

Fungal colonization of plant roots is resistant to nitrogen addition and resilient to dominant species losses

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Abstract. Global change drivers, such as nitrogen (N) deposition and non-random species extinctions, may shift interactions among aboveground and belowground communities. However, tightly coupled interactions between aboveground and belowground organisms may buffer ecosystems to global change. Here, we test how four years of organic and inorganic N addition and removal of a dominant plant species, *Festuca thurberi*, independently and interactively influences fungal colonization patterns and performance in a co-dominant plant species, *Helianthella quinqueneris*. Surprisingly, we found N addition and *Festuca* removal had no measurable effects on the colonization of arbuscular mycorrhizal fungi (AMF) and dark septate endophyte (DSE) or the performance of *Helianthella* seedlings grown with field-collected inoculum in a companion greenhouse experiment. However, the proximity of *Helianthella* to *Festuca* predicted fungal colonization: DSE colonization decreased by 1% for each cm of distance *Helianthella* was from *Festuca*, although the differences in fungal inoculum potential had no effect on *Helianthella* seedling survival or biomass. Our results suggest that plant–fungal interactions can be resistant to N addition and resilient to the loss of dominant plant species. Additionally, our results suggest that soil legacies, mediated through surviving symbiont communities or changes in soil properties, can shape ecosystem resistance and resilience to disturbance and perturbations.

Key words: arbuscular mycorrhizal fungi; dark septate endophytes; *Festuca thurberi*; fungal colonization; *Helianthella quinqueneris*; plant–plant interactions; plant–soil interaction.

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INTRODUCTION

Soil resource availability and dominant species both independently and interactively influence the structure and function of plant and soil fungal communities (Suding et al. 2004, McLaren and Turkington 2010, Souza et al. 2010, Lavorel 2013). For example, in nitrogen (N)-limited

terrestrial ecosystems, N addition increases aboveground productivity but decreases plant diversity (Suding et al. 2004, McLaren and Turkington 2010, Borer et al. 2014, Fay et al. 2015). Plant responses to N addition impact symbiotic partners like arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE; Egerton-Warburton et al. 2007, Henning et al. 2018),

which can feed back to affect plant productivity (van der Heijden et al. 1998), access to soil nutrient resources (Smith and Read 2008), and plant–plant interactions (Scheublin et al. 2007, Jiang et al. 2017). Thus, root-associated fungi may mediate the response of plant community composition and ecosystem function to global change drivers such as N deposition and non-random plant species losses (Urcelay et al. 2009, Marshall et al. 2011).

The composition and abundance of root-associated fungal communities are influenced by soil nutrient availability (Johnson et al. 2003, Egerton-Warburton et al. 2007, Henning et al. 2018), plant host identity (Bever et al. 1996, Vandenkoornhuyse et al. 2003, Verbruggen et al. 2012), and plant neighbors (Hausmann and Hawkes 2009). For instance, in N-limited ecosystems, N addition can shape fungal community composition directly (Egerton-Warburton et al. 2007, Leff et al. 2015) as well as through changes in plant carbon allocation and plant community composition (Westover et al. 1997, Vandenkoornhuyse et al. 2003, Blanke et al. 2011). Typically, direct and indirect effects of N addition result in a shift in the dominant fungal taxa colonizing plant roots (Treseder 2004, Egerton-Warburton et al. 2007, Henning et al. 2018). However, fungal response to N addition depends on the relative ratio of N to phosphorus in the ecosystem (Johnson et al. 2003, 2015, Egerton-Warburton et al. 2007) and likely differs by the type of N resource added (reviewed in Hodge and Storer 2015). Changes in the relative abundance of mutualistic and pathogenic fungi can influence the growth and the survival of plant hosts (Bever 1994, Bever et al. 2010, van der Putten et al. 2016). Thus, plant growth response to N addition is likely driven by the site-level soil properties as well as interactions among the fungi and plants in the community.

Fungal community members have a suite of traits that can influence plant productivity and community composition. These fungal functional groups often co-occur, and their relative abundance in plant roots influences the ability of a plant to acquire soil resources (Michelsen et al. 1996, Upson et al. 2009, Newsham 2011). For instance, in ecosystems with higher organic N availability, DSE, ectomycorrhizal fungi, and ericoid mycorrhizal fungi are typically more prevalent than AMF (Näsholm et al. 1998, Upson et al.

2009, Alberton et al. 2010, Newsham 2011, Hodge and Storer 2015). As a result, these systems often contain more DSE-associated grass and ectomycorrhizal and ericoid mycorrhizal-associated shrub species (Read 1991). In contrast, increasing inorganic N availability may favor AMF (Hawkins et al. 2000), leading to higher abundance of AMF-associated forb and grass species, a pattern that is exacerbated by phosphorus co-limitation (Smith and Read 2008). Thus, changes in nutrient availability and the type of soil resources available may shift the relative abundance of fungal functional groups in the ecosystem and the performance of their plant hosts.

To date, few studies have simultaneously investigated the abundance and growth response of plants and root-associated fungi to changes in plant community composition and soil resource availability (Manning et al. 2008). Here, we report on a 4-yr experiment that manipulated plant species composition (dominant species removal) and N addition (organic and inorganic N fertilizer treatments) in a montane meadow in Colorado, USA. We investigate how the removal of dominant plant species (*Festuca thurberi*) and N addition simultaneously and interactively influenced root-associated fungal colonization patterns in the field. We measured the response of the plant community and root colonization by DSE, AMF, and potential pathogenic fungi to the N addition and species removal treatments. To test how shifts in plant and fungal communities influenced the performance of seedlings establishing within these sites, we conducted a growth assay experiment using field-collected soils in the greenhouse. In both studies, we measured the performance and the colonization of root fungi in co-dominant plant species (*Helianthella quinquenervis*) to understand how dominant species removal and shifting nutrient inputs alter plant performance and the relative abundance of root-associated fungi. Here, we asked four inter-related questions: (1) Does the presence of a dominant plant species, *F. thurberi*, affect the AMF and DSE colonization patterns of co-dominant *H. quinquenervis*? (2) Do organic and inorganic N fertilization treatments alter fungal colonization patterns of *H. quinquenervis*? (3) Do the effects of a dominant plant species on colonization of *Helianthella* roots depend on soil N or proximity to the dominant plant species? and (4)

Do changes in fungal colonization patterns shape the performance of *Helianthella* seedlings?

METHODS

N addition and *Festuca* removal experiment

The experimental design and site characteristics are outlined in detail in Read et al. (2017). Briefly, in the summer of 2012, we established 24 permanent 1.5×1.5 m plots in Maxfield Meadow, a montane meadow on the property of the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA ($38^{\circ}56'58''$ N, $107^{\circ}59'34''$ W), at 2910 m above sea level. The two most abundant plant species in the meadow are *F. thurberi* (mean cover 25.2%; range 3–65%), a large, heavily DSE-associated, perennial bunchgrass (Poaceae) and heavily AMF-associated *H. quinquenervis* (Asteraceae; mean cover 14.0%; range 3–30%). However, in this system, both *Festuca* and *Helianthella* can be co-colonized by AMF and DSE. Other common species include *Erigeron speciosus* (Asteraceae), *Heliomeris multiflora* (Asteraceae), and non-native *Bromopsis inermis* (Poaceae).

Within the 24 plots, we established a full factorial cross ($n = 4$) of N addition and dominant species removal. We had three N treatments: organic, where we added 10 g urea, $(\text{NH}_2)_2$, $\text{N} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$; inorganic, where we added 10 g ammonium nitrate, NH_4NO_3 , $\text{N} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$; and a control with no N added. We overlaid a two-factor dominant species (*F. thurberi*) removal treatment on the nutrient manipulation treatment. In the *Festuca* removal treatment, we removed all aboveground *Festuca* biomass within the 1.5×1.5 m plot. After *Festuca* removal, we used a paintbrush to apply a dilute mixture of glyphosate herbicide to kill the remaining belowground biomass. To maintain the treatments, we added N and removed *Festuca* each spring. Negligible amounts of *Festuca* were observed growing back into the plots after the initial removal. After initial treatment establishment, we observed a decrease in total plant cover between removal and control plots, but after four growing seasons, other species increased in cover and compensated for the loss of *Festuca* (t test, $t_{11} = -0.083$, $P = 0.94$). Furthermore, *Festuca* was replaced by plant species with similar traits, such as *Poa pratensis*, so that the community-weighted

trait means of removal plots were similar to control plots (Read et al. 2017). Additionally, plant community composition did not respond to *Festuca* removal (PERMANOVA, $P > 0.1$) or N addition (PERMANOVA, $P > 0.1$; Read et al. 2017).

Measuring root-associated fungal communities in co-dominant *Helianthella*

In the summer of 2015, following four years of experimental manipulation, we measured the colonization of root-associated fungi in co-dominant species, *H. quinquenervis*. Within each of the 24 plots, we identified all *Helianthella* and *Festuca* individuals and haphazardly selected three similarly sized individual *Helianthella* plants to sample. Across all 24 plots, sampled *Helianthella* height was similar across removal and N addition treatments (*Festuca* removal, $F_{1,59.687} = 0.99$, $P = 0.32$; N addition, $F_{1,59.408} = 0.95$, $P = 0.39$). Additionally, we measured the proximity to the closest *Festuca* neighbor, either intact or removed, for each *Helianthella* individual (Fig. 1). The removed, dead *Festuca* tussocks were still visible even after four years of removal. From each focal *Helianthella*, we collected a single soil core (2.5×15 cm) from directly under the individual host plant (Fig. 1). All soil cores were transported on ice and stored at 4°C until processed within 24 h.

Mycorrhizal colonization

To measure fungal colonization, soil cores were dry-sieved at 2 mm to remove all soil from the live intact roots. We collected ~ 1 g subsample of live roots, clipped them into 2-cm segments, and placed them in Fisher brand Histosette II tissue cassettes (Thermo Fisher Scientific, Waltham, MA, USA). The remaining live roots were added back to the sieved soil samples and were frozen at -20°C to use as inoculum for a subsequent growth assay experiment. We bleached root subsamples in a 10% KOH solution overnight. We then acidified roots in a 2% HCl solution for 60 min and stained them with 0.05% trypan blue solution overnight (Koske and Gemma 1989). Following staining, we submerged roots in a 1:1 deionized H_2O glycerol solution for 3 d to allow any residual trypan blue stain to diffuse from root tissues. We mounted the stained root segments on glass microscope slides using polyvinyl-lacto-glycerol glue (INVAM 2017). We quantified the AMF hyphae, AMF exchange and

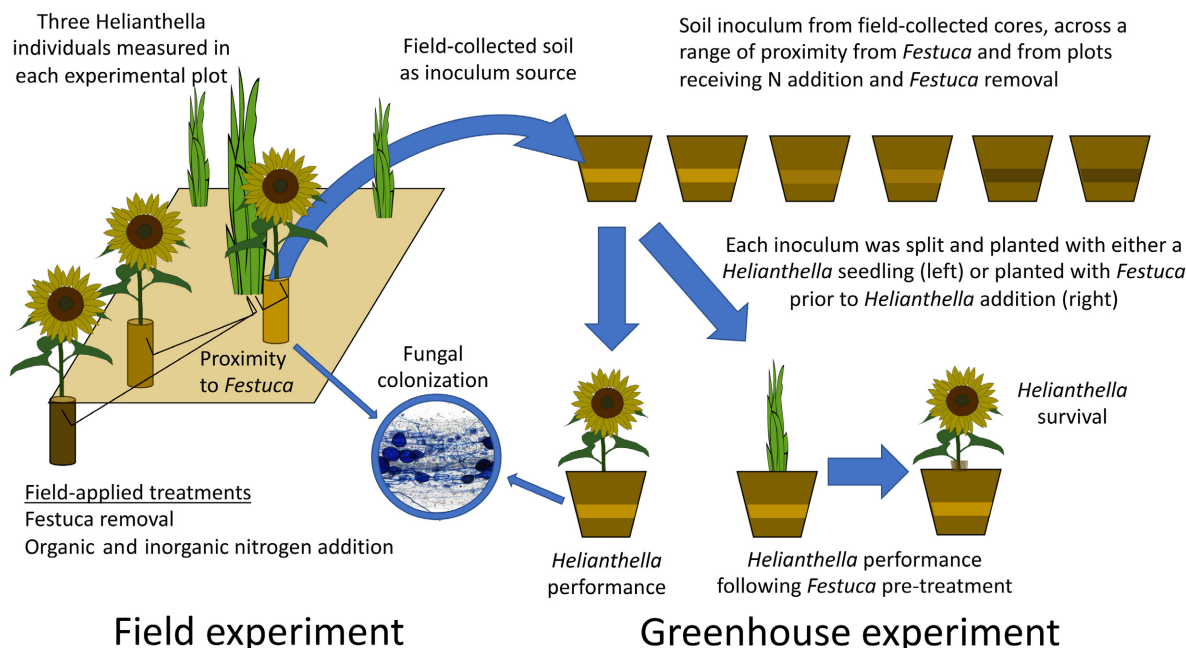


Fig. 1. Experimental design of the field experiment and companion greenhouse experiment. Field experiment consisted of a factorial *Festuca* removal and nitrogen addition experiment (organic nitrogen or inorganic nitrogen) at $n = 3$, for 24 total plots. Within each plot, we measured fungal colonization in three *Helianthella* individuals at various proximity to *Festuca* and collected soils for greenhouse experiment. Soil samples were then used as inoculum in a greenhouse experiment in which samples were split and planted with either a *Helianthella* or receiving a *Helianthella* seedling following *Festuca* pre-treatment. Next, we measured *Helianthella* survival, performance, and measured fungal colonization.

storage organs (arbuscules, coils, and vesicles), DSE hyphae, DSE structures (microsclerotia), and any potential root pathogens, using the magnified intersection method (McGonigle et al. 1990). We inspected a minimum of 50 randomly chosen root intersections on each slide and determined the percentage of AMF and percentage of DSE colonization as the proportion of root segments with AMF hyphae, arbuscules, and vesicles, or DSE hyphae and microsclerotia. The presence of arbuscules, coils, vesicles, microsclerotia, and fungal pathogens were extremely rare, so those data are presented within the Appendix S1: Table S1 and we present only AMF hyphal and DSE hyphal colonization data within the results.

Helianthella growth assay experiment

To explore how *Festuca* removal, N addition, and proximity to *Festuca* shaped the survival and productivity of *Helianthella* seedlings, we used

the soils from our colonization study as inoculum for a greenhouse growth assay experiment (Fig. 1). We selected soil cores that had a minimum of 100 mL of soil and roots remaining as an inoculum source, leaving us with 46 of 72 original soil cores. The 46 cores originated from all possible combinations of N addition and *Festuca* removal treatments and were oriented along a continuum of proximity to *Festuca*. Next, we split each soil sample (~50 mL) into two equal parts and randomly assigned one subsample to directly be planted with an individual *Helianthella* seedling or planted with an individual *Helianthella* seedling following a 12-week conditioning phase by *Festuca* (Fig. 1). The two treatments allowed us to distinguish (1) how prior field-applied N addition and *Festuca* removal, as well as proximity to *Festuca*, shaped survival and performance of *Helianthella* seedlings; and (2) how active *Festuca* conditioning of soils interacted with soil and soil communities to

shape *Helianthella* survival and performance. Thus, our greenhouse experiment consisted of 92 total pots (2 soil treatments $\times$ 46 original soil cores).

To establish the *Festuca* conditioning pre-treatment, we added each of our 46 soil inoculums to 1-L pots that contained 750 mL of a well-mixed, twice-autoclaved field-collected soil, followed by another 200 mL of twice-autoclaved field soils to minimize greenhouse contamination and splashing of soil communities between pots. Our field soil was collected approximately 50 m from the N addition and *Festuca* removal experiment described above. Next, a single pre-germinated *Festuca* seedling was transplanted and grown for 12 weeks. After 12 weeks of growth, we clipped, oven-dried, and weighed all *Festuca* above-ground biomass (Fig. 1). Next, we transplanted a single pre-germinated *Helianthella* seedling into each of the 46 pots. At this same time, we set up the final 46 pots which received only the *Helianthella* seedling using the same setup described above (Fig. 1). Our 92 pots were fully randomized, watered daily, and grown in the greenhouse for 4 months. To understand how biotic and abiotic factors mediate *Helianthella* response to N addition and conditioning by a dominant plant species, we measured survival bi-weekly among the two soil treatments. After 4 months, we harvested and oven-dried the *Helianthella* plants and recorded aboveground and belowground biomass and calculated root:shoot ratios. We also took a $\sim$ 1 g subsample of the roots to measure fungal colonization as described above.

Statistical analysis

All statistical analyses were conducted in R version 3.3.2 (R Development Core Team 2016) and RStudio version 1.0.44 (RStudio Team 2013), with packages cited where applicable. R code is available in Data S1. To explore the influence of N addition, *Festuca* removal, and the proximity to *Festuca* on fungal colonization, we fit linear mixed-effects models using the “lme4” package (Bates et al. 2015). Within the mixed models, we tested for differences in AMF colonization and DSE colonization response variables and modeled N addition (three levels, organic N, inorganic N, and none), *Festuca* removal (two levels, *Festuca* removed and *Festuca* intact), and their

interaction as fixed effects with the proximity to *Festuca* and plot both modeled as random effects. To test for the effects of N addition and *Festuca* removal, we constructed models with and without fixed effects, as well as an interaction between N addition and *Festuca* removal, and performed a likelihood ratio test. If including fixed factors (N addition and species removal) significantly improved model fit ($P < 0.05$ in likelihood ratio test), then we assessed model fit by calculating the proportion of variance explained by fixed effects alone (marginal R^2) and then the proportion of variance explained by both fixed and random effects (conditional R^2) using the *r.squaredGLMM* function in the “MuMIn” package (Bartoń 2018). In any analyses where we found a significant interaction between N addition and *Festuca* removal, we used the *glht* function in the “multcomp” package (Hothorn et al. 2008) to determine differences across the treatments.

To examine how field treatments, *Festuca* removal and N addition, and greenhouse treatment, *Festuca* conditioning, shaped *Helianthella* survival, we fit a generalized mixed-effects model with a binomial distribution using the *glmer* function (“lme4,” Bates et al. 2015). Within our models, *Festuca* conditioning, N addition, *Festuca* removal, proximity of inoculum from *Festuca* in the field, and sampling date were incorporated as fixed factors and individual inoculum identity and the plot that inoculum was collected in were treated as random effects. We constructed models with and without fixed effects and performed a likelihood ratio test and compared Akaike’s information criterion (AIC) scores to determine the best-fit model.

To analyze *Helianthella* final aboveground biomass, belowground biomass, and belowground:aboveground biomass in the growth assay experiment, we fit linear mixed-effects models which included *Festuca* removal, N addition, the proximity of inoculum from *Festuca*, and *Festuca* conditioning modeled as fixed effects and the plant inoculum identity and plot modeled as random effects. This allowed us to test how field-applied soil treatments (dominant removal and N addition) influenced *Helianthella* performance relative to greenhouse-applied *Festuca* conditioning. For aboveground biomass, belowground biomass, and biomass root:shoot ratio, we constructed a

full model that incorporated *Festuca* conditioning, N addition, *Festuca* removal, and the proximity of inoculum from *Festuca*, as well as simplified models removing fixed factors. Dead plants were removed from these analyses. Next, we determined best-fit model, by comparing models with and without fixed effects using AIC scores.

RESULTS

Field experiment

Surprisingly, neither N addition nor *Festuca* removal altered AMF or DSE colonization in *Helianthella* (Fig. 2; Appendix S1: Table S2). However, proximity to *Festuca* was retained in the model that best predicted the variation in DSE colonization among *Helianthella* individuals. Proximity to *Festuca* and plot number explained ~34% of the variation in DSE colonization in *Helianthella* (Appendix S2: Table S1). *Helianthella* growing within a few centimeters of *Festuca* had a relatively high DSE colonization rate (~70%), but DSE colonization reduced with increasing distance from *Festuca*, with DSE

colonization ~25% when *Helianthella* was 30 cm from any *Festuca* plant (Fig. 2). The positive effect of *Festuca* proximity on DSE colonization in the field, combined with the lack of a *Festuca* removal effect, suggests that there is a strong legacy of *Festuca* on DSE colonization patterns. Arbuscular mycorrhizal fungi colonization of *Helianthella* was unaffected by the proximity to *Festuca* (Fig. 2; Appendix S2: Table S1) and was consistently high (~80%), independent of N addition, *Festuca* removal, or proximity to *Festuca* (Fig. 2).

Growth assay experiment

Overall, we found that the survival of *Helianthella* declined by approximately 30% over the duration of our experiment (Fig. 3). We found that sampling date, *Festuca* conditioning, and field-applied *Festuca* removal treatments were all retained in the best model predicting *Helianthella* survival (AIC = 463.80; Appendix S2: Table S1). Seedling survival was higher early in the experiment in soils with *Festuca* conditioning; Survival was ~98% in *Festuca* conditioned soils compared to ~91% in unconditioned soils (Fig. 3). However, the differences in *Helianthella* survival with *Festuca* conditioning diminished over time (Appendix S2: Table S2). By the end of the experiment, there were weak survival differences between treatments (Fig. 3). There was no clear survival pattern with the field-applied *Festuca* removal treatment even though it was retained within our best-fit model (Fig 3).

Overall, fungal colonization in the greenhouse was much lower than in the field. Colonization rates of AMF ranged from 30% to 60% in the greenhouse compared to 80% in the field, and DSE colonization varied between 5% and 20% in the greenhouse and 20% and 60% in the field (Appendix S2: Table S1). Similar to the field results, we observed no difference in AMF colonization with field-applied N addition, *Festuca* removal, or the proximity of the inoculum source from *Festuca* (Appendix S2: Table S1). Additionally, we found that the greenhouse treatment, *Festuca* conditioning, had no effect on AMF colonization in *Helianthella* roots (Appendix S2: Table S1). We found that N addition, the proximity of the inoculum source from *Festuca*, and greenhouse-applied *Festuca* pre-treatment were retained in our best-fit

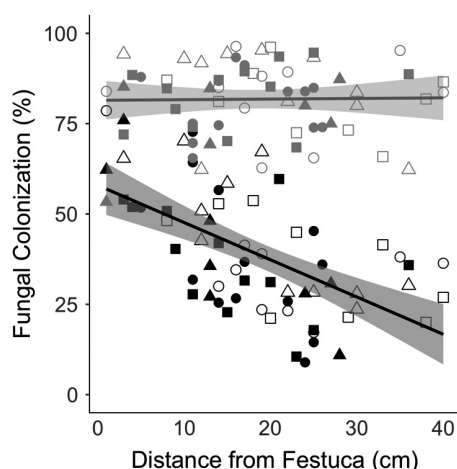


Fig. 2. Observed arbuscular mycorrhizal fungal (gray) and dark septate endophyte (black) colonization of *Helianthella quinquenervis* across varying proximity to co-dominant *Festuca thurberi* in a nitrogen addition and *Festuca* removal experiment. Black filled in dots indicate *Festuca* removal, and white filled dots indicate intact *Festuca* plants. $\square$ = inorganic N addition, Δ = Organic N addition, and $\circ$ = no N addition.

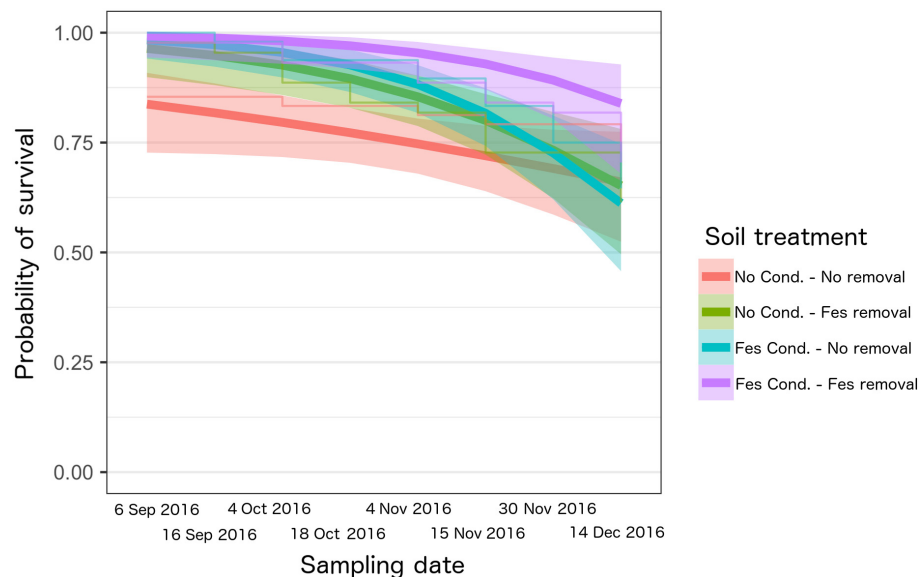


Fig. 3. *Helianthella quinquenervis* seedling survival across the duration of our growth assay experiment in response to greenhouse treatment *Festuca* conditioning (blue and purple) or non-*Festuca* conditioning of soil inoculum (red and green), with soil inoculum collected from either field-applied *Festuca* removed plots (green and purple) or *Festuca* intact plots (red and blue). Overall, *Festuca* conditioning ($P = 0.0005$) and *Festuca* removal ($P = 0.01$) increased survival of *Helianthella*; however, these effects diminished over time (Sampling date $\times$ *Festuca* conditioning, $P = 0.0054$; Sampling date $\times$ *Festuca* removal, $P = 0.024$, full results are located in Appendix S2: Table S2).

greenhouse DSE model (Appendix S2: Tables S1, S2). Specifically, the lowest rates of DSE colonization were in soils that originated from inorganic N-amended plots (~9% colonization), relative to organic N-amended plots (12% colonization), and control plots (~19% colonization; Appendix S2: Table S2, Fig. S1). Dark septate endophyte colonization was lowest in soils that were collected closest to *Festuca* in the field opposite of the pattern we observed in the field colonization experiment (Appendix S2: Table S2, Fig. S1). However, colonization only increased 2% per 5 cm of spatial distance from *Festuca*.

Total *Helianthella* biomass and biomass allocation between roots and shoots were unrelated to N addition, *Festuca* removal, or the proximity of the inoculum to *Festuca*. Although early *Helianthella* survival was influenced by *Festuca* conditioning, we found that *Festuca* conditioning had marginal effects on plant total biomass and no overall effect on biomass allocation in seedlings grown in the greenhouse (Appendix S1: Table S2, Fig. S2). *Festuca* conditioning marginally

reduced plant biomass ($P = 0.055$) by 13%. However, *Festuca* conditioning only explained about 4% of the variation in total plant biomass (Appendix S1: Fig. S2). *Festuca* conditioning, field-applied N addition, and *Festuca* removal were not retained as predictors in models to explain aboveground biomass, belowground biomass, or root:shoot biomass of *Helianthella* (Appendix S1: Table S2). Although we observed clear differences in fungal colonization patterns and early survival with both field and greenhouse treatments, if plants were able to survive, shifts in fungal inoculum potential did not alter plant biomass. We also tested for a correlation between total *Festuca* biomass collected during the conditioning phase and total *Helianthella* biomass and found no relationship ($r^2 = 0.02$, $P = 0.193$).

DISCUSSION

Overall, we found that a high elevation montane meadow ecosystem was resistant to N

addition, resilient to plant species losses, and that seedlings establishing within these manipulated soils were resilient to relative abundance differences of potential symbionts within the soil inoculum pool. The proximity to a dominant species, *F. thurberi*, had a long-lasting impact on fungal colonization within the co-dominant *H. quinquenervis*, even after the dominant (*Festuca*) had been removed from the community for 4 yr. Proximity to *Festuca* was a strong predictor of DSE colonization in *Helianthella*. The lasting impact *Festuca* had on the fungal community in adjacent species was surprising, especially since we found no direct or interactive impacts of the treatments—removal of a dominant species (*F. thurberi*) and the addition of organic or inorganic N—on fungal colonization of co-dominant *H. quinquenervis*. The strong effect of proximity to *Festuca* on root colonization combined with the lack of removal effects suggests a strong legacy of *Festuca* on the surrounding plant community members even four years after removal. However, proximity of the soil inoculum from *Festuca*, *Festuca* removal, and N addition had no measurable effects on survival, growth, total biomass, or biomass allocation of *Helianthella* seedlings in a companion greenhouse growth assay experiment. Early within the greenhouse experiment, *Festuca* removal in the field as well as the greenhouse-implemented *Festuca* conditioning weakly, but significantly, increased survival of *Helianthella*; however, the differences in survival diminished by the end of the experiment or initial differences were the result of transplant mortality that happened to occur within the non-conditioned, non-removed soil treatments.

Resistance and resilience of ecosystems to global change drivers

Four years of maintained N addition and *Festuca* removal resulted in no measurable effects on fungal colonization patterns or performance of seedlings of the co-dominant plant species *Helianthella* in the field and a companion greenhouse experiment. Although ecosystem function often changes drastically with N addition and plant removal (Wardle et al. 1999, Suding et al. 2004, Elser et al. 2007, McLaren and Turkington 2010, Fay et al. 2015, Kardol et al. 2018), many ecosystems likely contain some ability to resist or recover from disturbance (Urcelay et al. 2009,

Read et al. 2017). The role of resistance and resilience to N addition and plant removal in montane ecosystems has been documented at this site (Read et al. 2017): Plant community composition, plant trait composition, and soil respiration were resilient to *Festuca* removal and resistant to N addition, even though N addition increased plant productivity. After 4 yr of *Festuca* removal, the community-weighted leaf and root trait values converged toward pre-removal trait values, as grass taxa with similar trait values to *Festuca* increased in abundance over time (Read et al. 2017). Additionally, the grass species replacing *Festuca* in the removal plots were primarily associating with DSE (Ranelli et al. 2015; J. A. Henning, *unpublished data*). This suggests that soil microbial communities may contribute to the resilience of ecosystems to non-random plant species losses (Merryweather and Fitter 1998, Urcelay et al. 2009, Marshall et al. 2011).

Ghosts of neighbors past

The proximity of *Helianthella* plants to *Festuca* was positively related to DSE colonization of *Helianthella* roots independent of whether *Festuca* was intact or whether *Festuca* had been experimentally removed. Thus, *Festuca* influenced fungal colonization patterns to the same extent whether it was actively growing next to *Helianthella* or was removed from the community four growing seasons prior to sampling. Additionally, in our greenhouse experiment, *Helianthella* survival was initially ~12% greater when seedlings were grown following *Festuca* conditioning and in soils with *Festuca* removal relative to control plants in soil from *Festuca* intact plots. However, these effects diminished over the course of the experiment. Thus, *Festuca* has a strong impact on the fungal communities within *Helianthella* and a weak impact on survival of *Helianthella*, even four years after removal from the plant community. Soil legacies, mediated through surviving symbiont communities or changes in soil properties, may shape ecosystem resistance and resilience to disturbance and perturbations. Evidence from other ecosystems suggests that soil legacies can reside in soils for more than 40 yr following plant removal (Wardle et al. 2008, Urcelay et al. 2009); thus, the ghosts of neighbors past may influence ecosystem function long after plants die.

Although plant–soil feedback studies investigate how soil legacies (conditioning phase) shape the performance of contemporary plants on shorter timescales, a major knowledge gap exists in our understanding of the duration, spatial extent, and persistence of neighbor legacies in intact ecosystems.

Changes in root-associated fungal colonization but no change in host performance

Helianthella individuals near *Festuca* contained more DSE than individuals growing farther away from *Festuca*. Additionally, all *Helianthella* individuals were highly colonized by AMF taxa as well. Our working hypothesis was that maintaining a higher load of AMF and DSE when growing near *Festuca* would likely have growth or survival trade-offs for seedlings with a limited carbon supply available to symbionts. However, there was no apparent tradeoffs in survival or biomass while maintaining a higher endophyte load. It is possible that *Helianthella* do not allocate carbon resources to DSE, and DSE do not provide resources to *Helianthella*, or that any resources that are transferred to DSE are offset by marginal growth benefits for *Helianthella*; however, carbon stocks and transfer between plants and symbionts were not measured in the current study. There is mixed evidence documenting a relationship between the amount of carbon being allocated from plant to fungi and the extent of fungal colonization (Treseder 2013). Fungal taxa differ in their colonization strategies between root and soil leading to differences in colonization and allocation patterns (Hart and Reader 2002, Powell et al. 2009). Our approach ignores some of the idiosyncratic foraging style differences among fungal taxa and compared community-level fungal colonization responses to biotic and abiotic drivers (Johnson et al. 2003, Egerton-Warburton et al. 2007, Hausmann and Hawkes 2009, Ranelli et al. 2015, Henning et al. 2018).

Drivers of fungal colonization patterns

A wide variety of biotic and abiotic drivers regulate the colonization rates of root fungal symbionts. For instance, root colonization rates by AMF and DSE differ among plant functional groups, with grasses in this montane ecosystem commonly supporting higher colonization of

DSE relative to AMF, whereas forbs are typically more heavily colonized by AMF (Newsham 2011, Mandyam and Jumpponen 2014, Ranelli et al. 2015). Additionally, plant nutrient status and soil nutrient availability are strong determinates of fungal colonization rates (Bruce et al. 1994, Johnson et al. 2003, Egerton-Warburton et al. 2007, Henning et al. 2018). It is likely that plant selection of these symbionts ultimately shapes the rates of fungal colonization in root space in response to biotic and abiotic stressors, although this remains debated within the literature (Bever 2015). However, unlike these previous studies, our study found that changing plant community composition through the removal of the dominant plant species and increasing nutrient availability had no measurable influence on root colonization patterns of AMF and DSE within focal host, *Helianthella*. We found the proximity to a neighbor that preferred DSE associations over AMF associations (*Festuca*) was the most important factor determining fungal colonization rates within *Helianthella*. Our results suggest that studies investigating how plant–symbiont interactions are shaped by changing biotic or abiotic contexts should incorporate some measure of the plant community surrounding their focal host plant. Incorporation of community contexts can be accomplished by explicitly incorporating plant–plant interactions, devising spatially explicit sampling strategies, or simply measuring the composition and diversity of the supporting plant community around the focal host. The incorporation of community contexts may be especially important in systems where multiple functional groups of fungi, such as AMF, DSE, ericoid mycorrhizal fungi, or ectomycorrhizal fungi, co-exist within co-occurring plant taxa.

CONCLUSIONS

Contrary to a large literature showing the strong response of both plant-associated fungal and plant community response to N addition and plant species loss, we found that the colonization patterns of root symbionts, seedling survival, and seedling performance were resistant to N addition and resilient to the loss of dominant plant species. Even though removing *Festuca* from the ecosystem had no measurable effects on

fungus colonization or *Helianthella* survival and biomass, we found that proximity to *Festuca* was negatively correlated with DSE colonization in *Helianthella* roots. However, the relationship between maintaining a higher load of DSE and *Helianthella* survival and productivity is unclear since we observed no difference in plant biomass and survival of *Helianthella* seedlings at different proximities to *Festuca*. Our results, combined with previous studies from this system, suggest that plant–soil interactions may buffer ecosystem-level responses to global change. However, the mechanisms that regulate the buffering capacity require further study.

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