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Do plant–soil interactions influence how the microbial community responds to environmental change?

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Abstract. Global change alters ecosystems and their functioning, and biotic interactions can either buffer or amplify such changes. We utilized a long-term nitrogen (N) addition and species removal experiment in the Front Range of Colorado, USA to determine whether a codominant forb and a codominant grass, with different effects on nutrient cycling and plant community structure, would buffer or amplify the effects of simulated N deposition on soil bacterial and fungal communities. While the plant community was strongly shaped by both the presence of dominant species and N addition, we did not find a mediating effect of the plant community on soil microbial response to N. In contrast to our hypothesis, we found a decoupling of the plant and microbial communities such that the soil microbial community shifted under N independently of directional shifts in the plant community. These findings suggest there are not strong cascading effects of N deposition across the plant–soil interface in our system.

Key words: community ecology; dominant species; global change; microbial ecology; nitrogen fertilization; plant removal; plant–microbe interactions; soil bacteria; soil fungi.

Introduction

Global change, such as warming and nitrogen (N) deposition, directly alters plant (Walker and Wahren 2006, Payne et al. 2017) and microbial communities (Castro et al. 2010, Ramirez et al. 2010). It also influences interactions within and between plants and microbes, resulting in indirect effects (De Long et al. 2016, Shao et al. 2018, Chen et al. 2019). Importantly, these indirect effects may buffer or amplify the impacts of global change on ecological communities (Holling 1973, Brooker 2006, Suttle and Thomsen 2007, Tylianakis et al. 2008, Classen et al. 2015, Pires and Srivastava 2018). For example, the negative effects of warming on plants may be buffered if warming also favors the expansion of other plant species that form favorable microclimates (Anthelme and Cavieres 2014), thereby promoting facilitative interactions. On the other hand, altered plant–plant or plant–pathogen interactions may amplify the detrimental effects of warming on plant populations if interactions shift from facilitative to competitive (Olsen et al. 2016) or if disease outbreak is enhanced under the warmer conditions (Burgess 2017). It is vital to quantify the importance of indirect effects in order to better understand how plant and microbial communities are responding and will continue to respond to their shifting environment.

Human-induced perturbations to the global N cycle have increased the reactive N (e.g., NH_3, NO_X) deposited across the globe (Bobbink 2010, Liu 2013). Nitrogen deposition often affects plant communities by altering community composition, reducing species richness, and increasing aboveground biomass (Bowman et al. 2006, Lebauer and Treseder 2008, Cleland and Harpole 2010), and affects soils by reducing pH, increasing nitrate (NO_3^-), and ammonium (NH_4^+) concentrations, decreasing base cations and buffering capacity, and increasing toxic elements such as aluminum (Bowman et al. 2006, 2008, Lieb and Darrouzet-Nardi 2011). While N deposition has declined in some parts of the world due to legislation (Du et al. 2014), the return of ecosystem properties, including plant composition, nutrient cycling, and soil chemical characteristics, to their previous state has been slow (Street and Burns 2015, Bowman et al. 2018, Crawford and Hinckley 2020).

Shifts in plant communities do not occur in isolation, and changes in plant composition can cascade to affect soil bacterial and fungal communities. While edaphic properties, such as pH and resource availability, are typically considered the major drivers of soil microbial community distribution (Fierer and Bradford 2007, Lauber et al. 2009, Tedersoo 2014), there are several ways in

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which changes in plant community composition can impact microbial communities. Plants influence the soil microbial community through the quantity and quality of resources (e.g., litter, root exudates) they produce (Wardle et al. 2004, Ward et al. 2015), as well as through their effects on other soil physicochemical characteristics (e.g., pH and water holding capacity (Stefanowicz et al. 2018)). Plants can have effects on specific microbial taxa (e.g., through allelopathic compounds; Lankau and Strauss 2007) and entire communities (e.g., via alterations in nutrient cycling; Rodrigues et al. 2015). Therefore, N deposition can affect the soil microbial community through changes to the soil, such as enhanced nutrient availability or lower pH (Ramirez et al. 2010, Chen et al. 2019), and through changes to the plant community, through shifts in the biomass or relative abundance of plant species (Suding et al. 2008, Yuan et al. 2016, Zeng et al. 2016, Shao et al. 2018). In particular, changes in dominant plant species are theorized to be a strong driver of belowground shifts because these species represent a considerable amount of community biomass and shape ecosystem and community properties (Grime 1998, Gaston 2011). The response of dominant plant species and the overall plant community to N deposition could buffer or amplify the effects of N addition on the soil microbial community, hence making it important to investigate both responses and their interaction.

Our study utilizes an 18-yr N addition and codominant plant removal experiment in the Front Range of Colorado, USA to ask if and how different dominant plant species shape bacterial and fungal response to simulated N deposition. *Geum rossii* (hereafter *Geum*; a slow-growing rosaceous forb that declines precipitously with N addition; Suding et al. [2008]) and *Deschampsia cespitosa* (hereafter *Deschampsia*; a fast-growing bunchgrass) were annually removed from plots for 18 yr. Previous work in these plots has demonstrated that N addition and codominant removals impact the remaining codominant as well as forbs and graminoids (Suding et al. 2006, 2008). We hypothesize that N addition will increase available soil N, increase the abundance of *Deschampsia*, and reduce the abundance of *Geum* (Suding et al. 2008). We also predict that the removal of *Deschampsia*, as a strong competitor in the system, will result in the competitive release of *Geum* and other less abundant species. The removal of *Geum*, which some past work indicates as having a facilitative effect on other forbs (Suding et al. 2006), will result in an increase of *Deschampia* and a decline in other forb species. Furthermore, we expect that the removal of *Geum*, whose litter slows N cycling (Steltzer and Bowman 1998), will have a compounding effect on N addition, causing even greater shifts toward increased N availability and the dominance of *Deschampsia*.

Resultant shifts in the plant community should indirectly shape how the soil microbial community responds to N addition. Specifically, we hypothesize that the codominant species will indirectly influence how the soil microbial community responds to N addition by altering the input of plant materials to the soil, but that the two dominant species will have distinct effects due to their different characteristics. This indirect effect could either amplify or counteract the direct effects of simulated N deposition on the soil microbial community. We predict that the presence of *Geum*, which has a high phenolic content and is a driver microbial N immobilization (Steltzer and Bowman 1998, Bowman et al. 2004), will buffer the effects of N addition by depressing the response of fast growing, copiotrophic and nitrophilic organisms, while the presence of *Deschampsia*, a driver of N mineralization (Steltzer and Bowman 1998), will amplify the effects of N addition and the response of the microbial community. A lack of an interaction between simulated N deposition and the plant community would suggest that the soil microbial community responds to N independently of the plant community.

Methods

Study design

We conducted our study at Niwot Ridge in the Front Range of the Rocky Mountains, Colorado, USA (40.05° N, 105.59° W) in a long-term N addition and codominant species removal experiment initiated in 2001 (Suding et al. 2008). The experiment consists of seven experimental blocks within an ~5-km^2 region on Niwot Ridge, ranging in elevation from 3,397 to 3,544 m above sea level. Plots (1 m^2) within the seven blocks were chosen such that they were codominated (~30% each) by *Geum* and *Deschampsia*. The N (NPK 40-0-0, using urea-N) was added as slow-release fertilizer pellets. Starting in 2001, 144 g fertilizer/m^2 was applied at the start of each growing season. In 2008, N addition was reduced to 72 g/m^2 and in 2011, to a rate of 25 g/m^2. Previous work with these pellets in our system indicates that the average N inputs were 28.8 g $N{\cdot}m^{-2}{\cdot}yr^{-1}$ from 2001–2007, 14.4 g $N{\cdot}m^{-2}{\cdot}yr^{-1}$ from 2008–2010, and 5 g $N{\cdot}m^{-2}{\cdot}yr^{-1}$ from 2011–2018 (Bowman et al. 1993). The reduction in applied N accounts for the cumulative effects of N addition. All rates of N addition were well above the proposed saturation rate in the area, estimated at 1 g $N{\cdot}m^{-2}{\cdot}yr^{-1}$ (Bowman et al. 2006). At Niwot Ridge, wet and dry ambient N deposition is 0.6 g $N{\cdot}m^{-2}{\cdot}yr^{-1}$ (Sievering 2001). Removal of the codominant species began in 2001 and was achieved through clipping the aboveground biomass. The removal treatment consisted of *Geum* removal, *Deschampsia* removal, or no removal. Annual clipping was done one to three times throughout the growing season as needed. Plots were trenched to a depth of 10 cm one to three times per growing season to limit the influence of the codominant species growing on the periphery of the plot. By 2008 the average amount of biomass being clipped was around 1.2% of the original biomass

removed from the plots, indicating a successful removal of the selected codominant species (Appendix S1: Fig. S1). Had we assessed soil microbial community composition in the early years of the experiment when large amounts of biomass were clipped for the removal treatment, we could not have been sure whether interactive effects of the codominant removal treatment were a result of the plant community responding to disturbance due to biomass removal or to the absence of the codominant plant species. Hence, it was important to wait until the codominant biomass removed had tapered off before we could assess the potential indirect effects of our codominant plant species on the soil microbial community. Additionally, sampling at this later stage of the experiment ensured that the plant and microbial communities were experiencing a long-term press disturbance, which allows us to better predict their response to chronic N deposition. There were a total of 42 plots (6 treatments across 7 blocks).

Plant community composition data was collected annually from 2002 until 2018 during peak biomass using the point intercept method. Only top hits (the tallest species at each point) were recorded, but species that were present in the plot and not hit were assigned a value of 0.5. Before calculating plant community response to treatment, non-vascular plants as well as rocks and litter were removed from the data set. Aboveground biomass was harvested in 20 cm × 20 cm square areas within each plot every other year from 2004 to 2018. The collected biomass was sorted into biomass accumulated that year and litter. The location of the square within the plot was shifted each year to minimize impact.

Soil properties

From 26 July to 3 August 2018, soils were collected by homogenizing ten soil cores per plot taken to depth of 10 cm using a core 2 cm in diameter. Samples were kept on ice and transported to the lab within 8 h of collection. A subset was immediately aliquoted for DNA extraction and kept at −20°C for a maximum period of 2 months. Additionally, sieved soil was aliquoted for gravimetric soil moisture, pH, and K_2SO_4 extractions for NH_4^+ and NO_3^-. We measured pH using a SevenCompact pH meter S210 (Mettler Toledo, Greifensee, Switzerland) on soils that had been mixed 2:1 with deionized water and allowed to equilibrate with atmospheric CO_2 for 30 minutes. We extracted N from 10 g of soil using 50 mL of 0.5 mol/L K_2SO_4 and shaking for 2 h at 140 rpm. The following day, this mixture was filtered, frozen, and delivered to the Colorado State University Soil, Water and Plant Testing Laboratory (Fort Collins, Colorado, USA). Negative values indicate that the N content was below the detection limit and were thus set to 0. All roots collected during the sieving processes were washed over a 250-μm sieve, dried at 60°C for 72 h, and weighed for root biomass.

Soil sequencing

For the bacterial and fungal community analysis, DNA was extracted from 0.25 g of moist homogenized soil using a Qiagen DNeasy PowerSoil Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). Extracted genomic DNA was diluted 1:10 in sterile culture grade water (Sigma-Aldrich, St. Louis, Missouri, USA). PCR was then used to amplify the V4 hypervariable region of the 16S rRNA gene using barcoded 515f and 806r primers and the first internal transcribed spacer region (ITS) of the rRNA gene using barcoded ITS1-F and ITS2 primers to assess the structure of bacterial and fungal communities, respectively (Leff et al. 2017). PCR was conducted with Promega HotStart Mastermix (Promega, Madison, Wisconsin, USA) in a 25-μL reaction. The thermal cycling conditions were as follows: 3-minute initial denaturation at 94°C, 35 cycles of 45 s denaturation at 94°C, 1-minute annealing at 50°C, 1.5-minute elongation at 72°C, and a 10-minute final elongation at 72°C. Amplicons were purified and normalized with the SequalPrep Normalization Kit (Invitrogen, Carlsbad, California, USA). Samples were then pooled into single 16S and ITS amplicon libraries and sequenced on an Illumina MiSeq2000 (pair-end 2 × 300 bp) at the University of Colorado BioFrontiers Institute (Boulder, Colorado, USA). Sequences were demultiplexed using idemp[4] and sequencing adapters were removed using cutadapt (Martin 2011). Reads were then processed using the DADA2 pipeline (Callahan et al. 2016). First, reads were quality filtered and dereplicated. Then, exact sequence variants (ESV) were inferred and paired-end reads were merged. Next, chimeras were removed and taxonomy was assigned using SILVA (Quast et al. 2013) for the bacterial sequences and UNITE (Abarenkov 2010) for the fungal sequences. Two samples from the ITS data were dropped due to low-quality reads. The 16S sequences were rarefied to 13,283 sequences per sample and the ITS sequences were rarified to 14,946 sequences per sample.

Data analysis

To test for effects of the dominant plant species and N addition on soil properties and aspects of the plant community, we ran linear mixed effects models with block as a random effect and N addition, dominant plant removal, and their interaction as fixed effects (function lmer, package *lme4*; Bates et al. 2015). When required, variables were log or square-root transformed to meet assumptions of normality and homogeneity of variance. Pairwise treatment comparisons were assessed via Tukey's honest significant difference (function emmeans, package *emmeans*; Lenth et al. 2018). To test for treatment effects on the plant community composition in 2018, we ran a permutational multivariate analysis of

[4] https://github.com/yhwu/idemp/blob/master/idemp.cpp

variance (PERMANOVA) on Bray-Curtis dissimilarity matrices calculated on square-root transformed relative abundances with codominant plant removal, N addition, and their interaction as predictors (function adonis, package *vegan*; Oksanen 2019). Block was included as strata (a blocking variable), which restricted permutations to within blocks. Plant species present in fewer than 5% of plots (two plots) were removed from the compositional data prior to the PERMANOVA. We conducted a similarity percentage analysis to identify plant species contributing to treatment differences (function simper, package *vegan*). We expected that both N addition and the codominant plant species would have strong effects on soil properties and the plant community as those components of the system are the pathways through which we would expect buffering or accelerating processes to occur.

To test the response of the soil microbial community, we ran two partial distance-based redundancy analysis (dbRDA), described below. Data were relativized and then subset before analysis; we retained ESV with a mean relative abundance greater than 0.05% (e.g., Oliverio and Bradford 2017), thereby removing rare taxa. Before this filtering there were 6,336 bacterial and 6,199 fungal ESV. To test for the effects of our categorical treatment variables on microbial community composition, we first conducted a partial dbRDA on the bacterial and fungal communities with block as our condition (function dbrda, package *vegan*) and N addition, plant removal, and their interaction as predictors. We then ran a partial dbRDA on both microbial communities with a suite of continuous edaphic and plant-related variables as predictors. The continuous predictor variables of interest were *Geum* and *Deschampsia* relative abundance, forb and graminoid abundance, plant richness, live aboveground biomass, soil NO_3^-, soil NH_4^+, and soil pH (see Appendix S1: Fig. S2 for a principal coordinates analysis with these predictors [functions cmdscale and envift, package *vegan*]). Where *Geum* and *Deschampsia* were removed from plots, their relative abundances were set to NA when calculating forb and graminoid abundance. Of *Deschampsia*, *Geum*, forb, and graminoid abundance, only graminoid abundance was retained in the dbRDA due to collinearity ($r > 0.7$). All continuous variables were scaled to have a mean of 0 and a standard deviation of 1. For the dbRDA containing the continuous predictors, we used forward selection to choose the best model based on adjusted R^2 (function ordiR2step, package *vegan*; Blanchet and Legendre 2008). *P* values were adjusted using a Holm correction to reduce the risk of Type I error resulting from conducting multiple significance tests during forward selection.

We followed up on significant effects of our categorical variables on the soil microbial community with the nonparametric Kruskal-Wallis test (function taxa_summary_by_sample_type, package *mctoolsr*; Leff 2017); the Benjamini-Hochberg correction was used to account for the multiple comparisons. We ran the Kruskal-Wallis test at the phylum and ESV level for both bacteria and fungi. We also ran the test at the genus level for fungi to test for effects on the response of arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE), both of which are associated with plant nutrient uptake (Johnson et al. 2010, Newsham 2011). Arbuscular mycorrhizal fungi genera were defined as genera within the phylum Glomeromycota (Schüßler and Schwarzott 2001, Tedersoo et al. 2018). The DSE genera were defined as those described in reports of known DSE (Jumpponen and Trappe 1998, Newsham 2011). We also looked for AMF and DSE in the compositional data containing rare taxa to more accurately determine the number of detected genera. All statistical analyses and visualizations were performed in R ver. 3.4.2 (R Core Team 2020).

Results

Both dominant plant removal and N influenced the plant community

The codominant plant species had a strong effect on most aspects of the plant community, which was similar in magnitude to the effect of N addition, but there were no interactive effects on any of the measured plant variables. The removal of *Geum* and the addition of N decreased live aboveground biomass by 34% and 20%, respectively (Table 1; Fig. 1a; Appendix S1: Table S1). On the other hand, root biomass was 37% higher with N addition (Table 1; Fig. 1b).

As expected, N addition caused *Geum* to decline by 81% while *Deschampsia* increased by 61% (Table 1; Fig. 1c, d). Forbs declined with N addition (Table 1; Fig. 1e), including *Artemisia scopulorum*, *Erigeron simplex*, and *Castilleja occidentalis* ($P < 0.05$). Graminoids increased with N addition (Table 1; Fig. 1f), including *Carex scopulorum* and *Carex nova* ($P < 0.05$).

The removal treatments indicated a general facilitative relationship between *Geum* and other forbs, and competitive relationships between *Deschampsia* and *Geum*. The removal of *Geum* led to a decline in other forbs and an increase in graminoids (Table 1; Fig. 1e, f; Appendix S1: Table S1). *Deschampsia* abundance increased 52% under *Geum* removal (Table 1; Fig. 1d). On the other hand, the removal of *Deschampsia* led to a decline in other graminoids and an increase in forb abundance (Table 1; Fig. 1 e, f; Appendix S1: Table S1), with a 32% increase in *Geum* abundance under *Deschampsia* removal (Table 1; Fig. 1c).

Nitrogen addition significantly decreased plant species richness from an average of 14 species/m^2 to 12 species/m^2 (Table 1; Fig. 1g), but there was not an effect of removal. As indicated by the PERMANOVA, plant community composition was shaped by both N addition and codominant plant removals, with the latter explaining a greater amount of variation, but there was no interaction (Table 1; Fig. 2a).

TABLE 1. Effects of N addition, dominant plant removal, and their interaction on plant and soil variables.

				N Addition (df = 1)			Plant Removal (df = 2)			Interaction (df = 2)		
Dataset	Response Variable	Model	*n*	Stat	R^2	*P*	Stat	R^2	*P*	Stat	R^2	*P*
Plants	Aboveground Biomass	LME	42	5.5		**0.02**	9.8		**0.007**	4.8		0.09
	Root Biomass	LME	42	5.5		**0.02**	0.3		0.85	2.3		0.32
	Geum abundance	LME	28	18.8		**<0.001**	9.4		**0.002**	1.2		0.28
	Deschampsia abundance	LME	28	15.2		**<0.001**	10.2		**0.001**	0.9		0.35
	Forb abundance	LME	42	25.9		**<0.001**	79.8		**<0.001**	1.8		0.42
	Graminoid abundance	LME	42	26.1		**<0.001**	80.1		**<0.001**	1.8		0.41
	Richness	LME	42	7.9		**0.01**	4.0		0.14	5.1		0.08
	Composition	PERMANOVA	42	3.0	0.06	**0.003**	6.0	0.23	**0.001**	0.6	0.02	0.40
Soils	pH	LME	42	6.0		**0.02**	0.3		0.87	8.4		**0.02**
	Ammonium	LME	42	6.9		**0.01**	3.3		0.19	3.7		0.16
	Nitrate	LME	42	30.0		**<0.001**	1.2		0.55	0.4		0.82
	Moisture	LME	42	1.2		0.27	1.8		0.41	3.5		0.17

Notes: The Stat column refers to the test statistic (χ^2 for LME, pseudo-F for PERMANOVA). Bolded values highlight significant effects ($P < 0.05$). $n = 42$ for soils, plants, and bacterial statistics; $n = 28$ for *Deschampsia* and *Geum* abundance as the respective removal treatments were first subset out (df = 1 for the Plant Removal and Interaction for these two variables).

Dominant plants had little effect on soil properties

Our results showed a weak effect of the codominant plant species on soil properties. The soil was acidic with a pH that ranged from 4.4 to 5.1 (mean ± SD; 4.8 ± 0.2). There was an interaction between N addition and codominant plant removals such that soil pH was lower with *Geum* removals under added N relative to ambient N (Table 1; Fig. 3a). As a main effect, N addition caused a slight decline in pH; the mean pH in N addition plots (4.8 ± 0.2) was 1.3 times more acidic than the pH in the control plots (4.9 ± 0.2; Table 1). The available soil NO_3^- increased by over 1,000% with a mean of 3.5 ± 7.3 μg/g of soil under N addition (Table 1; Fig. 3b). The available soil NH_4^+ increased by 37% with a mean of 50 ± 16 μg/g of soil under N addition (Table 1; Fig. 3c). Contrary to our expectations, there was no effect of our codominant plants on soil N (Table 1). Soil moisture ranged from 18% to 84% (38% ± 18%), but it was not affected by treatment (Table 1; Fig. 3d).

The bacterial community was not influenced by the plant community, and shifts in plant composition did not mediate bacterial response to N

A total of 407 bacterial ESV were left following the removal of rare taxa, with a mean richness of 231 ± 28 ESV per sample. At the phylum level, we found that Acidobacteriota (27% of sequences), Proteobacteria (22%), and Chloroflexi (16%), dominated the bacterial communities. The class Acidobacteriae constituted 93% of the Acidobacteriota reads, Gammaproteobacteria and Alphaproteobacteria represented 66% and 34% of Proteobacteria reads, respectively, and Ktedonobacteria comprised 84% of Chloroflexi reads.

Contrary to our hypothesis that the plant community would mediate bacterial response to simulated N deposition, we found no interaction between N addition and plant removal (Table 2). Only N addition alone (Table 2; Fig. 2b), driven by increased soil NO_3^- (Table 3; Fig. 2d), shaped the soil bacterial community. The relative abundance of 11 bacterial phyla differed significantly between ambient and added N plots ($P < 0.05$; Fig. 4), including Acidobacteriota (declined 28% under N; Fig. 4a), Verrucomicrobiota (declined 53% under N addition; Fig. 4j), and Bacteroidota (increased 89% under N addition; Fig. 4c). There were 182 out of 407 bacterial ESV that were significantly affected by N addition with 77 ESV increasing with added N (including 20 ESV only detected in N addition plots (Appendix S1: Table S2)) and 105 declining with added N (including three ESV not detected in N addition plots (Appendix S1: Table S2)).

The fungal community was influenced by the plant community, but shifts in plant composition did not mediate fungal response to N

A total of 340 fungal ESV were left following the removal of rare taxa, with a mean richness of 126 ± 14 ESV per sample. We found that that Ascomycota (66% of sequences) and Basidiomycota (17%) dominated the fungal communities. Leotiomycetes and Archaeorhizomycetes made up 49% and 25% of Ascomycota reads, respectively, and Agaricomycetes made up 76% of Basidiomycota reads.

In contrast to the bacterial community, the fungal community was shaped by both N addition and plant removal, but contrary to our predictions, there were no interactive effects (Table 2; Fig. 2c). These effects were driven by soil NO_3^- and graminoid abundance (Table 3; Fig. 2e). Under N addition, the relative abundance of

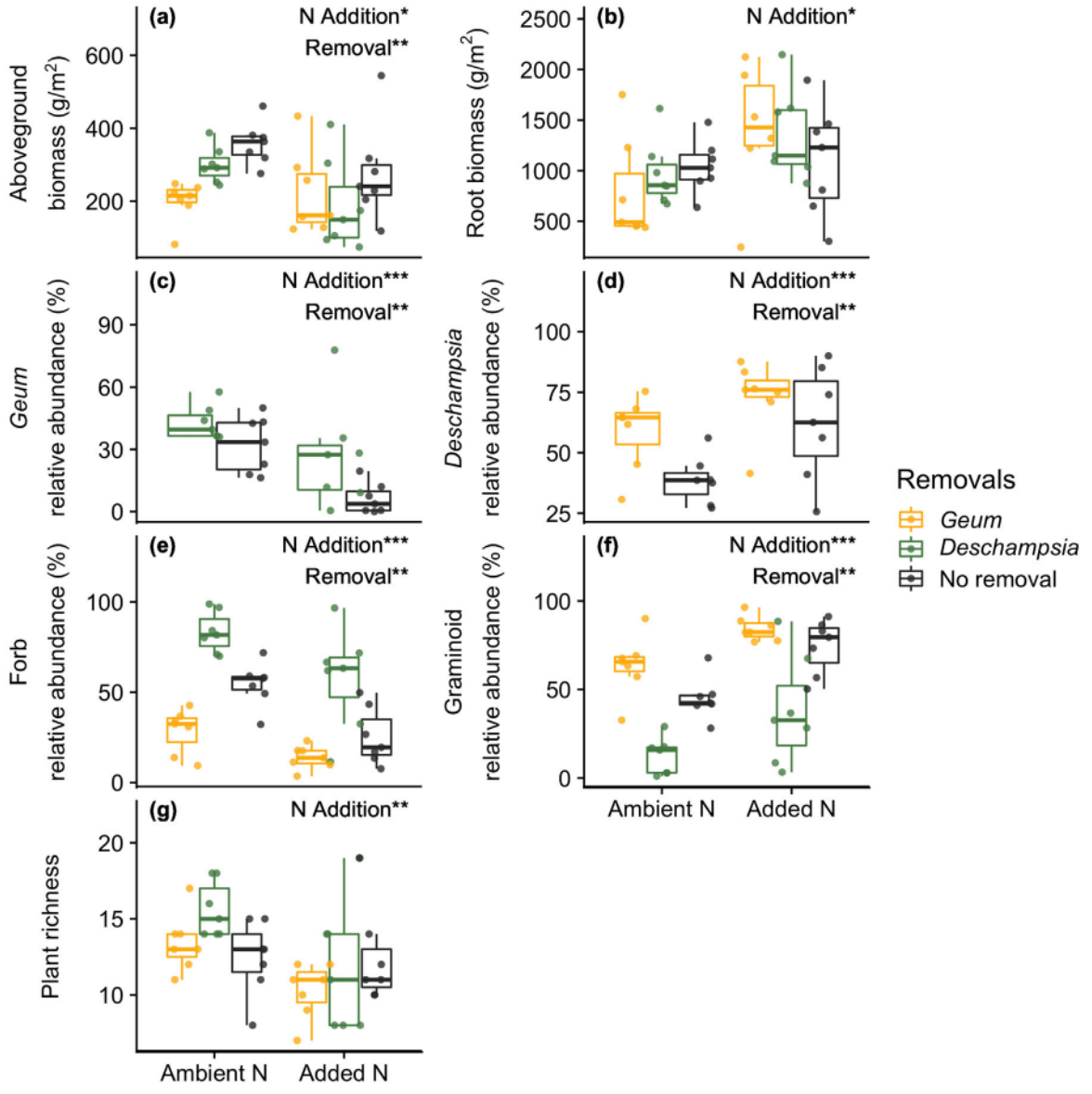

FIG. 1. Under ambient N, (a) aboveground biomass was lower with *Geum* removal relative to the control. (b) Root biomass was higher with N addition. (c) *Geum* declined under N addition and increased under *Deschampsia* removal, there was no interaction. (d) *Deschampsia* increased under both N addition and *Geum* removal, there was no interaction. (e) Forb abundance declined with N addition and *Geum* removal but increased with *Deschampsia* removal. (f) Graminoid abundance increased with N addition and *Geum* removal but decreased with *Deschampsia* removal. (g) N addition lowered plant species richness. Box plot components are mid line, median; box edges, first and third quartile (the 25th and 75th percentile); and whiskers, smallest and largest value no greater than 1.5 x the interquartile range. ***$P < 0.001$, **$0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$.

Ascomycota decreased by 11% ($P = 0.003$; Fig. 4l) while the relative abundance of Rozellomycota increased by 140% from 0.28% to 0.69% ($P = 0.01$; Fig. 4n). Twenty-seven out of 340 fungal ESV were significantly impacted by N addition with 17 of those ESV experiencing an increase in relative abundance (including one ESV only detected in N-addition plots that belonged to *Pseudogymnoascus destructans*) and 13 experiencing a decline in relative abundance ($P < 0.05$). There were no phylum-level differences in fungal composition between plots without codominant plant removals and plots with *Geum* removal. However, the relative abundance of Olpidiomycota declined 61% and 80% in *Deschamspia* removal plots relative to plots without codominant plant removals and plots with *Geum* removal, respectively ($P < 0.05$; Fig. 4m). There were three AMF genera detected in the data set subjected to the relative abundance threshold and 14 in the data set containing rare

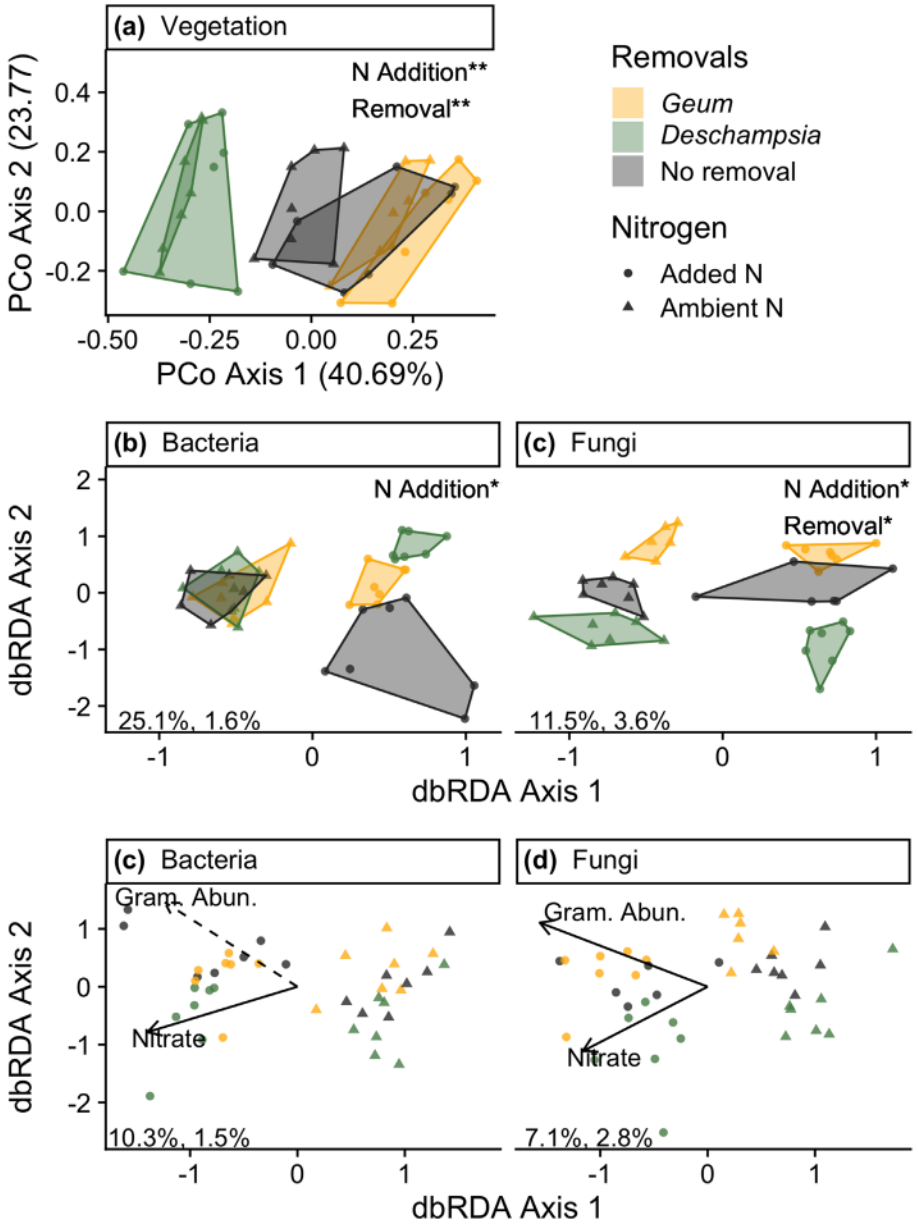

FIG. 2. (a) Both N addition and dominant plant removal influence plant community composition, but there was no interaction. (b) Only N addition shaped bacterial community composition while (c) both N addition and codominant plant removal impacted fungal community composition. Nitrate was a driver of both (d) bacterial and (e) fungal community composition, and the latter was also shaped by graminoid abundance. Percentages in the bottom left corner of each panel represent the variation explained by the first and second dbRDA axes, in that order. The dashed arrow indicates the predictor was not significant ($P > 0.05$). *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$.

taxa, but only one genus (an unidentified genus in the Ambisporaceae family) had significantly lower relative abundance under added N, declining by 64% from 0.39% to 0.14%. We found seven DSE genera in the data set subjected to the relative abundance threshold and 16 in the data set containing rare taxa, but only one genus (*Leptodontidium*) saw significantly lower relative abundance under added N, declining by 60% from 0.80% to 0.32%.

DISCUSSION

Understanding the cascading effects of the plant community on soil microbial communities under simulated N deposition is important for parsing apart the drivers of microbial community change resulting from this chronic stressor. While it is widely thought that biotic communities may have feedbacks that can amplify or buffer the effects of external drivers (reviewed by

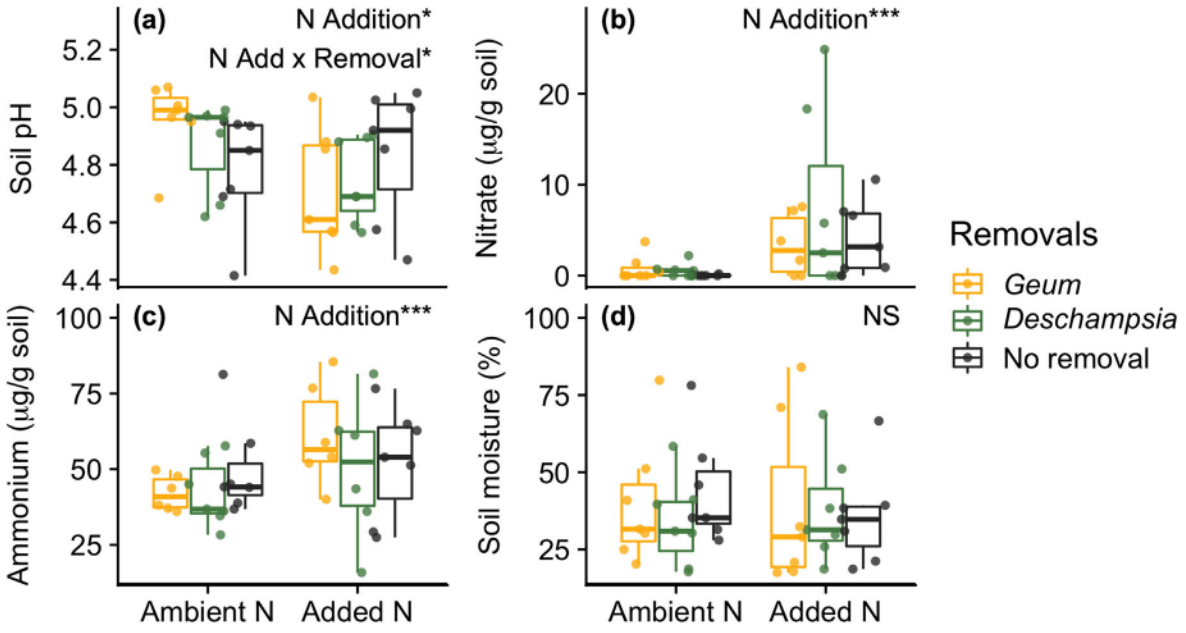

FIG. 3. (a) Soil pH was lowered by N addition only when the dominant plant *Geum* was removed. N addition drove an increase in (b) soil nitrate and (c) ammonium. (d) There were no significant effects on soil moisture. ***$P < 0.001$, **$0.001 \leq P < 0.01$, *$0.01 \leq P < 0.05$.

TABLE 2. N addition significantly impacted both the bacterial and fungal communities.

	Bacterial community			Fungal community		
Predictor variable	df	*F*	*P*	df	*F*	*P*
N addition	1	21.2	**0.001**	1	6.8	**0.001**
Plant removal	2	0.93	0.44	2	1.7	**0.02**
Interaction	2	0.88	0.53	2	0.90	0.66

Notes: Fungal communities were additionally shaped by the codominant plant removal treatment.

Values in boldface type highlight significant effects ($P < 0.05$).

TABLE 3. Nitrate and graminoid abundance were the continuous predictors selected via model selection from a suite of continuous predictors (nitrate, ammonium, pH, root biomass, aboveground biomass, and plant richness).

	Bacterial community			Fungal community		
Predictor variable	df	*F*	*P*	df	*F*	*P*
Nitrate	1	5.0	**0.02**	1	2.3	**0.04**
Graminoid abundance	1	2.27	0.40	1	3.0	**0.04**

Notes: Available nitrate significantly impacted both the bacterial and fungal communities. Fungal communities were additionally shaped by graminoid abundance.

Values in boldface type highlight significant effects ($P < 0.05$).

Tylianakis et al. 2008), our results suggest little evidence for these interactions between taxa at our site. The long-term application of N (at a level meant to saturate the system) and removal of codominant species represents a press disturbance where communities are set onto a new trajectory rather than being allowed to return to their predisturbance state. This design allowed us to assess how the alteration of plant communities under this disturbance may influence the assembly of the soil microbial community. After 18 yr of press disturbances our results demonstrate that though codominant plants influenced aboveground biomass and plant community composition, the plant community response to N did not mediate how the soil microbial community responded to simulated N deposition. Instead, bacterial communities were altered only by N addition while fungal communities were affected by both N addition and codominant plants, but without an interaction. This suggests that the altered quality and quantity of plant inputs as a result of N addition were not as important as external changes to resource availability in shaping soil microbial community response to N deposition.

In line with our hypothesis, we saw strong effects of 18 yr of codominant plant removals and N addition on plant community composition and productivity. The plant removal treatments effectively eliminated *Deschampsia* and *Geum* where intended and resulted in alterations to aboveground biomass and plant community composition. The removal of each codominant species released from

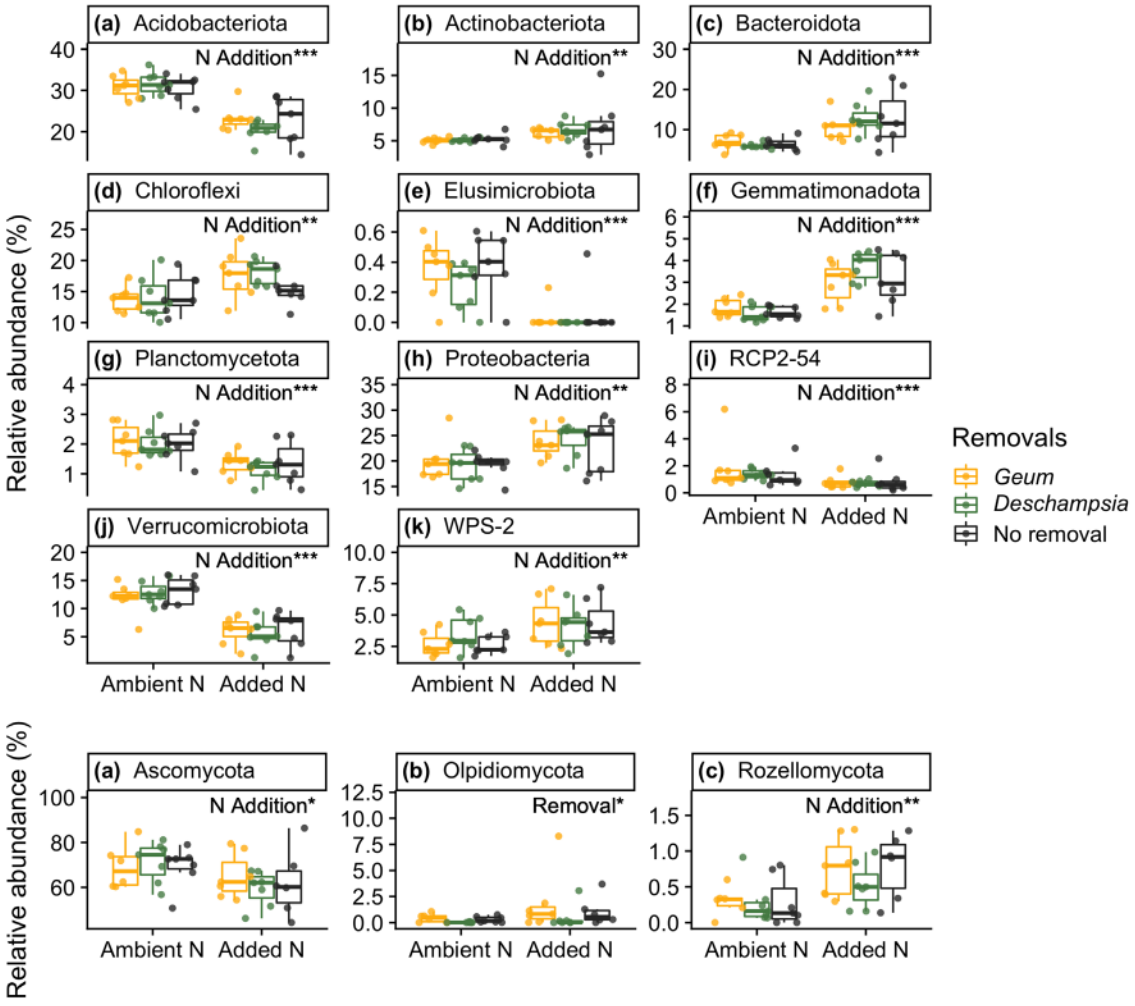

FIG. 4. There were (a–k) 11 bacterial phyla that differed significantly between N addition and control plots, (l and n) 2 fungal phyla that different significantly between N addition and control plots, and (m) one fungal phylum that was affected by dominant plant removal. *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$.

competition the functional type of the remaining codominant, primarily driven by an increase in abundance of the dominant itself, with both *Geum* removal and N addition increasing *Deschampsia* and overall graminoid abundance, as hypothesized.

In contrast to our hypothesis, we detected no effect of the codominant plant removal on N availability. That the presence of *Geum* or *Deschampsia* did not influence soil N availability was surprising given that previous work demonstrated strong effects of these two codominant species on nutrient cycling (Steltzer and Bowman 1998). However, Steltzer and Bowman (1998) documented nutrient cycling in patches where each of the codominant species were separately highly abundant; they appear to play a smaller role in nutrient cycling when they are part of more diverse plant communities, as is the case in our plots (~30% initial abundance of each of the codominant species).

We anticipated that the removal of these codominant species would alter soil N processes by altering the input of litter that slows (*Geum*) or hastens (*Deschampsia*) N cycling, thereby establishing a plant-microbial interaction that might mediate microbial response to N. In contrast to our hypothesis, we found similar responses of the soil microbial community to N despite shifts in the abundance of the codominant species and other aspects of the plant community, such as root biomass, aboveground biomass, and forb and graminoid abundance. This suggests that N acted directly on the soil bacterial and fungal communities rather than acting indirectly through buffering or amplifying effects of the plant community. The response of the microbial community generally indicated an environment that was more resource rich, which aligns with our finding of higher N availability and a significant effect of NO_3^- availability on the soil bacterial and fungal communities. The relative abundance of copiotrophic taxa,

which thrive in resource rich environments, such as Bacteroidota (Fierer et al. 2007), increased and the relative abundance of taxa on the other end of the spectrum, oligotrophic taxa, such as Acidobacteriota and Verrucomicrobiota, declined (Nemergut et al. 2008, Fierer et al. 2012, Ramirez and Craine 2012).

In the fungal community, the response was slightly less predictable. Because NO_3^- availability significantly affected the soil fungal community, we expected a decline in fungi that aid in plant nutrient uptake, such as AMF (Johnson et al. 2010) and DSE (Newsham 2011), because plants generally devote less photosynthate carbon (C) to such fungi under higher resource scenarios (Read 1991). The weak response of AMF and DSE genera to N addition may be because N addition led to higher demand for phosphorus, maintaining the need for relationships with fungi that aid in nutrient acquisition. This possibility is supported by other work at our site that demonstrated that neither AMF nor DSE genera within the roots of *Geum* and *Deschampsia* declined with N addition (Dean et al. 2014) and that the total amount of C allocated to Ascomycota (the phylum containing most DSE) by *Geum* and *Deschampshia* did not decline under N addition (Farrer et al. 2013). It is also possible that the lack of AMF response was due to their overall low abundance and/or because specific mycorrhizal primers were not used, hence limiting our conclusions regarding this group. Altogether, there were large shifts in both the soil bacterial and fungal communities as a result of N addition, which occurred independently of shifts in the plant community.

The lack of mediating effects of the codominant plant species on soil microbial response to N may be because the presence of *Geum* or *Deschampsia* did not strongly influence soil N availability or root biomass. Hence, the role of the plant community in shaping soil chemistry and resource availability is likely an important factor that determines whether the plant community mediates soil microbial response to N addition (Yuan et al. 2016, Zeng et al. 2016) or whether N addition acts directly on the soil microbial community (Ramirez et al. 2010, Wardle et al. 2013). While we did not find mediating effects of the plant community on microbial response to N, the presence of the codominant plant species did affect the fungal community, driven by the abundance of graminoids, which was positively correlated with the abundance of *Deschampsia* and negatively correlated with the abundance of *Geum* and other forbs. The decrease in Olpidiomycota, which contains known plant pathogens (Tedersoo et al. 2018), in the *Deschampsia* removal plots may suggest that there are pathogens specific to *Deschampsia* that decline in its absence. These results indicate that plant inputs, such as litter and root exudates, as well as specific plant–microbe associations influenced the soil fungal community though they did not shape how the soil fungal community responded to N addition.

Conclusions

This manipulative experiment demonstrates that the effects of simulated N deposition on the soil bacterial and fungal community were not mediated by the plant community but were instead manifest through increased resource availability. Dominant plant species, despite their impacts on aboveground biomass and plant community composition, neither buffered nor amplified the response of the soil microbial community to N addition. Changes to the soil microbial community occurred independently of directional shifts in the plant community, suggesting there are not strong cascading effects of N addition across the plant–soil interface in our system. More broadly, our results highlight the importance of understanding when indirect effects shape community response to global change in order to improve our ability to predict how biodiversity will respond to change.

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Literature Cited

Abarenkov, K., et al. 2010. The UNITE database for molecular identification of fungi—recent updates and future perspectives. New Phytologist 186:281–285.

Anthelme, F., L. A. Cavieres, and O. Dangles. 2014. Facilitation among plants in alpine environments in the face of climate change. Frontiers in Plant Science 5:1–15.

Bates, D., M. Mächler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Blanchet, F. G., P. Legendre, and D. Borcard. 2008. Forward selection of explanatory variables. Ecology 89:2623–2632.

Bobbink, R., et al. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications 20:30–59.

Bowman, W. D., A. Ayyad, C. P. Bueno de Mesquita, N. Fierer, T. S. Potter, and S. Sternagel. 2018. Limited ecosystem recovery from simulated chronic nitrogen deposition. Ecological Applications 28:1762–1772.

Bowman, W. D., C. C. Cleveland, Ĺ. Halada, J. Hreško, and J. S. Baron. 2008. Negative impact of nitrogen deposition on soil buffering capacity. Nature Geoscience 1:767–770.

Bowman, W. D., J. R. Gartner, K. Holland, and M. Wiedermann. 2006. Nitrogen critical loads for alpine vegetation and terrestrial ecosystem response: are we there yet? Ecological Applications 16:1183–1193.

Bowman, W. D., H. Steltzer, T. N. Rosenstiel, C. C. Cleveland, and C. L. Meier. 2004. Litter effects of two co-occurring alpine species on plant growth, microbial activity and immobilization of nitrogen. Oikos 104:336–344.

Bowman, W. D., T. A. Theodose, J. C. Schardt, and T. Richard. 1993. Constraints of nutrient availability on primary production in two alpine tundra communities. Ecology 74:2085–2097.

Brooker, R. W. 2006. Plant–plant interactions and environmental change. New Phytologist 171:271–284.

Burgess, T. I., et al. 2017. Current and projected global distribution of *Phytophthora cinnamomi*, one of the world's worst plant pathogens. Global Change Biology 23:1661–1674.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods 13:581–583.

Castro, H. F., A. T. Classen, E. E. Austin, R. J. Norby, and C. W. Schadt. 2010. Soil microbial community responses to multiple experimental climate change drivers. Applied and Environmental Microbiology 76:999–1007.

Chen, D., W. Xing, Z. Lan, M. Saleem, Y. Wu, S. Hu, and Y. Bai. 2019. Direct and indirect effects of nitrogen enrichment on soil organisms and carbon and nitrogen mineralization in a semi-arid grassland. Functional Ecology 33:175–187.

Classen, A. T., M. K. Sundqvist, J. A. Henning, G. S. Newman, J. A. M. Moore, M. A. Cregger, L. C. Moorhead, and C. M. Patterson. 2015. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: what lies ahead? Ecosphere 6:art130.

Cleland, E. E., and W. S. Harpole. 2010. Nitrogen enrichment and plant communities. Annals of the New York Academy of Sciences 1195:46–61.

Crawford, J. T., E. L. S. Hinckley, and J. C. Neff. 2020. Long-term trends in acid precipitation and watershed elemental export from an alpine catchment of the Colorado Rocky Mountains, USA. Journal of Geophysical Research: Biogeosciences 125:1–17.

De Long, J. R., E. Dorrepaal, P. Kardol, M. C. Nilsson, L. M. Teuber, and D. A. Wardle. 2016. Contrasting responses of soil microbial and nematode communities to warming and plant functional group removal across a post-fire boreal forest successional gradient. Ecosystems 19:339–355.

Dean, S. L., E. C. Farrer, D. Lee Taylor, A. Porras-Alfaro, K. N. Suding, and R. L. Sinsabaugh. 2014. Nitrogen deposition alters plant-fungal relationships: linking belowground dynamics to aboveground vegetation change. Molecular Ecology 23:1364–1378.

Du, E., W. De Vries, J. N. Galloway, X. Hu, and J. Fang. 2014. Changes in wet nitrogen deposition in the United States between 1985 and 2012. Environmental Research Letters 9: 095004.

Farrer, E. C., D. J. Herman, E. Franzova, T. Pham, and K. N. Suding. 2013. Nitrogen deposition, plant carbon allocation, and soil microbes: changing interactions due to enrichment. American Journal of Botany 100:1458–1470.

Fierer, N., M. A. Bradford, and R. B. Jackson. 2007. Toward an ecological classification of soil bacteria. Ecology 88:1354–1364.

Fierer, N., C. L. Lauber, K. S. Ramirez, J. Zaneveld, M. A. Bradford, and R. Knight. 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME Journal 6:1007–1017.

Gaston, K. J. 2011. Common ecology. BioScience 61:354–362.

Grime, J. P. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. Journal of Ecology 86:902–910.

Holling, C. S. 1973. Resilience and stability of ecological systems. Annual Review of Ecology, Evolution, and Systematics 4:1–23.

Johnson, N. C., G. W. T. Wilson, M. A. Bowker, J. A. Wilson, and R. M. Miller. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences USA 107:2093–2098.

Jumpponen, A., and J. M. Trappe. 1998. Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytologist 140:295–310.

Lankau, R. A., and S. Y. Strauss. 2007. Mutual feedbacks maintain both genetic and species diversity in a plant community. Science 317:1561–1563.

Lauber, C. L., M. Hamady, R. Knight, and N. Fierer. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75:5111–5120.

Lebauer, D. S., and K. K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89:371–379.

Leff, J. W. 2017. mctoolsr: microbial community data analysis tools.

Leff, J. W., R. C. Lynch, N. C. Kane, and N. Fierer. 2017. Plant domestication and the assembly of bacterial and fungal communities associated with strains of the common sunflower, *Helianthus annuus*. New Phytologist 214:412–423.

Lenth, R., H. Singmann, J. Love, P. Buerkner, and M. Herve. 2018. Emmeans: estimated marginal means, aka least-squares means.

Lieb, A. M., A. Darrouzet-Nardi, and W. D. Bowman. 2011. Nitrogen deposition decreases acid buffering capacity of alpine soils in the southern Rocky Mountains. Geoderma 164:220–224.

Liu, X., et al. 2013. Enhanced nitrogen deposition over China. Nature 494:459–462.

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10–12.

Nemergut, D. R., A. R. Townsend, S. R. Sattin, K. R. Freeman, N. Fierer, J. C. Neff, W. D. Bowman, C. W. Schadt, M. N. Weintraub, and S. K. Schmidt. 2008. The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: implications for carbon and nitrogen cycling. Environmental Microbiology 10:3093–3105.

Newsham, K. K. 2011. A meta-analysis of plant responses to dark septate root endophytes. New Phytologist 190:783–793.

Oksanen, J., et al. 2019. vegan: community ecology package. R package version 2.5-6. https://cran.r-project.org/web/packages/vegan/index.html

Oliverio, A. M., M. A. Bradford, and N. Fierer. 2017. Identifying the microbial taxa that consistently respond to soil warming across time and space. Global Change Biology 23:2117–2129.

Olsen, S. L., J. P. Töpper, O. Skarpaas, V. Vandvik, and K. Klanderud. 2016. From facilitation to competition: temperature-driven shift in dominant plant interactions affects population dynamics in seminatural grasslands. Global Change Biology 22:1915–1926.

Payne, R. J., N. B. Dise, C. D. Field, A. J. Dore, S. J. M. Caporn, and C. J. Stevens. 2017. Nitrogen deposition and plant biodiversity: past, present, and future. Frontiers in Ecology and the Environment 15:431–436.

Pires, A. P. F., D. S. Srivastava, and V. F. Farjalla. 2018. Is biodiversity able to buffer ecosystems from climate change? What we know and what we don't. BioScience 68:273–280.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41:590–596.

R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org

Ramirez, K. S., J. M. Craine, and N. Fierer. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology 18:1918–1927.

Ramirez, K. S., C. L. Lauber, R. Knight, M. A. Bradford, and N. Fierer. 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology 91:3463–3470.

Read, D. J. 1991. Mycorrhizas in ecosystems–Nature's response to the "Law of the minimum". Pages 101–130 *in* D. L. Hawksworth, editor. Frontiers in mycology. CAB International, Regensburg, Germany.

Rodrigues, R. R., R. P. Pineda, J. N. Barney, E. T. Nilsen, J. E. Barrett, and M. A. Williams. 2015. Plant invasions associated with change in root-zone microbial community structure and diversity. PLoS ONE 10:1–19.

Schüßler, A., D. Schwarzott, and C. Walker. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycological Research 105:1413–1421.

Shao, Y., T. Liu, N. Eisenhauer, W. Zhang, X. Wang, Y. Xiong, C. Liang, and S. Fu. 2018. Plants mitigate detrimental nitrogen deposition effects on soil biodiversity. Soil Biology and Biochemistry 127:178–186.

Sievering, H. 2001. Atmospheric chemistry and deposition. Pages 32–44 *in* W. D. Bowman, and T. R. Seastedt, editors. Structure and function of an alpine ecosystem: Niwot Ridge, Colorado. Oxford University Press, New York, New York, USA.

Stefanowicz, A. M., M. L. Majewska, M. Stanek, M. Nobis, and S. Zubek. 2018. Differential influence of four invasive plant species on soil physicochemical properties in a pot experiment. Journal of Soils and Sediments 18:1409–1423.

Steltzer, H., and W. D. Bowman. 1998. Differential influence of plant species on soil nitrogen transformations within moist meadow alpine tundra. Ecosystems 1:464–474.

Street, L. E., N. R. Burns, and S. J. Woodin. 2015. Slow recovery of High Arctic heath communities from nitrogen enrichment. New Phytologist 206:682–695.

Suding, K. N., I. W. Ashton, H. Bechtold, W. D. Bowman, K. N. Suding, I. W. Ashton, H. Bechtold, W. D. Bowman, M. L. Mobley, and R. Winkleman. 2008. Plant and microbe contribution to community resilience in a directionally changing environment. Ecological Monographs 78:313–329.

Suding, K. N., A. E. Miller, H. Bechtold, and W. D. Bowman. 2006. The consequence of species loss on ecosystem nitrogen cycling depends on community compensation. Oecologia 149:141–149.

Suding, K. N., J. G. Smith, L. M. Brigham, and NWT-LTER. 2021. Co-dominant removal and N and C fertilization experiment for moist meadow tundra from 2002 to 2018 ver 4. Environmental Data Initiative. https://doi.org/10.6073/pasta/256cc59654ffbc97fb56adf7688f02c0

Suttle, K. B., M. A. Thomsen, and M. E. Power. 2007. Species interactions reverse grassland responses to changing climate. Science 315:640–642.

Tedersoo, L., et al. 2014. Global diversity and geography of soil fungi. Science 346:1256688.

Tedersoo, L., S. Sánchez-Ramírez, U. Kõljalg, M. Bahram, M. Döring, D. Schigel, T. May, M. Ryberg, and K. Abarenkov. 2018. High-level classification of the Fungi and a tool for evolutionary ecological analyses. Fungal Diversity 90:135–159.

Tylianakis, J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. Ecology Letters 11:1351–1363.

Walker, M., and C. Wahren. 2006. Plant community responses to experimental warming across the tundra biome. Proceedings of the National Academy of Sciences USA 103:1342–1346.

Ward, S. E., K. H. Orwin, N. J. Ostle, M. J. I. Briones, B. C. Thomson, R. I. Griffiths, S. Oakley, H. Quirk, and R. D. Bardgett. 2015. Vegetation exerts a greater control on litter decomposition than climate warming in peatlands. Ecology 96:113–123.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science 304:1629–1633.

Wardle, D. A., M. J. Gundale, A. Jäderlund, and M. C. Nilsson. 2013. Decoupled long-term effects of nutrient enrichment on aboveground and belowground properties in subalpine tundra. Ecology 94:904–919.

Yuan, X., J. E. Knelman, E. Gasarch, D. Wang, D. R. Nemergut, and T. R. Seastedt. 2016. Plant community and soil chemistry responses to long-term nitrogen inputs drive changes in alpine bacterial communities. Ecology 97:1543–1554.

Zeng, J., X. Liu, L. Song, X. Lin, H. Zhang, C. Shen, and H. Chu. 2016. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. Soil Biology and Biochemistry 92:41–49.

Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3554/suppinfo

Open Research

Environmental data and metadata (Suding et al. 2021) are archived in the Environmental Data Initiative repository: https://doi.org/10.6073/pasta/256cc59654ffbc97fb56adf7688f02c0. Sequencing data are accessible on GenBank via BioProject accession no. PRJNA748005 at https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA748005