupled* when a perturbation (here, warming) systematically disrupts this coordinated response. Thus, as plant and fungal communities turnover with warming, we hypothesized that warming would strengthen coupling between plant and microbial diversity, including both alpha and beta diversity.

Because AM fungi can increase the amount and variety of nutrients necessary for plant growth, increases in AM fungal diversity are often positively coupled with plant diversity in observational studies (28, 29). In a manipulative study, Kivlin and Hawkes (30) found that increasing plant diversity led to higher diversity of both AM fungal and saprotrophic fungi belowground, experimentally confirming observational patterns. Thus, we predicted that warming-induced stress would strengthen the association between plants and mycorrhizal fungi relative to ambient conditions.

We further predicted that, under warming, saprotrophic fungi would become more tightly linked to plant communities as low-litter

quality shrubs come to dominate the vegetation. Because plant diversity affects the magnitude and chemical diversity of carbon compounds entering the soil, plant diversity is often positively coupled with belowground decomposer diversity (31). However, if plant and fungal turnover is inconsistent across plots, we would instead predict decoupling of above- and belowground communities.

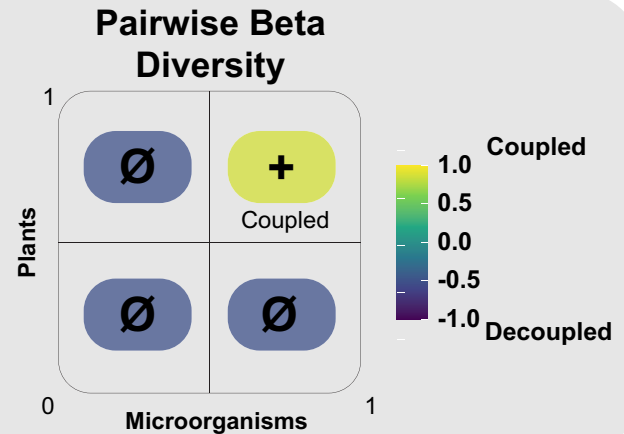
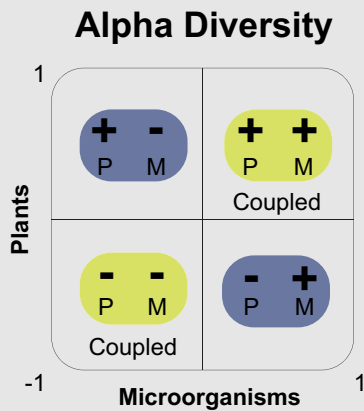
If mutualisms become more costly under warming, we expect this effect to be strongest for AM fungi, and for decomposer fungi if soil nutrients, carbon availability, and plant quality decline. For example, if plant and microbial alpha diversity remain coupled under both ambient and warmed conditions, increases or decreases in alpha diversity in one group should be mirrored by the other (Fig. 1). Similarly, coupled plant–microbial systems should exhibit concordant pairwise beta-diversity (i.e., turnover) patterns across plots under both ambient and warming conditions. Our experiment explicitly enables us to examine plant–microbial interactions across warming treatments without confounding shared responses to a third factor (e.g., soil moisture, other trophic levels).

Plant Cover and Traits. After 29 y of experimental manipulation, warming increased plant aboveground biomass by 64% (Fig. 2A and SI Appendix, Tables S2 and S3). As predicted, warming also shifted plant composition from a forb- and graminoid-dominated ecosystem to an *Artemisia* shrub-dominated ecosystem. Shrubs increased 150% in warmed vs. ambient plots (Fig. 3A and SI Appendix, Fig. S2 and Table S3). This pattern reflects an acceleration of the warming responses observed earlier in this experiment. After 4 y of experimental warming, the aboveground biomass and growth rate of sagebrush were higher in warmed plots (22, 32, 33), while the cover of herbaceous species declined (21). In the current study, aboveground plant biomass increased with warming (Fig. 2A), while belowground plant biomass did not respond (Fig. 2B). Thus, plant resource allocation shifted toward shoot over root investment (Fig. 2C and SI Appendix, Tables S2 and S3). Warming also did not alter rooting biomass at any of the depths measured (SI Appendix, Fig. S2 and Table S3). Together, these biomass and allocation patterns indicate that aboveground plant processes were more consistently sensitive to warming than those belowground, as observed in Edwards et al. (34).

Increases in shrubs under experimental warming also occur in tundra ecosystems (35). Biomass–temperature relationships of tundra vegetation can differ between above- and belowground components, with aboveground biomass increasing with warming but not belowground biomass (36, 37). Changes in above- vs. belowground plant biomass could occur as plants shift allocation to leaves vs. roots, or as communities change in composition with warming. Here, shifts in plant composition from *Festuca thurberi* to *Artemisia tridentata* determined patterns in shoot-to-root allocation under warming, but allocation remained fixed within plant species. Plants rarely use a single allometric strategy; instead, they adjust their biomass allocation under different environmental conditions (38, 39). However, *Artemisia* species can adapt to environmental variation through a variety of physiological traits, including carbon assimilation and evaporative demand, thus adhering to a relatively fixed allometric strategy (40–42). This reliance on physiological rather than anatomical responses to warming may facilitate *Artemisia*'s positive response to warming in this study.

Warming temperatures that extend the growing season (43) can either increase the window for plant growth (35) or reduce plant growth by drying soils (44); however, we did not detect changes in soil moisture under warming in this study (SI Appendix, Table S3). While typically unmeasured, belowground shifts in plant roots under warming can be large (35). We expected the observed shifts in herbaceous to woody plant functional types (32, 45) to increase

Theoretical Patterns



Observed Patterns

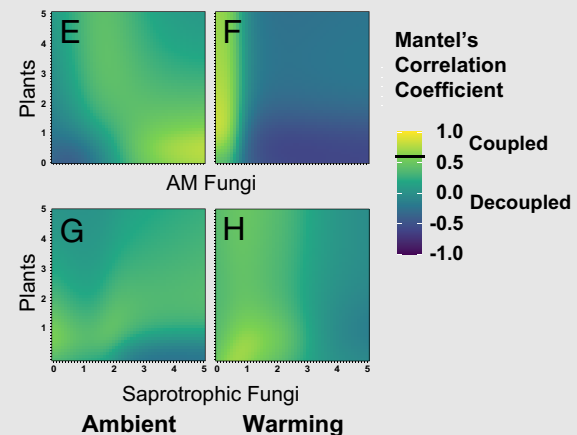
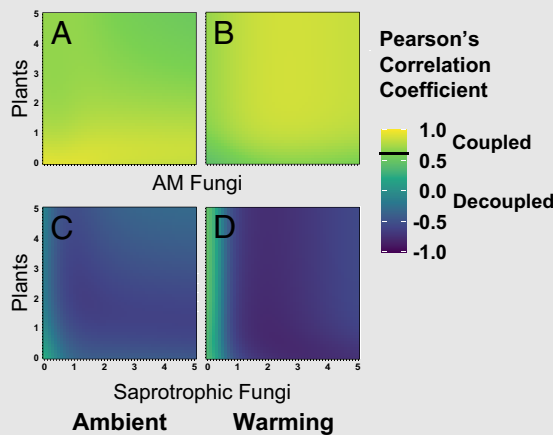


Fig. 1. (Top) Theoretical patterns of how coupling of plant and microbial alpha and beta diversity can shift following warming. Here, we define coupling as direct responses of plants and microorganisms to each other, regardless of their shared responses to the environment. Plant and microbial alpha diversity can either increase or decrease following disturbance. If both plant and microbial alpha diversity increase or decrease, plant-microbial alpha diversity remains coupled. However, if either plant or microbial alpha diversity increases while the other decreases, plant-microbial alpha diversity will be decoupled. Plant and microbial beta diversity can also be coupled if both communities experience large shifts in composition following disturbance. However, if plant communities have large shifts in composition while microbial communities do not, plant-microbial beta diversity would be decoupled. Similarly, decoupling of plant-microbial communities could also occur if microbial composition shifts whereas plant composition does not. (Bottom) (A–D). Plant and fungal (AM fungal and saprotroph) alpha diversity correlations at all levels of plant and fungal Hill numbers, here described as q , indicate contrasting relationships. Plant and AM fungal alpha diversities were consistently positively correlated under ambient and warmed conditions. Plant and saprotrophic fungal alpha diversities were consistently negatively correlated with the exception that at low levels of saprotrophic fungal q under warming, plants and saprotrophic fungal alpha diversity were strongly positively correlated (E–H). Plant and fungal (AM fungi and saprotroph) composition associations across levels of q for each group indicate cases for both decoupling and coupling under warming. Plant turnover positively correlated with AM fungal turnover under ambient temperature (Mantel tests) with the association decoupling at higher levels of AM fungal q with warming; yet the associations of plant turnover and saprotrophic fungal turnover were relatively consistently positive under ambient and warmed conditions.

coarser and shallower roots in warmed vs. ambient plots. Contrary to expectations, we did not detect shifts in the coarse-to-fine root ratio or rooting depth (SI Appendix, Figs. S2 and S3 and Table S3) under warming. We did observe that several root traits, including area and length, declined under warming at both intermediate and deeper soil depths, but not at shallower surface depths (SI Appendix, Fig. S3). As seen in other warming experiments (35), we found that plant height increased with warming (SI Appendix, Fig. S1 and Table S3). Plant communities also had more resource-conservative aboveground traits, with 22% lower specific leaf area and 17% greater leaf dry matter content (LDMC; Fig. 4A and B) than ambient plots. Shifts in plant community traits, as measured by community-weighted means (CWM), reflected increases in plant abundance within species rather than a shift in individual trait values. For CWM of LDMC, both increases in species relative abundance and increases in trait values under warming resulted in more conservative traits; whereas, CWM of SLA reflected increases

in species relative abundance, with no significant changes in trait values with warming (SI Appendix, Fig. S4). These shifts were similar in direction and magnitude to other studies in alpine and arctic ecosystems documenting increases in conservative traits under warmer, and often drier, conditions (46).

Plant and Microbial Coupling. Shifts in plant abundance were mirrored by changes in fungal abundance, diversity, and compositional dissimilarity. Changes in plant functional types and traits should affect plant-associated microbes, but these interactions are often unmeasured in global change studies. Surprisingly, and contrary to our prediction, warming weakened the coupling between plant and arbuscular mycorrhizal (AM) fungal abundance and composition, while strengthening coupling between plants and saprotrophic fungi (Fig. 3). Specifically, warming decreased the abundance of forbs and graminoids (Fig. 3B and C), which was associated with a 48% reduction in symbiotic AM fungi in

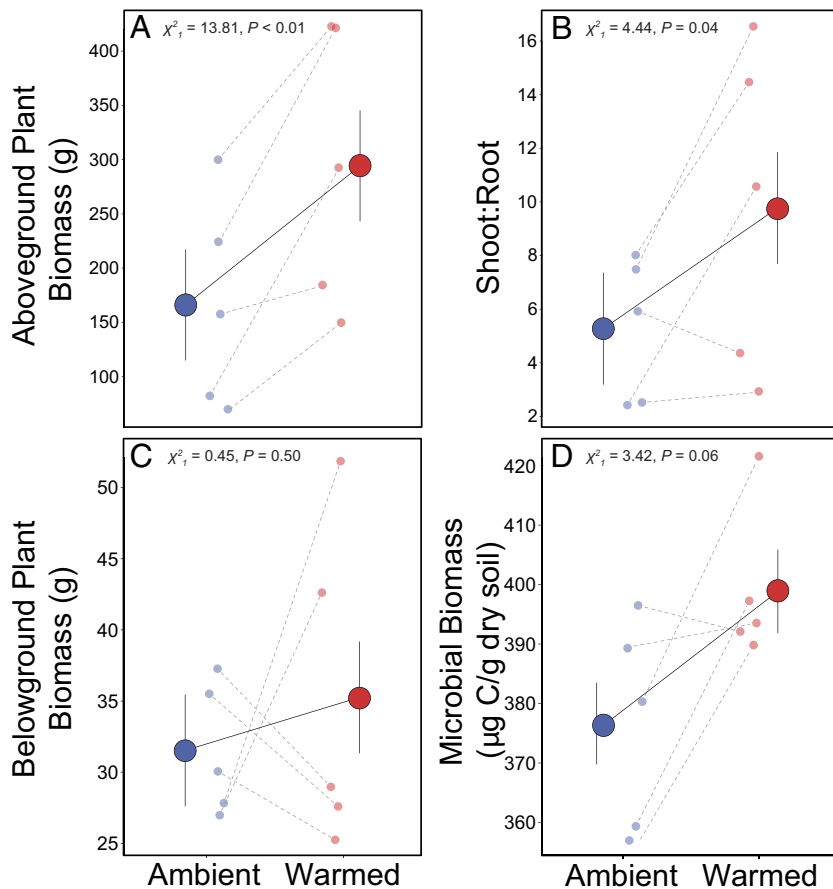


Fig. 2. Warming increased the community weighted means of aboveground biomass (A), allocations of shoot vs. roots (B), but not the community weighted mean of belowground biomass (C). Warming increased microbial biomass (D). Community weighted means of other plant root traits (e.g., coarse: fine root ratio) did not vary with warming (SI Appendix, Fig. S1). Means and SE of warmed and ambient plots are presented in large circles and bars whereas individual plots are presented in smaller, lighter circles. Dashed lines connect plots within the same block.

soils measured as relative abundance of AM fungal sequence reads at the plot level (Fig. 3 D and F–H). Furthermore, microscopically counted AM fungal colonization of individual plants was 17% lower in warmed plots compared to ambient plots (Fig. 4C). Despite the use of dissimilar methods, these trends are congruent because microscopic counts and sequence read abundances of AM fungi were significantly positively correlated in our study (Pearson's $r = 0.44$, $P < 0.05$). Warming can also directly affect plant-associated fungal communities; however, soils may buffer root-associated fungi, limiting this effect (34).

Saprotrophic fungal root colonization was lower in warmed plots (Fig. 4D), yet saprotrophic fungal abundance (Fig. 3E) and microbial biomass (Fig. 2D) increased in bulk soil with warming. This pattern suggests that plant–microbial coupling may be more disrupted for fungal symbionts than other microbial guilds, particularly in soils. These microbial responses may be linked to the 35% increase in soil phosphate availability observed in warmed plots (Fig. 4E), since AM fungi are often more abundant when soil nutrients are limited (47).

Given declines in AM fungal abundance in soils and in plant roots with warming (Fig. 2), it is notable that plant and AM fungal richness patterns were unaffected. Plant and AM fungal alpha diversity remained consistently coupled under both ambient and warmed conditions (Fig. 1 A and B). In contrast, the low correlation between plant and saprotrophic fungal diversity in ambient plots reversed at low Hill numbers ($q = 0$ to 0.5) of saprotrophic fungi under warmed conditions, resulting in a positive correlation between plant and saprotrophic fungal diversity (Fig. 1 C and D). Plant composition shifted most strongly with warming, followed by fungal composition; however, bacterial composition did not change with warming (SI Appendix, Fig. S5).

As we predicted, plant composition was strongly coupled with dominant AM fungal composition under ambient conditions but decoupled under warmed conditions (Fig. 1 E and F). The positive association between saprotrophic composition and plant composition under ambient conditions strengthened under warmed conditions (Fig. 1 G and H). Overall, the rhizosphere fungal community was highly sensitive to warming, with fungal symbionts decoupling from plants while plant–saprotrophic fungal interactions strengthened. These results mirror a previous root-associated fungal study at this site and two others, where the relative abundance of mutualist fungal taxa declined with warming, while the relative abundance of pathogens and saprotrophs increased (34). Although this single sampling point cannot determine causality [passenger vs. driver hypothesis (48)], the pattern is nonetheless strong.

The decoupling of plant and AM fungal composition, but not richness, under warming potentially represents a key transitional phase between herbaceous and more conservative woody-dominated ecosystems, which are often associated with different symbiotic microorganisms such as dark septate endophytes (49, 50). This decoupling can reduce nutrient transfer from soils to plants, shifting ecosystems to a more conservative nutrient and carbon economy (13). Evidence from our study supports slower nutrient and carbon cycling: Warming increased soil phosphate availability and saprotrophic fungal abundance but reduced bulk soil enzyme activity by 10%, likely due to more recalcitrant shrub-derived substrates (SI Appendix, Table S3). Interestingly, 29 y of warming did not alter soil organic matter (SOM) (Fig. 4F), even at different depths in the soil profile (SI Appendix, Fig. S3). These results suggest that earlier reductions in soil carbon pools after 14 y due to warming (45, 50) were transient in this study system, as observed in other long-term studies (18).

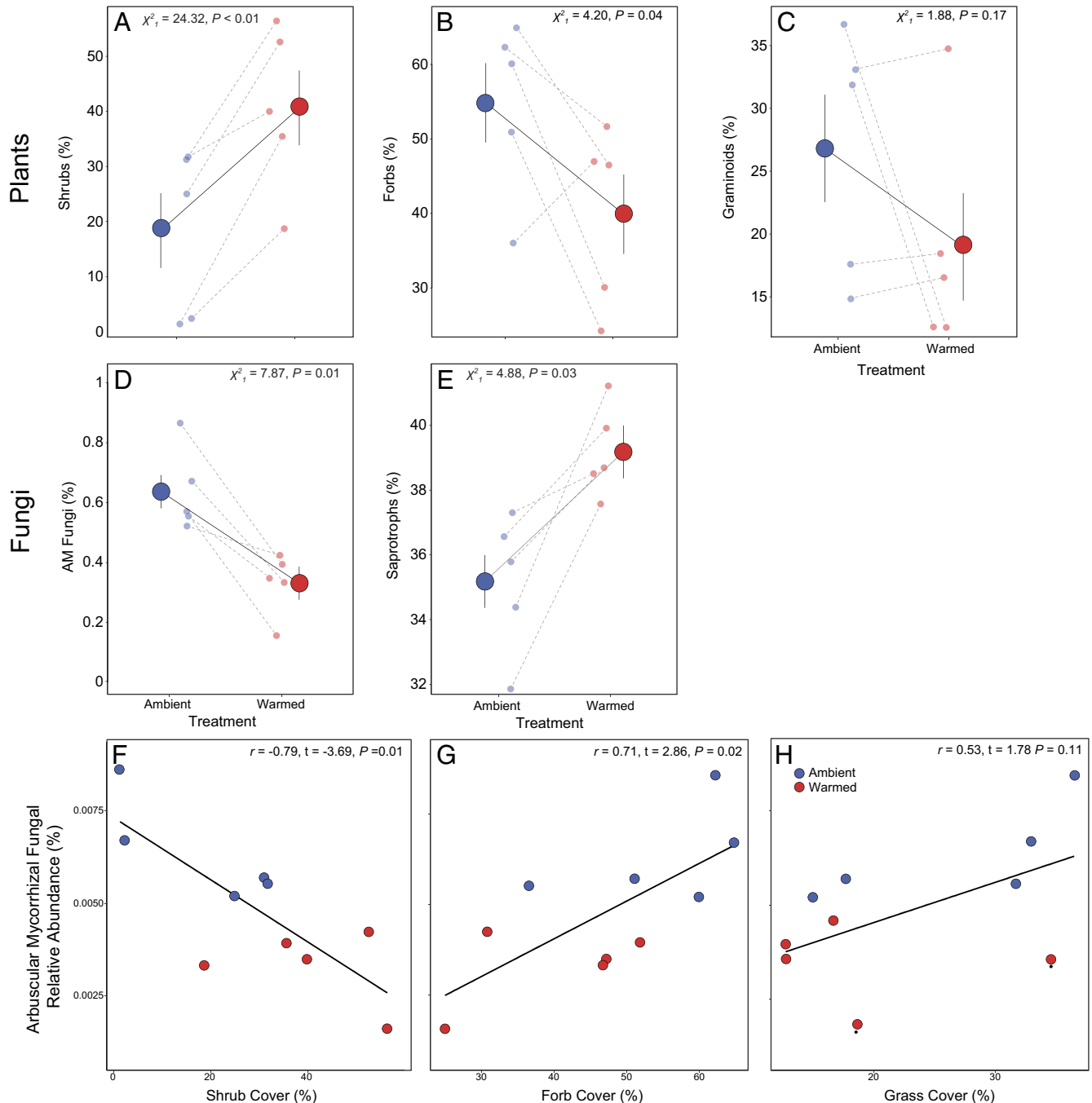


Fig. 3. Plant percent cover (A–C) and fungal relative abundance (D and E) by functional group in warmed vs. ambient plots. Means and SE of warmed and ambient plots are presented in large circles and bars whereas individual plots are presented in smaller, lighter circles. Dashed lines connect plots within the same block. Pearson's correlation of functional groups with AM fungal relative abundance in soil vs. shrub cover (F), forb cover (G), or grass cover (H). Red points are warmed plots and blue points are ambient plots. AM fungal abundance in soils was calculated as the relative proportion of sequence reads, which was correlated with microscopic counts of AM fungal abundance in soil (Pearson's $r = 0.44, P < 0.05$).

Conclusions

Plants at this site have been accumulating biomass for over a century (43) and are often subjected to disturbances such as avalanches and small mammal grazing that leads to significant soil disruption (51). Therefore, we expected this ecosystem to be relatively resistant to perturbations. However, after only 29 y of a 2 °C warming press experiment, we observed an ecosystem-level shift from a forb–graminoid, resource-acquisitive, mycorrhizal-dependent ecosystem to a resource-conservative, shrub-dominated ecosystem associated with saprotrophic fungi. The aboveground compositional changes

observed here mirror regional vegetation transition from alpine grassland to shrubland under recent climate change (32, 52–54). This “shrubification” is ongoing, as summer and fall temperatures at our site have increased over the past 48 y [1975 to 2022; (55)], accompanied by rising aridity. Soil moisture has declined from 18 to 28% in 1991/1992 to around 12% measured in this study (56). Additionally, growing degree days have increased by an average of 11 d per year since 1975 (57). Mountain ecosystems vary in plant composition, nutrient availability, climate, and climate change velocity, so these patterns may not generalize across ecosystems (58). Nonetheless, long-term warming presses can have ecosystem-level

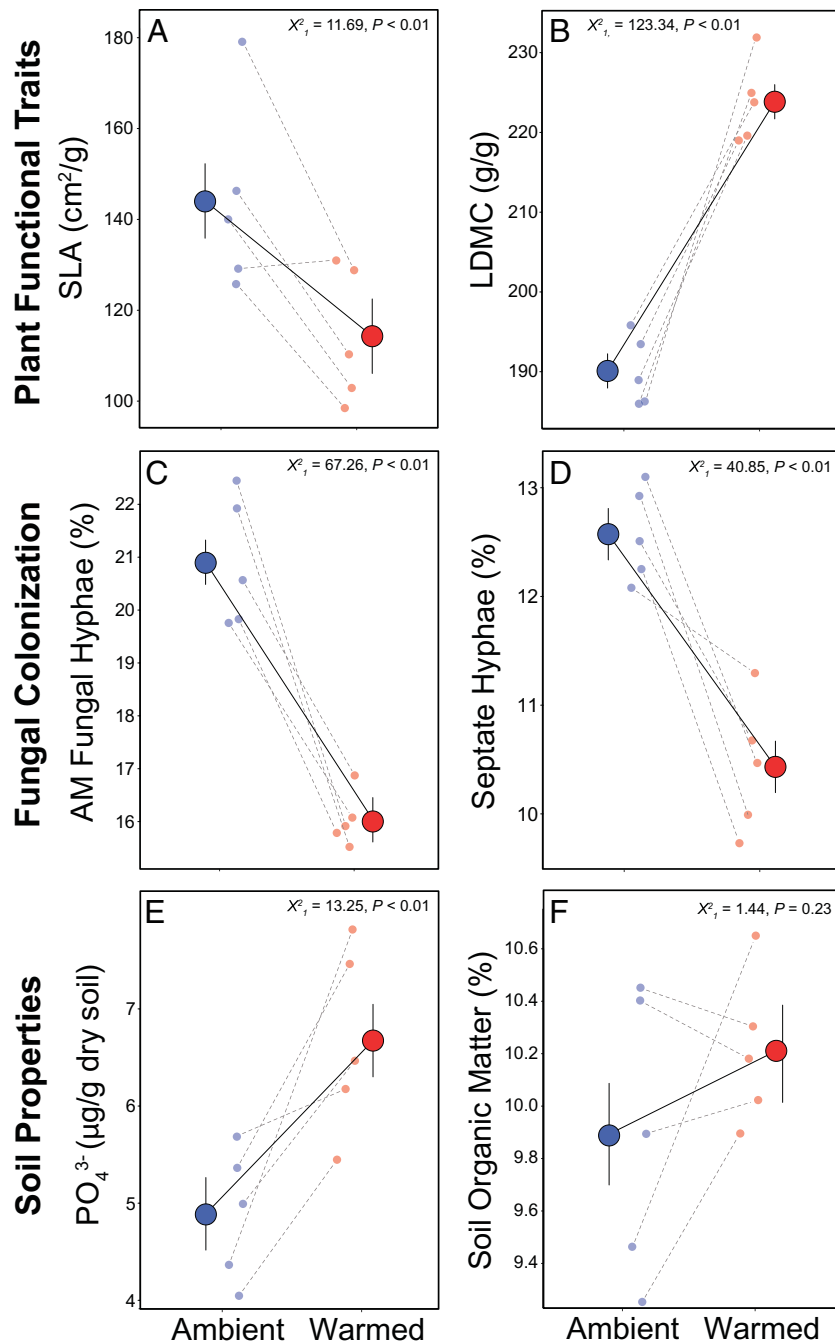


Fig. 4. Plant functional trait community weighted means were more conservative with thicker, denser leaves for specific leaf area (A) and leaf dry matter content (B) in warmed relative to ambient plots. Fungal root colonization was reduced for both AM fungi (C) and other fungal phyla including saprotrophs (septate hyphae) (D) in warmed relative to ambient plots. Soil phosphorus availability increased (E) and carbon sequestration remained constant in warmed relative to ambient plots (F). Plant functional traits were calculated using community weighted means of plants, but fungal root colonization and soil data are raw values. Means and SE of warmed and ambient plots are presented in large circles and bars, whereas individual plots are presented in smaller, lighter circles. Dashed lines connect plots within the same block.

consequences. Within three decades, warming at our site has shifted both above- and belowground community composition and traits, moving this ecosystem from an herbaceous alpine grassland toward a more conservative arid-shrubland. Regardless of ecosystem, shrubification will likely shift nutrient cycling and carbon sequestration with the reassortment of plant-microbial associations.

Methods

Study Site and Experimental Design. This study was conducted at the Rocky Mountain Biological Research Laboratory, near Crested Butte, Colorado, United States of America (38°57' N; 106°59'W; 2,920 m above mean sea level (MSL) in a subalpine grassland (for details see ref. 32). Briefly, ten parallel 3 × 10 m plots were established so that their length spanned the slope of the moraine. This study focuses on the 3 × 3 m upper zone (zone C), at the top of each plot, which is warmer and drier than the lower zone (zone A) at the plot's base. This upper zone typifies the common dry-meadow habitat in the area (32, 33) and thus is the focus of this

study. At the start of the experiment, the sagebrush biomass (*Artemisia tridentata*) and the herbaceous plant community composition were similar across all plots.

Half of the plots ($n = 5$) were continuously warmed from January 1991 until July 2019 using 1.6 m long infrared heaters (Kalglo, Inc. Lehigh, PA, USA) suspended approximately 1.5 m above the treatment plots, alternating in space with the ambient plots. The heaters operated 24 h d⁻¹, emitting a radiation flux of 22 W m⁻². A reflective shield around each heater ensured uniform radiation over the plot. The top 10 cm of soil warmed by ~2 °C (20), but soil moisture remained similar among plots (~12%). Winter snowfall, which accounted for approximately 80% of annual precipitation, melted 10 to 14 d earlier in warmed plots compared with ambient plots. No heaters were present over ambient plots, and heater shadows affected less than 0.5% of the plot area during less than one third of daylight hours (59). Prior work from this experiment has reported on the response of microclimate (22), plants (21, 32, 56, 57, 59–67), ecosystem function (20, 61, 68), and microbial communities (4, 5, 21, 23) to warming. Although results from global change studies can be underpowered by small sample sizes (69), long-term studies like this can provide insights of a consistent warming press on ecosystems (70).

In 2019, we harvested the Warming Meadow experiment. To avoid potential edge effects of the treatments, we focused our plant and soil measurements on a 2 × 2 m from the edge of each plot within the 3 × 3 m upper zone area. After the plants were removed (see below), we used a backhoe to excavate each plot, digging two meters in from the edge of the plot and 100 cm from the soil surface. This allowed us to collect root and soil samples at 10 cm intervals to a 1-m depth (10 total samples across depth for each plot).

Community-Weighted Means, Plant Biomass, Plant Traits. We determined plant composition by identifying plant species and estimating species-specific relative abundance (foliar cover and biomass) in each 2 m × 2 m plot. We selected nine species (*SI Appendix, Table S2*) that on average represented 80% and 68% of total cover in the warmed and ambient plots, respectively. For each of the nine targeted plant species, we randomly selected three individuals in each plot and measured their above- and belowground traits and biomass (71). To determine if plant communities shifted from resource acquisition to conservation strategies, we quantified aboveground-specific leaf area (cm² g⁻¹) and leaf dry matter content (LDMC, mg g⁻¹) and belowground traits (*SI Appendix, Table S2*) (72, 73). We harvested each individual by clipping the aboveground plant and used a soil core over the stem (2.5 cm diameter × 15 cm deep) to harvest the roots. We calculated the community-weighted mean of biomass and each plant trait by scaling the weights of the three individuals harvested from each plot by their relative cover in that plot. For each plant trait measured, we standardized trait values by multiplying them by the relative cover of each focal plant species and then summing the standardized values across species within each plot to obtain community-level weighted means.

Fungal Colonization of Roots and Soils. All plants in our plots, except sedges, can associate with AM fungi. We measured percent colonization of AM fungal hyphae (aseptate hyphae) and other fungi (septate hyphae), as well as AM fungal organelles (arbuscules and vesicles), in 100 fields of view for each individual plant root system via the crosshair intercept method (74). Fungal hyphal length of AM fungi (aseptate hyphae) and other fungi (septate hyphae, which encompass saprotrophs and pathogens) were counted in 5 g of soil using the grid line-intercept method with 50 fields of view per sample (75).

Microbial Composition. From each collected soil sample, we extracted microbial DNA using Qiagen DNEasy PowerSoil extraction kits using a bead-beating technique known to yield consistent results across taxa (76). We combined 20 samples per MiSeq lane and sequenced 250 PE bases for 20,000 reads/sample. Kivlin et al. (77) previously optimized this sequencing depth as well as sample preparation and analysis pipelines for this ecosystem. Fungal sequences were taxonomically assigned using the UNITE database (78) and matched to functional groups using the Fungal Traits database (79). Sequences are deposited in the NCBI SRA (PRJNA1400617).

Soil Properties. Soil resources are often influential drivers of symbiotic AM fungal (80) and heterotrophic microbial abundance and composition (81). Thus, we quantified plant-available soil nutrients (phosphate, nitrate, and ammonium), soil organic matter, soil pH, and gravimetric soil moisture of each soil sample collected from each of the 10-cm increment samples collected above. Soil nutrients were assayed following standard colorimetric techniques (82, 83). Soil organic matter was calculated using the loss on ignition at 360 °C for 3 h (84). Gravimetric soil moisture was measured on soil dried at 100 °C for 48 h. Soil pH was measured in a 1:2 ratio of weight to volume of soil to water slurry.

Soil Microbial Biomass C and Enzyme Activity. We extracted microbial biomass from each soil sample using the standard chloroform fumigation direct extraction method (85). We assayed for the potential activity of six ecologically relevant microbial enzymes involved in carbon, nitrogen, and phosphorus cycling (86).

Statistics. We analyzed all univariate variables (microbial biomass, fungal colonization, fungal read abundance, plant cover, plant SLA, LDMC and height, soil nutrients, and organic matter) using linear mixed effects models with warming treatment as the main fixed effect and block as a random effect using the lmer function in the lme4 package in R v 4.3.0 (87). Soil variables collected along the soil depth gradient (nutrients, organic matter, enzymes) were analyzed with linear mixed effects models with warming treatment and depth (and their interaction) as fixed effects and block as a random effect. Differences in composition among treatments were analyzed using a dbrda framework on quantitative Jaccard (Ružička) dissimilarity matrices conditioned on block using the dbrda function in the vegan R package (88). Block 4 is consistently different from the other blocks, with block 3 occasionally following the opposing pattern. We explicitly account for this variation by including block as a random effect in all models.

The associations of plant and fungal community composition were analyzed using pairwise Pearson correlations for alpha diversity at all levels of Hill numbers (q) from 0 to 5 in increments of 0.1 for plants and fungal guilds in the hillR package (89). Multiple levels of q were tested for each group given differential species definitions for plants vs. microorganisms. Here, plants were identified using typological species concepts, whereas microorganisms were defined as separate species using Amplicon Sequence Variants (ASVs) defined as unique sequence variants, leading to 600–700x more microbial species compared to plant species (*SI Appendix, Fig. S5*). Differences in composition were analyzed using pairwise Mantel tests (mantel function in vegan with Pearson correlations) at Hill numbers from 0 to 5 in 0.1 increments using the HillR package in R (90). Hill numbers preferentially weight dominant taxa as the Hill number increases, allowing us to understand how plant and fungal communities change relative to each other at different taxonomic grains. For example, because ASVs are calculated at 100% similarity, ASVs of fungi and bacteria will be hyperdiverse compared to plants that are defined by morphological species concepts. If plants were sequenced and grouped as we do for microbial ASVs, the results may be more akin to genotypes. For microorganisms, increasing orders of q will down weight rare sequence variants when characterizing diversity. Plants and microorganisms were considered to be coupled when Pearson correlations were significantly positively correlated (here $r > 0.575$) and otherwise they were considered decoupled.

Data, Materials, and Software Availability. Sequences data have been deposited in NCBI SRA (PRJNA1400617) (91). Other data are included in the article and/or *SI Appendix*.

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Author affiliations: ^aSchool of Biological Sciences, University of Oklahoma, Norman, OK 73019; ^bThe Rocky Mountain Biological Laboratory, Crested Butte, CO 81224; ^cEcology and Evolutionary Biology Department, University of Michigan, Ann Arbor, MI 48109; ^dDepartment of Biology, University of New Mexico, Albuquerque, NM 87131; ^eDepartment of Microbiology, University of Tennessee, Knoxville, TN 37996; ^fDepartment of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996; and ^gThe Energy and Resources Group, University of California, Berkeley, CA 94720

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