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PLANT AND MICROBE CONTRIBUTION TO COMMUNITY RESILIENCE IN A DIRECTIONALLY CHANGING ENVIRONMENT

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Abstract. To understand the role biota play in resilience or vulnerability to environmental change, we investigated soil, plant, and microbial responses to a widespread environmental change, increased nitrogen (N). Our aim was to test the plant–soil threshold hypothesis: that changed biotic structure influences resilience to accumulated changes in N. For six years, we removed one of two codominant species, *Geum rossii* and *Deschampsia caespitosa*, in moist-meadow alpine tundra in Colorado, USA. We also manipulated nutrient availability by adding carbon (C) or N, separately and in combination with the species removals.

Consistent with our hypothesis, *Geum* was associated with soil feedbacks that slowed rates of N cycling and *Deschampsia* with feedbacks that increased rates of N cycling. After a four-year initial resilience period, *Geum* dramatically declined (by almost 70%) due to increasing N availability. In contrast, *Deschampsia* abundance did not respond to changes in N supply; it only responded to the removal of *Geum*. Forbs and graminoids responded more positively to *Deschampsia* removal than to *Geum* removal, indicating stronger competitive effects by *Deschampsia*. The changed biotic interactions appear to have community-level consequences: after six years of *Geum* (but not *Deschampsia*) removal, evenness of the community declined by over 35%.

Increased N affected the soil–microbial feedbacks, particularly in association with *Geum*. Microbial biomass N declined at higher N, as did the activities of two C-acquiring and one N-acquiring extracellular microbial enzymes. In the presence of *Geum*, N fertilization slowed the activity of phenol oxidase, a tannin-degrading enzyme, suggesting that microbes shift from degrading *Geum*-derived compounds. In the absence of *Geum*, acid phosphatase activity increased, suggesting increased phosphorus limitation in association with *Deschampsia*.

With continued N deposition forecast for this system, these results suggest that initial resilience of *Geum* to increased N will be overwhelmed through elimination of microbial feedbacks. Once *Geum* declines, the loss will indirectly facilitate *Deschampsia* via competitive release. Because *Deschampsia* exerts strong competitive effects on subordinate species, increased *Deschampsia* abundance may be accompanied by a community-wide drop in diversity. We conclude that plant–soil feedbacks through the microbial community can influence vulnerability to exogenous changes in N and contribute to threshold dynamics.

Key words: alpine tundra; Deschampsia caespitosa; *environmental change;* Geum (Acomastylis) rossi; *microbial extracellular enzyme activity; nitrogen deposition; Niwot Ridge, Rocky Mountains, Colorado, USA; plant competition; plant–soil feedbacks; resilience; species loss; thresholds.*

INTRODUCTION

Human activities are altering, at unprecedented rates, many of the processes that structure ecological communities (Vitousek et al. 1997, Chapin et al. 2000, Tilman et al. 2001). For instance, many ecosystems are experiencing changes in climate and disturbance regimes, resource availability, and regional species pools. While it is clear that these changes are occurring and that they can have profound ecological impacts, it is often difficult to predict the future structure of communities due to the complexity of direct and indirect interactions affected by the changes. In particular, the sensitivity of systems to a sustained change (i.e., a press sensu Underwood [1994]) in the environment may depend on the resilience conferred by their biota (Walker et al. 1999, Allison 2004, Nystrom 2006).

Plant species can either stabilize the effects of environmental change or, if they are pushed beyond their tolerances, their initial resilience and then sudden demise can lead to strongly nonlinear threshold dynamics (van Nes and Scheffer 2004, Chapin et al. 2006). Biotic vulnerability to environmental change has been ascribed to food webs with few links or low interaction

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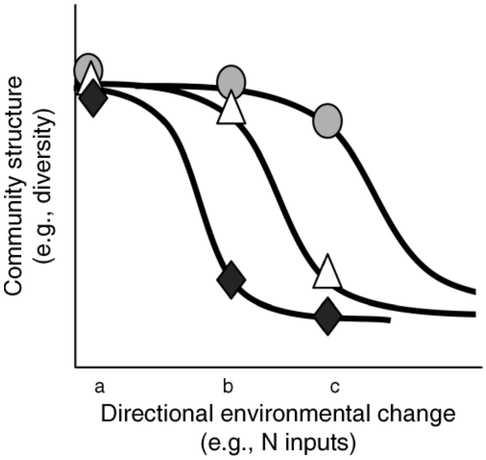

FIG. 1. Biotic feedback could influence how a system responds to directional environmental change, either by accelerating or counteracting the change. We assume a threshold response, where community structure is altered and diversity decreases in response to environmental change. If environmental change is accelerated due to changed species interactions, the system would be less resistant to environmental change, and exogenous forces would have stronger-than-anticipated effects on community structure (solid diamonds). Alternatively, dominant species could exert counteracting feedbacks on the local environment and increase resistance to change. In this case, the exogenous forcing would have less-than-anticipated effects on community structure over a given time period (gray circles). We use this framework to depict our predictions about effects of biotic structure and N availability. Symbols refer to effects of species due to manipulations of biotic interactions: solid diamonds, removal of an N-conservative species (*Geum rossii*); open triangles, ambient conditions; gray circles, removal of an N-exploitative species (*Deschampsia caespitosa*). Our N-availability manipulations simulate a nitrogen gradient that could occur over time due to increased N deposition: a = C addition to reduce N availability; b = ambient N conditions; and c = N addition.

strengths (Ruesink and Srivastava 2001, O'Connor and Crowe 2005), changes that disproportionately affect a keystone or dominant species (Allison 2004, Chapin et al. 2006), low functional redundancy or response diversity (Walker et al. 1999, Elmqvist et al. 2003, Nystrom 2006), or lack of strong internal stabilizing feedbacks (Ehrenfeld 2003, Bezemer et al. 2006). Here we investigate whether plant–soil feedbacks through the microbial community can accelerate or slow vulnerability to exogenous environmental change, contributing to threshold dynamics.

It is often thought that the plant species can play a particularly critical role in feedback loops that stabilize important environmental variables such as the supply of soil nitrogen (N) (Bowman and Steltzer 1998, Evans et al. 2001, Reynolds et al. 2003) as well as influence competitive interactions (Casper and Castelli 2007, Kardol et al. 2007). Changes in the abundance of plant species in such feedbacks could lead to disproportionately large threshold changes in community structure. Abundant species, because they account for the most biomass, and keystone species, because they have ecological impacts disproportionate to their biomass, are most likely to influence threshold dynamics (Power et al. 1996, Grime 1998). If these species are lost due to environmental change, soil feedback loops may shift so substantially that the repercussions to the rest of the community are dramatic and sudden. For instance, if N-conservative species that exert counteracting feedbacks on the local environment are replaced by nitrophilic species, then exogenous increases in N availability may have stronger-than-anticipated effects on community structure due to changed species interactions (Fig. 1). Alternatively, the presence of N-conservative species could increase community resilience and resistance to change. In that case, exogenous forcing would have less-than-anticipated effects on community structure. Such changes may be key sources of surprise in management because they are hard to predict based on effects of the environmental change prior to the biotic collapse (Suding et al. 2004, Groffman et al. 2006).

Virtually all systems are experiencing increased N availability due to atmospheric deposition (Matson et al. 2002, Vitousek et al. 2002). Because the availability of N limits the productivity of many terrestrial temperate systems (Vitousek and Howarth 1991), changes in N supply can strongly influence plant abundance and diversity (Bobbink et al. 1998, Matson et al. 2002, Fenn et al. 2003, Stevens et al. 2004). Nitrogen fertilization has been a common experimental approach to study these effects, and most fertilization studies have shown that increased N supply alters diversity and composition (Gough et al. 2000, Shaver et al. 2001, Bowman et al. 2006). In our present study we both increase and decrease the ambient supply of N to examine how species loss alters composition and function along a gradient in N availability.

Microbial community structure and function is emerging as one key link between exogenous changes in N and plant–soil effects. Increased N availability can directly affect microbial structure (Treseder 2004, Sinsabaugh et al. 2005, Stursova et al. 2006). In addition, plant effects on N cycling in soils can occur indirectly via microbial composition and activity (Hobbie 1992, Aerts 1999, Ehrenfeld et al. 2005). One mechanism by which microbes can influence soil feedbacks is the production of extracellular enzymes (Dakora and Phillips 2002). Extracellular enzymes mediate the decomposition of litter, the breakdown of organic matter, and the mineralization of N and phosphorus (Sinsabaugh 1994). Extracellular-enzyme activity can indicate the sensitivity of microbial function to plant species loss (Kourtev et al. 2002, Allison et al. 2006), contributing to resilience or vulnerability to environmental change.

Although ecologists recognize that plants and microbes may sometimes regulate and precipitate threshold dynamics (Chapin et al. 2004, Groffman et al. 2006), studies that examine the effects of altered nutrient supply rarely also investigate the role of biotic structure and feedbacks through the microbial community. For instance, little is known about how plant community structure contributes to the overall changes observed in N-fertilization experiments. Many of the studies that have focused on microbial and biogeochemical effects of N fertilization have been conducted in ecosystems where plant species turnover is slow and assumed not to be an important factor in ecosystem response (Waldrop et al. 2004, Sinsabaugh et al. 2005). In cases when both plant and microbial change occur, effects of altered nutrient supply on microbial structure and function (Mack et al. 2004, Schmidt et al. 2004, Bradley et al. 2006, Stursova et al. 2006, Rinnan et al. 2007) are often considered separately from concurrent changes in plant structure and function (Seastedt and Vaccaro 2001, Gough et al. 2002, Pauli et al. 2002). In the few experiments that have manipulated both N supply and biotic structure, direct limitation of environmental conditions appears to be more important than species interactions, with surprisingly little interaction among factors (Bret-Harte et al. 2004, Brooker 2006, Manning et al. 2006). While these results bring into question the assumed importance of plant species to system resilience, other work suggests that system vulnerability will vary depending on characteristics of biotic structure (Bardgett et al. 1999, Eviner and Chapin 2002, Scherer-Lorenzen et al. 2003, Allison 2004, Chapin et al. 2006). Thus, to understand the role biota play in resilience to environmental change, it is essential to understand the reciprocal interactive nature of the relationships between plant–soil feedbacks and limiting factors (Hobbie et al. 1999, Nilsson et al. 2002, Bret-Harte et al. 2004).

In this study we investigated soil, plant, and microbial responses to a widespread environmental change, increased N supply, and how its effects are mediated by changes in biotic structure. In the Front Range of the Rocky Mountains in Colorado, USA, there is evidence that atmospheric N deposition (currently ~6 $\mathrm{kg \cdot ha^{-1} \cdot yr^{-1}}$; Sievering 2001) is affecting plant community structure and diversity via changes in N supply (Bowman et al. 2006). Over the past 20 years, the biotic structure of moist-meadow alpine tundra appears to be shifting from codominance of two plant species (*Geum rossii*, *Deschampsia* caespitosa) that are associated with divergent effects on net N cycling (decreased and increased rates, respectively) (Steltzer and Bowman 1998, Bowman et al. 2004) to the dominance of *Deschampsia*. This is an ideal study system to test the importance of biotic resilience to changes in N supply due to the codominance of two plant species associated with very different soil effects (see Plate 1). The codominance allows us to conduct experimental manipulations within one system that has relevance to the general dichotomy of plant strategies and plant species effects common across systems (Chapin 1980, 2003, Craine et al. 2002, Diaz et al. 2004), thereby encompassing differences that could greatly influence plant–soil feedbacks in response to N fertilization.

Our experimental approach involved removing species and manipulating N supply. As long-term records indicate that changes in species composition have already occurred, we both decreased and increased N supply rates from ambient levels. We examined effects of N supply and species loss separately, and in combination, over six years to determine whether changes in biotic structure (in this case the loss of either one of the codominant species) influence the vulnerability of the system to accumulated changes in N. Specifically, we tested the following set of predictions:

1) Plant species vary in their sensitivity to sustained directional changes in a critical environmental factor (e.g., N supply). Species with strong soil feedbacks that act to slow N cycling may be particularly vulnerable to increases in N availability, while species that act to accelerate N cycling may benefit most from enhanced N.

2) Plant species associated with slow rates of N cycling may be able to counteract the effects of exogenous increases in N in the short term, maintaining their abundance and strong interactions with other species in the community. As a result, the community as a whole (e.g., in terms of diversity, evenness, composition) will initially be resilient to exogenous changes.

3) Eventual loss of the species associated with feedbacks that counteract the direction of exogenous change would dramatically curtail resiliency, causing abrupt and noncontinuous responses in microbial function and plant community characteristics (Fig. 1).

Together, we refer to these three predictions as the "plant–soil threshold hypothesis" because it posits that resiliency depends on species interactions mediated by sensitivities of plant–soil feedbacks to exogenous change. Alternatively, either environmental limitation or biotic control could determine community resilience, with few interactive effects. Environmental limitation would predict that sustained changes in a critical environmental factor (such as our manipulations of N availability) would change biotic structure but that these changes will not depend on plant species interactions within the community. Conversely, biotic control would predict that loss of particular plant species (which we simulate with species removal), regardless of underlying environmental cause, drives changes in resilience. Because we manipulated N availability and species loss separately and in all possible combinations over a six-year time course, we are able to distinguish among the plant–soil threshold hypothesis and these alternatives. By explicitly testing how plant–soil feedbacks and directional environmental change can interact to influence system resilience, we extend previously developed theory of threshold effects (May 1977, Gunderson 2000, Scheffer and Carpenter 2003, Bestelmeyer 2006, Groffman et al. 2006).

METHODS

We conducted our experiment in moist-meadow alpine tundra on Niwot Ridge (40°03′ N, 105°35′ W) in the Front Range of the Rocky Mountains, Colorado, USA. We focused on *Geum rossii* (R. Br) C. L. Hitchc. (formerly *Acomastylis rossii*), a roseaceous forb, and *Deschampsia caespitosa* (L.) P. Beauv., a tillering bunchgrass, the two most abundant species (each approximately 30% cover) at the study site (May and Webber 1982). While these two species are roughly equivalent in cover, they are functionally distinct, particularly in relation to N cycling. *Deschampsia* grows faster, has a lower foliar C-to-N ratio, and is associated with faster net N mineralization than *Geum*, a nitrogen-conservative species (Steltzer and Bowman 1998, Miller and Bowman 2002, Bowman et al. 2004). High phenolic concentrations produced by *Geum* appear to be associated with high microbial activity and high net N immobilization (Steltzer and Bowman 1998, Bowman et al. 2004).

Experimental design

In June 2001 we selected seven moist-meadow sites. The sites varied in elevation from 3397 m to 3544 m and were aligned across the ridge within an area of ~5 km^2. We used site as a replicate blocking factor due to the space limitations within any one moist-meadow area. Within each of the seven sites, we selected 10 1-m^2 plots that had equal proportions of *Geum* and *Deschampsia* cover. Cover was determined nondestructively with point-quadrant frame sampling. Plots were separated by at least 2 m and trenched to a depth of 15 cm three times during each growing season. Of the 10 plots, we randomly selected 3 plots in which we removed *Geum*, 3 plots in which we removed *Deschampsia*, 1 plot in which we removed biomass randomly in proportion to site productivity, and 3 plots where no biomass was removed. Removal treatments consisted of repeatedly clipping the selected species to ground level. All clipped biomass was collected, dried to a constant mass, and weighed. Clipping of these perennial species does not initially cause mortality of the individuals, and thus does not create a large flush of soil C from decaying roots. Instead, root death likely occurred slowly due to repeated clipping of aboveground biomass. Because root C (and root-associated organisms) likely have a stronger influence on microbial communities than aboveground litter (Brant et al. 2006), we expect that the effects associated with the focal species dissipated slowly in the first two years of the removal treatments. The removal treatments were initiated in 2001 and continued for the 2002, 2003, 2004, 2005, and 2006 growing seasons.

In combination with the removal treatments, we manipulated resource availability by adding N or C to one of each of the removal plots, with the exception of the random biomass control. We selected plots for each treatment randomly with the constraint that the N-addition plots were never within 5 m uphill from any other type of plot. Nitrogen was added at the beginning of the growing season each year, starting in 2001, using Osmocote slow-release fertilizer (urea 40-0-0 [N–P–K]) at a maximum rate of 16 g $N \cdot m^{-2} \cdot yr^{-1}$. We expect the actual N made available within each plot was <16 g $N \cdot m^{-2} \cdot yr^{-1}$ (we estimate 10 g $N \cdot m^{-2} \cdot yr^{-1}$) due to surface water flow (which is high at the beginning of the season due to snowmelt) and incomplete release of the slow-release pellets (Bowman et al. 1993). The application rate was lower than or equivalent to many other N-fertilization experiments previously conducted at the site (Bowman et al. 1993, 1995, Seastedt and Vaccaro 2001) but higher than estimated critical loads of N in an adjacent community type (Bowman et al. 2006). Carbon was added three times during the growing season at a rate of 900 g $C \cdot m^{-2} \cdot yr^{-1}$ in a mixture of equal parts glucose, cellulose, and lactose.

Response over time

In the five years following the initiation of the experiment (2002–2006) we measured resin-extractable N and species composition. To estimate plant-available N in soils, three ion-exchange resin tubes (2 cm length × 2 cm diameter cylinders wrapped in nylon mesh) were placed at a depth of 10 cm within each plot at the start of the growing season, collected after one year, extracted with 1 mol/L KCl, and analyzed on a continuous-flow autoanalyzer (QuikChem 8000 [Lachat Instruments, Milwaukee, Wisconsin, USA]) in the Kiowa Analytical Laboratory (Nederland, Colorado, USA) for nitrate, nitrite, and ammonium. Species composition was determined at the peak of the growing season using a point–intercept method where species presence was recorded at 100 points within a 1 × 1 m grid at regular 10-cm intervals. When species were present but did not occur at a grid point, they were counted with a value of 0.5 (corresponding to <0.5% cover). Relative abundance was estimated as the proportion of points with vegetation that contained both species. We also took initial measures of composition prior to the initiation of the experimental treatments to standardize starting conditions (in 2001, see *Experimental design*, above). However, we do not include that data point in the compositional time series (2002–2006) because it was taken early in the season rather than at peak growing season.

In addition to the annual measurements of estimated N supply and species composition, net primary productivity was estimated across all sites in 2006. In 2006 we collected aboveground biomass from three 20 × 20 cm subplots within each of the 70 plots. In all cases, aboveground biomass was sorted into litter, *Geum*, *Deschampsia*, and other species, dried at 70°C, and weighed.

Plant–soil feedbacks

We used two approaches to assess plant effects on soils: (1) measures of N cycling in monospecific patches dominated by either *Geum* or *Deschampsia* at each experimental site in 2003, and (2) measures in the treatment plots of N cycling and microbial extracellular enzyme activities in 2006.

In 2003 we measured in situ net N-mineralization rate and microbial biomass N in monospecific patches dominated by either *Geum* or *Deschampsia* at the experimental sites to confirm presumed species effects on N cycling. Because our main experimental design used areas where both species codominated (necessary for removal treatments), we used another set of plots (adjacent to our main experimental plots) for these measures. In addition, we replicated these measures both within and among sites, something we were not able to do in the main experiment due to space constraints. We selected four replicate patches of naturally occurring *Geum* or *Deschampsia* at each of the experimental sites, with the exception that we combined sampling of two sites that were in close proximity to each other. We took three soil cores (2 cm diameter × 10 cm deep) from each patch and pooled them, for a total of 48 samples (2 patch types × 6 sites × 4 replicates/site). The soil from the cores was gently split in half, and one part was returned to the hole inside a gas-permeable bag, while the other part was brought back to the laboratory on ice for extraction and analysis (Eno 1960). After ~30 days the soil incubated at the site was removed and brought back to the laboratory for analysis. After field collection the soils were sieved through a 2-mm^2 mesh screen. Soils were then extracted in 0.5 mol/L K_2SO_4 for inorganic N and total nitrogen (TN). We measured inorganic N colorimetrically on a continuous-flow autoanalyzer (Latchat QuikChem 8000) and extractable TN and $CHCl_3$-N colorimetrically as inorganic N following high-temperature persulfate digestion. We calculated N concentrations of extractable dissolved organic N as the extractable TN minus extractable inorganic N. Chloroform-labile N was determined using the chloroform-fumigation extraction technique and then soils were extracted with 0.5N K_2SO_4 as above (Robertson et al. 1999). We calculated microbial N as the $CHCl_3$-labile N minus extractable TN, divided by a factor of 0.54 to correct for chloroform-labile N extraction efficiency (Brookes et al. 1985). We calculated net N-mineralization rates as the difference in extractable inorganic N between the initial and final cores, divided by the incubation period of 30 days.

We also measured soil processes in the main experiment to determine the effects of species removals and fertilization treatments in 2006. We measured available inorganic N, dissolved organic N, and microbial biomass N in all treatment plots. Three soil cores (2 cm diameter × 10 cm deep) were taken from each of three areas within each plot and pooled, resulting in three samples from each of the 70 experimental plots. The soils were processed and analyzed similarly to our measurements of monospecific patch effects in 2003, with the exception that we did not measure in situ net N mineralization.

We used a portion of the soil samples collected in 2006 to assay the activity of microbial extracellular enzymes in five of the seven sites. These soil samples were frozen (0°C), shipped to University of California (Irvine, California, USA), and processed during winter 2007. We measured the activity of extracellular enzymes involved in the acquisition of C (α-glucosidase, β-glucosidase, β-D-cellobiohydrolase, β-xylosidase, phenol oxidase, and peroxidase), N (N-acetyl-β-glucosaminidase and leucine amino peptidase), and P (acid phosphatase) using 4-methylumbelliferyl (MUB) α-D-glucopyranoside, 4-MUB β-D-glucopyranoside, 4-MUB β-D-cellobiohydrolase, 4-MUB B-D-xylopyranoside, L-3,4-dihydroxyphenylalanine, 4-MUB N-acetyl-β-D-glucosaminide, L-Leucine-7-amido-4-methylcoumarin hydrochloride, and 4-MUB phosphate as substrates, respectively. We followed published protocols (Sinsabaugh et al. 2003). Briefly, soil slurries were prepared with 1 g of soil in 125 mL sodium acetate solution (50 mmol/L, pH 5) and homogenized for 1 min. The phenol oxidase and peroxidase assays were prepared in clear 96-well plates and the remainder in black 96-well plates, allowing for 16 analytical replicates per sample. Soils and labeled substrates were incubated at 13°C for ~18 h. Fluorescence was measured on a Bio-Tek FL600 microplate fluorescence reader (BioTek Instruments, Winooski, Vermont, USA) with excitation filters set at 360 nm and emission filters set at 460 nm. For phenol oxidase and peroxidase assays, absorbance was measured at 460 nm.

Thus, in our experimental plots we measured species composition and resin-available N each year (2002–2006) following the treatment initiation (in 2001) of nitrogen availability and species removal. In 2006 we additionally measured available inorganic, dissolved organic, and microbial biomass N, aboveground biomass production, and the activities of nine microbial extracellular enzymes, in the experimental plots. Because we chose our experimental plots to have approximately equal representation of *Geum* and *Deschampsia* prior to our experimental manipulations, to better assess species effects we also measured N cycling in monospecific patches dominated by either *Geum* or *Deschampsia* at each experimental site (adjacent to the experimental plots) in 2003.

Statistical analyses

Differences in net N mineralization and microbial biomass N in patches dominated by *Geum* and *Deschampsia* were assessed with a two-way ANOVA, with patch and site as fixed factors. In cases where there was a patch effect, we conducted post hoc contrasts of patch type with each site.

The amount of biomass clipped from the removal plots was examined to determine if the effects of *Geum* or *Deschampsia* removal would be confounded with the amount of biomass removed. We analyzed differences in total biomass removed using a repeated, linear, mixed-

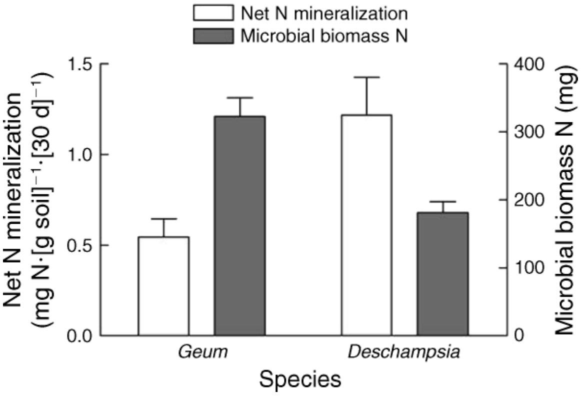

FIG. 2. N-cycling rates and microbial biomass N, measured in 2003 in monospecific patches (either dominated by *Geum* or *Deschampsia*) adjacent to the experimental treatments (that were established in co-dominated areas) at six of the seven moist-meadow alpine tundra sites on Niwot Ridge (Rocky Mountain Front Range, Colorado, USA). Across all sites, net N-mineralization rates were slower, and microbial biomass N higher in areas associated with *Geum* compared to *Deschampsia*.

effects model (lme) in R version 2.4.0 (R Development Core Team 2006) with year, removal treatment, and nutrient treatment as fixed effects and site and plot as random effects. Biomass measures were log transformed prior to analyses to meet assumptions of normality and heteroscedasticity. We used AIC criterion to determine the best-fit model and the best-covariance structure (Pinheiro and Bates 2000). To determine the degree to which effects of species removal were due to a reduction in general biomass rather than a change in species-specific interactions, we compared plots where biomass was randomly removed to *Deschampsia* removal, *Geum* removal and control plots using a linear mixed model.

To test for the effect of removal and nutrient treatments on resin-available N, we used a repeated, linear, mixed model with year, removal, and nutrient treatment as fixed effects and site and plot as a random effect. We tested for treatment effects on 2006 soil inorganic and dissolved organic N and on microbial biomass N using a nested, linear, mixed model with treatments as fixed effects and soil cores nested within subplot and site. We used a similar model for aboveground biomass production measured in 2006. For enzyme activities, we tested for treatment effects on mean activity of each enzyme for each plot in a linear mixed model with site and plot as random effects and microbial biomass N as a covariate. One of the enzymes, phenol oxidase, did not meet the assumptions of normality and so we used the Scheirer-Ray-Hare extension of a Kruskall Wallis test (Sokal and Rohlf 1994).

To examine the changes in species-abundance patterns over time and how these were affected by removal and fertilization treatments, we analyzed the species composition data in three ways: (1) a repeated-measures, linear model examining the effect of removal and nutrient treatments and all interactions on the relative abundance of our two focal species, *Geum* and *Deschampsia*, and of the major functional groups, forbs and graminoids; (2) a repeated-measures, linear model examining the effect of removal and nutrient treatments on total species number and evenness; and (3) a multivariate permutational analysis of variance based on Bray-Curtis dissimilarities of plant communities among plots. Details about each of these analyses follow.

First, to examine patterns in species composition over time, we analyzed changes in the relative abundance of our two focal species, *Geum* and *Deschampsia*, and changes in the relative abundance of the major functional groups, forbs and graminoids, using a repeated, linear, mixed model in R version 2.4.0 (R Development Core Team 2006) with removal and nutrient treatment and year as fixed effects, and site and plot as random effects. The forb group excluded *Geum* and the graminoids excluded *Deschampsia*. When analyzing *Geum* and *Deschampsia* abundance we excluded the corresponding removal treatment where abundances were kept close to zero for the length of the experiment. Relative abundances were log-transformed prior to analyses to meet assumptions of normality and heteroscedasticity. Second, we used the same model to test for effects of nutrient and removal treatments on species number and evenness.

Finally, multivariate analyses of the relative abundance of all species excluding *Geum* and *Deschampsia* were done using a permutational multivariate analysis of variance (PerMANOVA) (Anderson 2001, McArdle and Anderson 2001) with nutrient treatment, year, and removal treatment as main effects and 2006 productivity as a covariate. Relative abundances were fourth-root transformed and analyses were based on Bray-Curtis dissimilarities. Permutations were run 9999 times. Pairwise a posteriori comparisons were done using these permutations to examine differences among years and treatments. To determine the species most responsible for compositional differences, we used repeated linear models, as above, to test for treatment effects on the most abundant species.

RESULTS

Species effects in monospecific stands

Net N-mineralization rates measured in 2003 were higher in *Deschampsia* patches relative to *Geum* patches at our experimental sites (Fig. 2; $F_{1,36} = 7.951$, $P < 0.01$), consistent with past results (Steltzer and Bowman 1998). Microbial biomass N was higher in *Geum* patches relative to *Deschampsia* patches (Fig. 2; $F_{1,36} = 30.07$, $P < 0.001$). Microbial biomass N also varied significantly among sites ($F_{5,36} = 4.28$, $P < 0.01$), but this site variation did not affect the relative differences between patch types.

Evaluation of experimental manipulations

The amount of biomass clipped from the plots to maintain the removal treatments varied among years, declining with time ($F_{5,219} = 358.6$, $P < 0.001$). In the sixth year of our removals, 2006, we removed ~7 g biomass/m^2 from each plot, or <3% of the annual productivity at the sites, indicating that we had succeeded in reducing the presence of the focal species. While the abundance of the target-removal species was initially similar to each other, we removed different cumulative amounts of biomass among removal treatments ($F_{2,44} = 30.32$, $P < 0.001$) due to differences in their response to clipping. We removed more biomass from the random-removal plots than either *Geum* ($F_{1,24} = 55.2$, $P < 0.001$) or *Deschampsia* ($F_{1,24} = 51.9$, $P < 0.001$) removal plots and more from the *Geum* than *Deschampsia* ($F_{1,38} = 10.6$, $P = 0.002$) removal treatments. The amount of biomass removed did not differ among nutrient treatments ($F_{2,44} = 0.8$, $P = 0.463$).

Nutrient addition significantly affected resin-available N (Fig. 3; $F_{1,12} = 111.4$, $P < 0.001$) and this difference increased over time (Fig. 3; $F_{8,209} = 27.9$, $P < 0.001$). As intended, we increased N availability in the N-addition plots compared to ambient controls ($P < 0.001$) and C-addition plots ($P < 0.001$) and decreased N availability in the C-addition plots compared to controls ($P = 0.001$). Based on the species effects we documented in monospecific patches at the experimental sites (Fig. 2), we expected that species removal would also affect N cycling. However, resin-available N did not vary among removal treatments (Fig. 2; $F_{2,36} = 1.52$, $P = 0.232$).

Soil and microbial processes

Nutrient additions also caused large differences in soil inorganic, organic, and microbial N pools among the plots in 2006 ($F_{2,48} = 326.9$, $P < 0.001$; $F_{2,48} = 90.2$, $P < 0.001$; and $F_{2,48} = 14.0$, $P < 0.001$, respectively; Fig. 4a–c; Appendix). Similar to the resin-available N, soil inorganic N increased in the N-addition plots compared to ambient controls ($P < 0.001$) and C-addition plots ($P < 0.001$) and decreased N availability in the C-addition plots compared to ambient controls ($P < 0.001$). The difference in ambient and C-addition plots was due to higher soil inorganic N in the ambient *Geum* removal and non-removal plots relative to the C-addition plots where *Deschampsia* was also removed ($P = 0.006$, $P = 0.005$, respectively). Dissolved organic N was lower in the N-addition plots relative to ambient ($P < 0.001$) and C-addition plots ($P < 0.001$), but there was no effect of removals ($F_{2,48} = 0.29$, $P = 0.751$).

Microbial biomass N was higher in the C-addition ($P < 0.001$) and ambient plots ($P < 0.001$) relative to the N-addition plots. While overall there were no significant effects of removals ($F_{2,48} = 0.7$, $P = 0.510$) or significant removal × nutrient interactions ($F_{2,48} = 0.5$, $P = 0.723$) on microbial biomass N, post hoc tests show that microbial biomass N was significantly lower in the N-addition plots compared to ambient ($P = 0.016$) and C-addition ($P = 0.020$) treatments in the absence of species removal, as well as in the *Geum* removal, C-addition plots ($P = 0.013$).

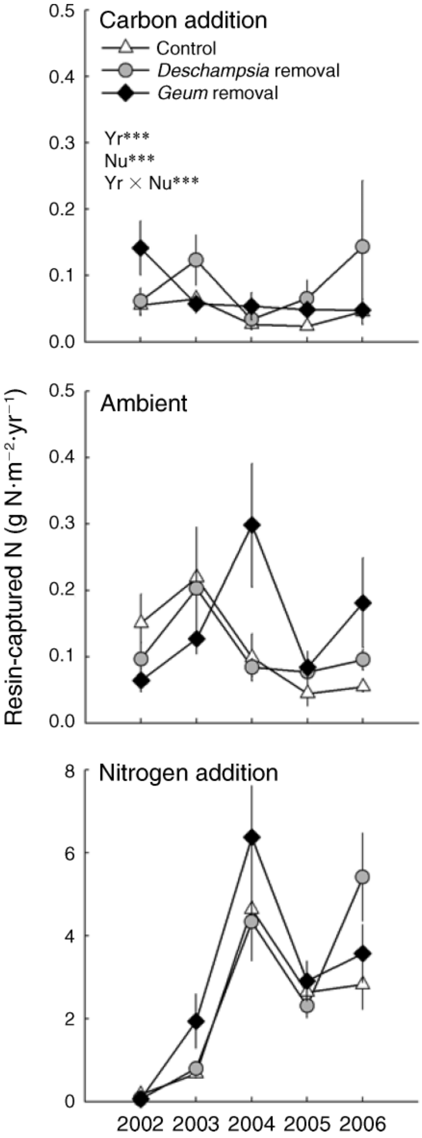

FIG. 3. The effect of nutrient additions and dominant species removals on resin-available N. Data are means ± SE. Treatments were initiated in 2001. There were no significant differences among removal treatments within each nutrient treatment. From Tukey hsd tests, ambient N conditions and the C- and N-addition treatments differ significantly from each another ($P < 0.05$). Factor abbreviations (all figures) are: Yr, year; Nu, nutrient-addition treatment; Rem, species-removal treatment.

*** $P < 0.001$; nonsignificant factors are not listed.

The activities of five out of the nine enzymes examined significantly responded to the experimental treatments (Fig. 5). The activity of two C-acquiring enzymes, α-glucosidase and β-xylosidase, and one N-acquiring enzyme, leucine amino peptidase, significantly decreased due to nutrient fertilization ($F_{2,30} = 9.1$, $P = 0.0008$; $F_{2,29} = 3.7$, $P = 0.038$; and $F_{2,30} = 3.4$, $P = 0.046$, respectively; Fig. 5a, c, g). In contrast, the activity of phenol oxidase,

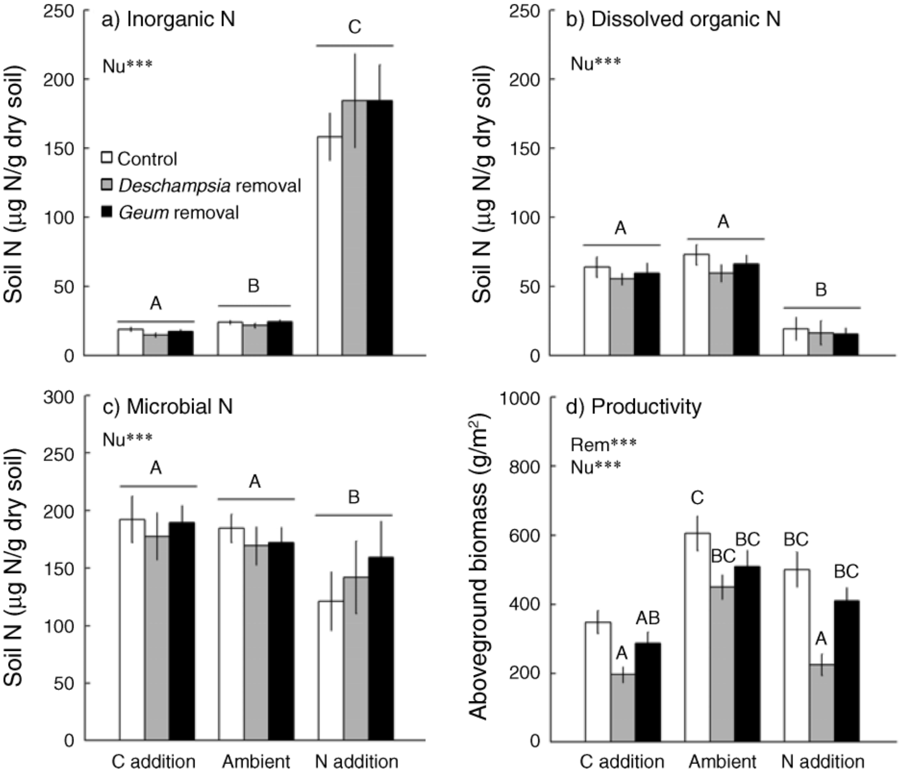

FIG. 4. The effect of nutrient additions and dominant-species removals on soil inorganic N, dissolved organic N, microbial biomass N, and aboveground productivity in 2006, the sixth growing season of the experiment. Data are means ± SE. Different uppercase letters indicate significant differences among means ($P < 0.05$). Abbreviations are as in Fig. 3.

*** $P < 0.001$; nonsignificant factors are not listed.

a tannin-acquiring enzyme, and acid phosphatase, a P-acquiring enzyme, showed a significant interaction between nutrient and removal treatments ($F_{4,30} = 3.0$, $P = 0.024$ and $F_{4,30} = 3.3$, $P = 0.024$; Fig. 5e, i). Phenol oxidase activity was highest in *Geum*-removal plots with N addition and lowest in *Deschampsia*-removal plots with C addition ($P < 0.05$; Fig. 5e). In non-removal plots, phenol oxidase decreased with N addition and increased with C addition ($P < 0.05$). *Geum* removal also increased the activity of acid phosphatase compared to non-removals in N-addition plots and relative to activity in *Deschampsia*-removal plots with no nutrient additions ($P < 0.05$; Fig. 5i). However, when C was added to *Geum*-removal plots acid phosphatase activity was significantly repressed ($P < 0.05$; Fig. 5e).

Aboveground biomass production

By 2006, nutrient and removal treatments had affected aboveground biomass ($F_{2,48} = 16.4$, $P < 0.001$ and $F_{2,48} = 13.6$, $P < 0.001$, respectively; Fig. 4d; Appendix). Standing biomass in plots where *Geum* had been removed had recovered to control (no removal) levels. However, aboveground plant biomass in plots where *Deschampsia* had been removed was significantly lower than control levels ($P < 0.001$); regrowth had not fully compensated for the biomass of *Deschampsia* in six years, likely due to the slow growth of *Geum* and other subdominant forbs. Production responses to N availability were surprising: the addition of N did not increase, and actually slightly decreased, aboveground biomass ($P < 0.001$). The negative effect of N on *Geum* strongly contributed to this effect. N addition decreased production most when *Deschampsia* was removed and had little effect when it was present. As expected, C additions decreased production.

Community structure

Species abundances in unmanipulated plots remained constant over the length of the experiment ($F_{4,20} = 0.02$, $P = 0.9992$). *Geum* was more abundant than *Deschampsia* ($F_{4,20} = 9.0$, $P = 0.006$), with average relative abundances of 39% and 30%, respectively. Forbs other than *Geum* constituted most of the remaining cover; other graminoids were rare and averaged only 5% relative abundance in unmanipulated plots. While the unmanipulated plots contained 4–13 species/m^2, there were no consistent trends in richness over time ($F_{4,24} = 1.8$, $P = 0.161$). The identity of the species present varied among plots and over time; within the course of the experiment we identified 47 species in our experimental plots.

Removal-treatment effects.—Competition intensity between the codominants was strong and fairly sym-

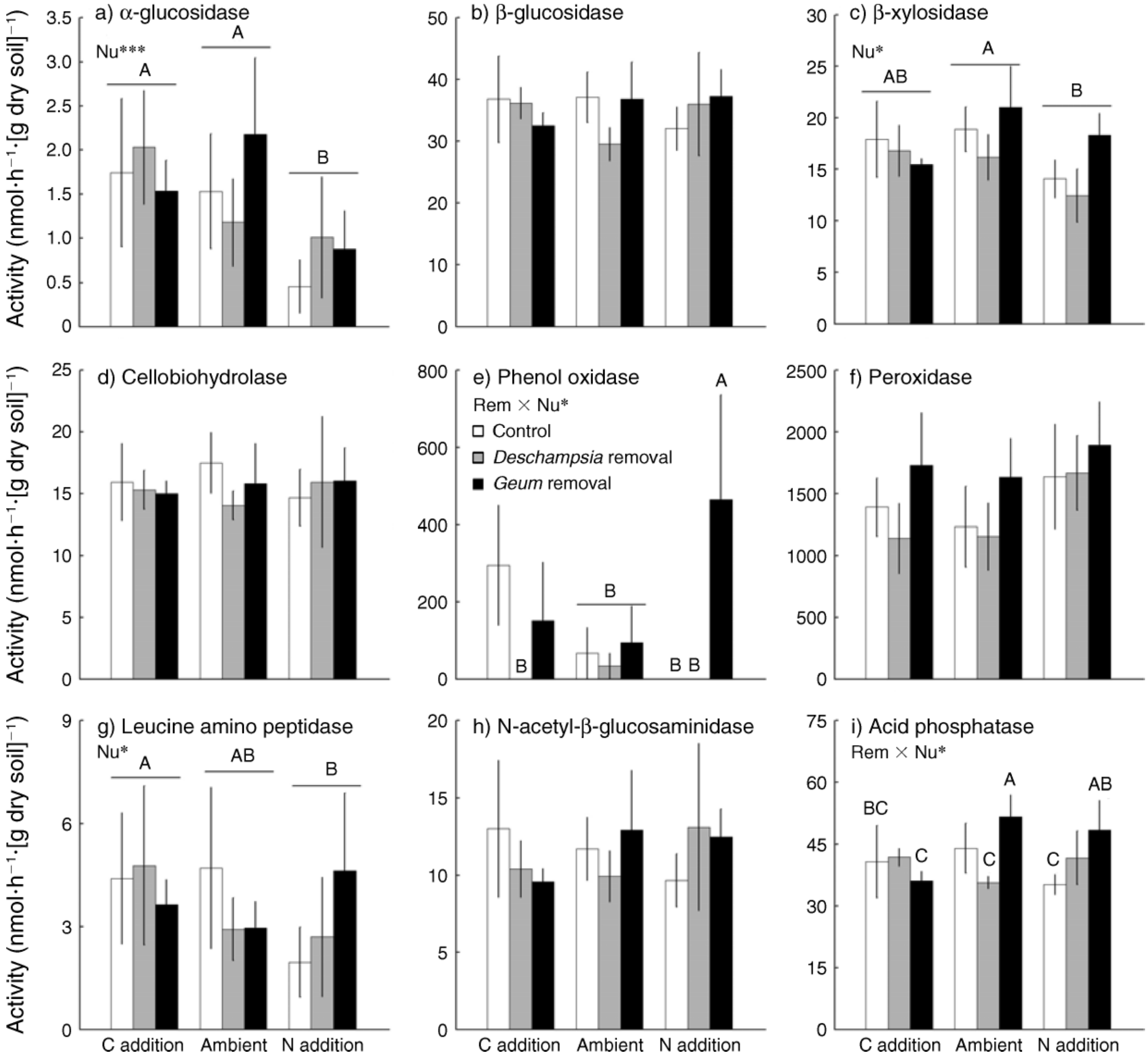

FIG. 5. The effect of nutrient additions and dominant-species removals on extracellular enzyme activity for enzymes involved in the breakdown of simple C (a–d), oxidative C (e, f), N (g, h) and phosphorus (i) compounds. Data are means ± SE. Different uppercase letters indicate significant differences among treatments at $P < 0.05$. Nutrient additions and species removals had no effect on the activity of β-glucosidase, cellobiohydrolase, peroxidase, and N-acetyl-β-glucosaminidase. Abbreviations are as in Fig. 3.

* $P < 0.05$; *** $P < 0.001$; nonsignificant factors are not listed.

metrical, with both codominants negatively affecting the other dominant species more than other subordinate species. *Geum* increased in relative abundance when *Deschampsia* was removed from plots (Fig. 6a; $F_{1,30} = 60.5$, $P < 0.001$). The response to *Deschampsia* removal was marginally greater than the response to the removal of vegetation from random species ($P = 0.062$). *Deschampsia* increased in abundance when *Geum* was removed from plots (Fig. 6b; $F_{1,30} = 89.6$, $P < 0.001$) and this response was significantly larger than the response to the random-removal treatment ($P < 0.001$).

While both codominants negatively affected other species in the community, *Deschampsia* appeared to exert a stronger competitive effect on many of the subordinates. Initially forbs responded positively to removal of *Geum* (Fig. 6c; $P < 0.001$) and *Deschampsia* (Fig. 6c; $P < 0.001$) and did not vary in their response to *Deschampsia* and *Geum* removals ($P = 0.942$) or from the response to random removals ($P = 0.861$, $P = 0.855$, respectively). However, the competitive effect of *Deschampsia* was apparent over time ($F_{8,215} = 3.0$, $P = 0.003$): by 2006 forbs were more abundant in the *Deschampsia*-removal plots than the *Geum*-removal plots ($P = 0.001$). The response of graminoids also varied among the species-removal treatments (Fig. 6d; $F_{2,48} = 6.6$, $P = 0.0030$). Similarly to the forbs,

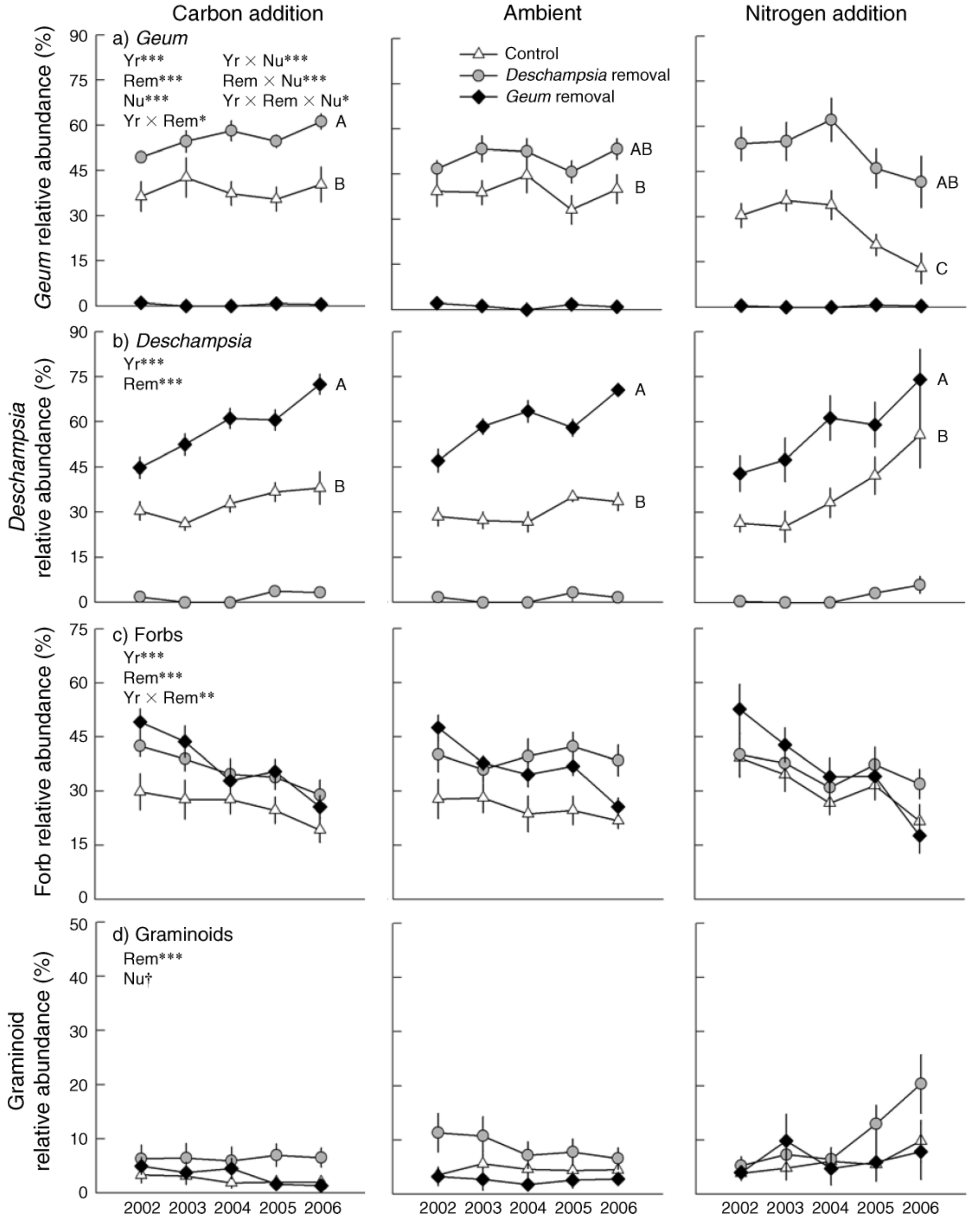

FIG. 6. The effect of nutrient additions and dominant-species removals on the peak-season relative abundance (%) of (a) *Geum*, (b) *Deschampsia*, (c) other forbs, and (d) other graminoids over six years (treatments were initiated in 2001). While we measured composition in 2001 prior to treatment initiation to ensure that plot composition was similar in all treatments, we do not include those data here because they were taken early in the growing season. Data are means ± SE. Different uppercase letters indicate significant differences among removal treatments in 2006 at $P < 0.05$. Statistical analysis of the abundance of *Geum* or *Deschampsia* did not include the treatment where species were experimentally removed. Abbreviations are as in Fig. 3.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; † $P < 0.10$; nonsignificant factors are not listed.

graminoids responded positively to *Deschampsia* removal compared to controls ($P < 0.001$) and *Geum* removals ($P < 0.001$), and marginally more than to plots with random species removed ($P = 0.081$). However, graminoids did not respond to *Geum* removal, as compared to the controls ($P = 0.438$) or to random-species removals ($P = 0.842$). *Artemisia scopulorum*, the third most abundant species in the moist-meadow sites after *Geum*

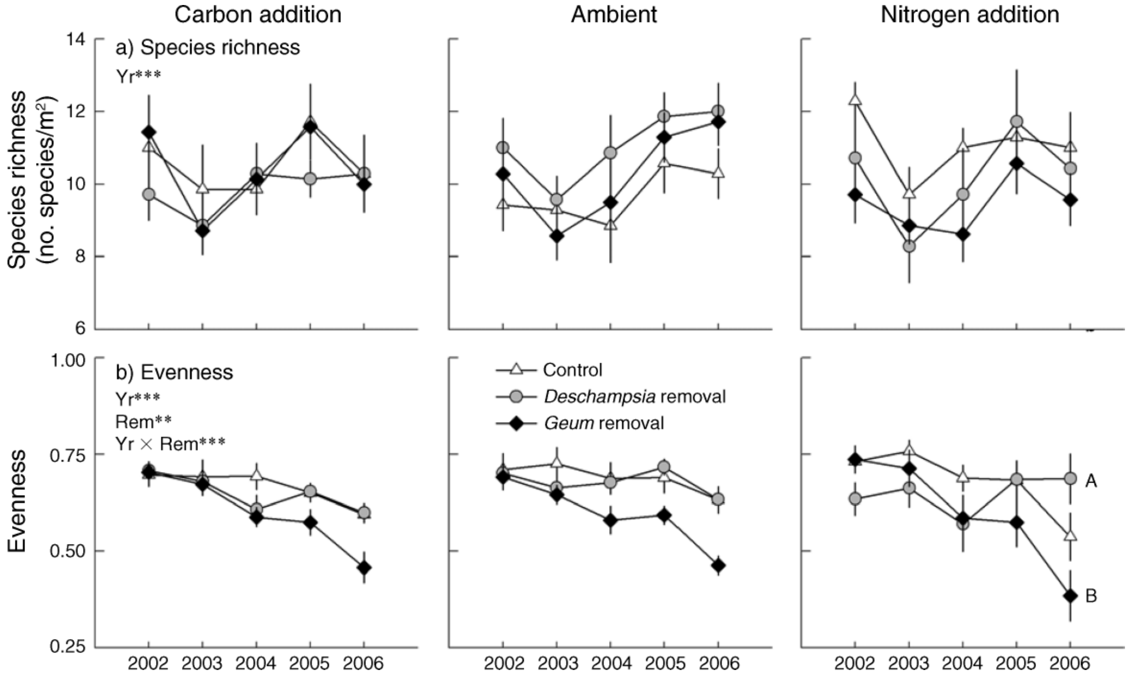

FIG. 7. The effect of nutrient additions and dominant-species removals on species number and evenness over six years. Treatments were initiated in 2001. Data are means ± SE. Different letters indicate significant differences from post hoc tests among removal treatments in 2006 at $P < 0.05$. Nutrient additions had no effect on species number or evenness. Abbreviations are as in Fig. 3.

** $P < 0.01$; *** $P < 0.001$; nonsignificant factors are not listed.

and *Deschampsia*, increased in abundance with the removal of either dominant ($F_{2,48} = 11.8$, $P = 0.0001$), as did another common forb, *Erigeron simplex* ($F_{2,48} = 6.5$, $P = 0.0031$). The abundance of *Carex scopulorum*, the most common graminoid, varied with removal treatments ($F_{2,48} = 6.6$, $P = 0.003$), increasing in abundance when *Deschampsia* was removed compared to when *Geum* was removed ($P < 0.001$).

Interactions with either of the codominants did not affect species richness (Fig. 7a; $F_{2,48} = 0.3$, $P = 0.718$) but *Geum* facilitated community evenness. Evenness showed a significant response to removal treatments (Fig. 7b; $F_{2,48} = 10.6$, $P < 0.001$) and the response changed over time ($F_{8,215} = 7.3$, $P < 0.001$). The removal of *Geum* caused a decrease in evenness compared to *Deschampsia* removals ($P < 0.001$) and to controls ($P < 0.001$). The difference in evenness between *Geum* removal and other plots increased over time: after six years evenness had declined by >35%.

Nutrient-treatment effects.—Species composition was generally less responsive to fertilization amendments than to species removals. *Geum* responded to C and N amendments differently (Fig. 6a; $F_{2,30} = 10.2$, $P < 0.001$). It decreased in abundance when plots were fertilized with N (Fig. 6a, $P < 0.001$) and the negative effect of N increased over time (Fig. 6a; $F_{8,215} = 7.2$, $P < 0.001$), but showed no response to C additions ($P = 0.853$). The abundance of *Deschampsia* and forbs showed no response to fertilization treatments (in Fig. 6b, $F_{2,30} = 0.29$, $P = 0.750$; in Fig. 6c, $F_{2,48} = 0.04$, $P = 0.959$, respectively). Graminoids responded to nutrient additions differently (Fig. 6; $F_{2,48} = 2.1$, $P = 0.064$), but due to large variances, the overall response was only marginally significant. Graminoids increased in abundance with N addition (Fig. 6d) compared to C-addition plots (Fig. 6k; $P = 0.004$), but there was only a marginal increase in N-addition plots compared to ambient (Fig. 6d; $P = 0.069$). A common forb, *Bistorta bistortoides*, was one of the only subordinate species that showed a strong response to nutrient treatments ($F_{2,46} = 15.4$, $P < 0.001$), decreasing in abundance with the addition of C ($P < 0.001$). Nutrient treatments did not significantly affect species richness ($F_{2,48} = 0.1$, $P = 0.874$) or evenness ($F_{2,48} = 0.5$, $P = 0.637$).

Interactive effects of nutrient and removal treatments.—Responses to nutrient addition and species removal were generally independent of one another, with the exception of the response of *Geum* (Fig. 6; significant effect of removal × nutrient). While *Geum* generally decreased when N was added, competition with *Deschampsia* compounded the decline. Conversely, *Geum* abundance was greatest when C was added and *Deschampsia* was removed (Fig. 6). In comparison, *Deschampsia* generally increased in relative abundance with the removal of *Geum*, and this effect did not change with time or nutrient additions (Fig. 6).

Multivariate analyses indicated that the composition of the plant community was affected by nutrient and

PLATE 1. Moist meadow alpine tundra at Niwot Ridge on the Front Range in Colorado, USA. In this community, two codominant plant species, the rosaceous forb (*Geum rossii*) and a bunchgrass (*Deschampsia caespitosa*), differentially affect soil processes. These plant–soil feedbacks may influence how the community responds to environmental changes such as increased nitrogen deposition. Photo credit: I. Ashton.

removal treatments. The community in the C-addition, N-addition, and no-nutrient-addition plots all differed significantly from one another ($F_{2,314} = 3.4$, $P < 0.01$), as did the community in all the removal treatments ($F_{2,314} = 2.5$, $P < 0.01$). There were no interactive effects of nutrient manipulations and species removals on community composition.

DISCUSSION

Consistent with the plant–soil threshold hypothesis, which posits that resiliency depends on species interactions mediated by sensitivities of plant–soil feedbacks to exogenous change, we found strong evidence that biota were initially resilient to the changed N supply but that this resilience broke down as N availability continued to increase. In particular, N fertilization did not affect the cover of *Geum* for the first four years of the study, but then caused strong declines in years five and six. After six years of fertilization, *Geum* cover declined from almost 40% cover to 10% cover. Species-removal manipulations indicate that the continued decline of *Geum* will result in changed community-level parameters, decreasing evenness and shifting species composition. Surprisingly, however, it appears as if the biomass of neither of the codominant species in this system, which has been generally assumed to be N limited, is directly limited by N. This result suggests that simple resource competition for N is not the sole controlling factor of plant–soil feedbacks in this system.

Resilience via plant–soil feedbacks

Soil microbial effects likely mediated the resilience effects conferred by *Geum*. *Geum* patches were associated with slow rates of net N mineralization and high microbial biomass N at every site in the experiment (Fig. 2); the lack of association between the removal of *Geum* and changes in N cycling characteristics (Figs. 3 and 4) is likely due to our sampling resolution in mixed-species plots. While we attribute the time lag as resilience due to these effects of *Geum*, it could also be due to the slow-growing perennial life history of these plants. However, we saw fairly rapid response of *Deschampsia* and *Geum* to our species-removal treatments; effects were apparent in the second year of manipulation compared to the delayed response to N fertilization. In addition, we have observed fairly rapid changes in species composition due to N fertilization in other experiments (Bowman et al. 1995, 2006). As such, this lag in response supports the idea that species that slow N cycling can counteract the effects of exogenous increases in N, maintaining their abundance and diversity of other species in the community in the short term.

The same plant–soil effects that lead to resilience also can lead to rapid changes after the system has been pushed past some buffering threshold. The decline in *Geum* at high N availabilities does not appear to be solely due to changes in competitive interactions mediated by changed nutrient availability. Rather, the decline occurred with or without the other codominant, *Deschampsia*, present. Most general models of fertilization effects assume that declines due to fertilization are due to changed competitive interactions (Rajaniemi 2003, Suding et al. 2005). However, our findings indicate that other processes mediate the sensitivity of *Geum* to increased N. One possibility is that our N additions acidified the soils (pH 5.8 in ambient-N plots and pH 5.7 in N-addition plots) and decreased the ability of *Geum* to acquire micronutrients. However, given the small change in pH and the general association of *Geum* with acidic soils (USDA-NRCS 2006), such a decline in pH is unlikely to explain the strong response to N addition. Another possibility is that increased N made *Geum* more susceptible to another stress like drought. However, given that 2002 was the only year during the experiment with below average precipitation (D-1 weather station [40°3′34″ N, 105°37′0″ W; 3739 m]; 1054, 893, 1081, 1108, 1159, and 1109 mm for 2001–2006, respectively) and that soil moisture did not differ in any of our experimental treatments, drought stress is unlikely to explain the strong decline of *Geum*.

We speculate that the mechanism behind the threshold decline is related to changes in the microbial community. *Geum* litter and root exudates, which are high in dissolved organic carbon and phenolic compounds (Bowman et al. 2004; Meier et al. 2008), may support a microbial community that enhances the success of the plant species. Under ambient N conditions, *Geum* may support a microbial community that either inhibits the growth of other species or directly facilitates its own growth via resource acquisition or pathogen resistance. After six years, microbial biomass N declined at higher N availabilities, as did the activities of two C-acquiring extracellular microbial enzymes, α-glucosidase and β-xylosidase and an N-acquiring enzyme, leucine amino peptidase. Alpine soils are often rich in organic matter, but after six years of N additions microbial activity may have consumed much of the labile soil C (Neff et al. 2002). At high N availability, decreased soil C may decrease microbial biomass and the activities of C-acquiring enzymes. These effects are consistent regardless of removal treatment. Thus, carbon availability may more strongly limit microbial activity in the N-addition plots compared to the ambient and C-addition plots.

Certain species, in this case *Geum*, could be more sensitive to changes in microbial function at higher N availabilities. In addition to its effects on microbial biomass N and C-degrading enzyme activities, N fertilization significantly slowed the activity of phenol oxidase, a tannin-degrading enzyme, in the presence of *Geum*. This change suggests that microbes might shift away from degrading *Geum* root-derived C and litter (which contain very high concentrations of tannins; Meier et al. 2008) under fertilized conditions. Furthermore, increases in acid phosphatase activity suggest that soils may become depleted of P in the absence of *Geum*, particularly when soils are also fertilized with N. If *Geum* growth is facilitated by a microbial community that is associated with low N availability, the decline in microbial biomass and change in microbial function in plots where N was added may explain the corresponding decline in *Geum* abundance.

The combination of competitive interactions with *Deschampsia* and high N levels caused the most dramatic declines in *Geum* cover. The removal of *Deschampsia* initially caused *Geum* to increase, indicating competition, but this response was only sustained at low N levels. At high N levels, *Geum* initially increased when *Deschampsia* was removed, but then declined in years five and six. The timing of the decline in response to high N was the same regardless of whether *Deschampsia* was present. The negative effects of N and the positive effects of *Deschampsia* removal counterbalanced each other, resulting in little overall change in *Geum* cover when the two factors were combined.

Our results also indicate that the decline of *Geum* will trigger further changes in plant community structure. The loss of *Geum* is associated with community-wide decreases in evenness and changes in composition. Species richness, on the other hand, was relatively robust to the species decline. Forbs in particular appear to be sensitive to the loss of *Geum*. The initial response of forbs is to increase in abundance when *Geum* is removed, but over time their abundance declines dramatically. This suggests that the large abundance of forbs in the tundra is dependent on the presence of *Geum*, perhaps due to alterations in the soil environment, changes in other limiting nutrients such as P, and/or through indirect competitive interactions where the presence of *Geum* limits the abundance of graminoids.

While the decline of *Geum* at high N availabilities is noteworthy, the mechanisms responsible for the decline could be general. Species turnover is common in N-fertilization experiments; however, most species that decline are presumed to be competitively excluded rather than particularly sensitive to changed microbial function at high N (Suding et al. 2005). We expect that species that slow N cycling through feedbacks with the microbial community may be most likely to exhibit similar responses, although feedbacks and responses to N have been studied so infrequently that more work is needed to test generality. One aspect of our results that is unusual for terrestrial N-fertilization studies is that aboveground production declined with N addition. Production likely decreased due to sensitivity of one of the most abundant species in the community, *Geum*, to N. We expect that similar species responses may occur to N fertilization but not affect the overall biomass result to such an extent, particularly if the vulnerable species were less abundant.

Species-level resource limitation

Similar to other studies that examined the interactive effects of biotic structure and changed N supply (Bret-Harte et al. 2004, Klanderud 2005, Manning et al. 2006), we found evidence for direct effects of enhanced N availability indicated by changes in *Geum* cover, graminoid cover, and multivariate measures of community composition. However, these changes were fairly minor compared to the interactions between biota and changes in N availability and, in the case of *Geum*, opposite in direction with what we predicted. In addition, we did not see direct abundance responses to N availability in *Deschampsia*, as we predicted.

In contrast to the strong environmental limitation shown by *Geum*, the other codominant, *Deschampsia*, was predominately limited by biotic interactions, namely, interactions with *Geum*. Based on its fast growth rate and effects on N cycling, we predicted *Deschampsia* would increase in abundance due to N fertilization and act to accelerate effects of increased N availability (Fig. 1). However, the abundance of *Deschampsia* did not increase due to N fertilization. While it did not increase due to N fertilization, *Deschampsia* cover strongly increased within one year after *Geum*-removal treatments were initiated. This response was relatively constant over time, resulting in a 20% increase in *Deschampsia* cover in the absence of *Geum*. In addition, possibly due to the decrease in *Geum* starting the year prior, there was an increasing trend in *Deschampsia* cover in year six due to N fertilization. Taken together, these results suggest that *Deschampsia* should continue to increase as N fertilization causes *Geum* cover to decline and that this change is largely under biotic control.

The potential effects of N deposition on plant community structure are generally considered strong due to the predominance of N limitation in temperate ecosystems (Vitousek et al. 2002). Previous work at Niwot Ridge and other alpine sites has indicated that production is largely limited by nitrogen or co-limited by N and P (Bowman et al. 1993, 1995, 2006, Theodose and Bowman 1997, Seastedt and Vaccaro 2001, Soudzilovskaia et al. 2005). In fact, Bowman et al. (1995) conducted a N-fertilization experiment in moist-meadow tundra very similar to the system we worked in and found a significant increase in productivity, predominately due to an increase in graminoid (including *Deschampsia*) biomass.

Although we assumed general N limitation based on this past work, our results indicate that only one functional group in the moist-meadow systems we studied was directly limited by N: non-*Deschampsia* graminoids. Because this group contributed a relatively small amount to overall biomass production (~5%) and because *Geum* biomass declined with N, we did not find evidence of a positive effect of N addition on productivity. A similar pattern was found in another fertilization experiment where the decline of lichens counterbalanced the increase in vascular plants, resulting in no community-wide change in biomass due to increased N (Soudzilovskaia et al. 2005).

Interestingly, these effects were not symmetrical in our N-reduction treatment, suggesting that effects of changed N availability may be strongly nonlinear. The C-addition treatment, while it decreased N availability to a smaller extent than the corresponding increase in N due to N-addition treatment, decreased aboveground biomass production. In contrast to the lack of increase due to N addition, this response to N reduction is what we would expect in an N-limited system. Again, there is evidence that this discontinuity is due to species responses. While *Geum* decreased due to elevated N, it did not increase due to lowered N availabilities. In addition, microbial associations with *Geum* appear to be more sensitive to increased N and remain fairly constant across low N availabilities. This nonlinear response, as well as the difference between the moist-meadow response to N that we and Bowman et al. (1995) found, is consistent with the idea that the study site has just reached the critical load of N due to N deposition (Bowman et al. 2006).

We speculate that one reason why *Deschampsia* did not respond to changes in N availability in this current study, even in the absence of its codominant competitor, is because it has become particularly dependent on P availability. This P limitation is a possibility for several reasons. First, although *Deschampsia* did not respond to increased N availability, it did respond to the removal of *Geum*. The differences in response between these two manipulations suggest that *Deschampsia* is competing with *Geum* for something other than just N. Second, *Geum* removal was associated with increased acid phosphatase activities, suggesting the enhancement of P limitation when *Geum* is lost and *Deschampsia* increases in abundance. Last, while past work generally finds evidence of N as the primary limiting resource limiting overall production, the studies that followed the response of *Deschampsia* found evidence of strong species-level P limitation (Theodose and Bowman 1997, Seastedt and Vaccaro 2001). Litaor et al. (2005) also documented a correlation between graminoid biomass and P availabilities at our study site. If *Deschampsia* is predominately limited by P availability in moist-meadow alpine tundra, further increased N deposition may cause little direct change in its abundance. Further changes in community structure may be fairly minimal until the system approaches the critical threshold in N supply, the point when *Geum* starts declining due to N, as we observed in year six of this study. The lack of responsiveness to N in a fast-growing species such as *Deschampsia* could also be an indicator of progressive P limitation of the plant community as a whole as it approaches its critical N load and becomes N saturated.

Conclusions

Plant–soil feedbacks through the microbial community can affect vulnerability to exogenous environmental change, contributing to threshold dynamics. Here we demonstrate that moist-meadow alpine tundra is initially resilient to increased N availability. During this resilience period, community structure was relatively stable, with few changes attributable to increased N-supply rates. However, five years after the manipulations were initiated the resilience of the community broke down, likely through changes in microbial feedbacks affecting *Geum*, one of the dominant species. This loss of resilience due to a directional environmental change may be a common phenomenon (Gunderson 2000, McClanahan et al. 2002, Chapin et al. 2006), and may be particularly strong if vulnerability is associated with changed species interactions. In this case, because the vulnerable species, *Geum*, was also a major facilitator of subordinate species, its loss increased the abundance of the other dominant, *Deschampsia*, and decreased community diversity (evenness). Improved understanding of dynamics of change, particularly the complex interactions between biotic interactions and environmental change, can be applied to managing ecological processes and feedbacks to avoid the occurrence of these threshold effects.

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LITERATURE CITED

Aerts, R. 1999. Interspecific competition in natural plant communities: mechanisms, trade-offs and plant–soil feedbacks. Journal of Experimental Botany 50:29–37.

Allison, G. 2004. The influence of species diversity and stress intensity on community resistance and resilience. Ecological Monographs 74:117–134.

Allison, S. D., C. Nielsen, and R. F. Hughes. 2006. Elevated enzyme activities in soils under the invasive nitrogen-fixing tree *Falcataria moluccana*. Soil Biology and Biochemistry 38:1537–1544.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46.

Bardgett, R. D., J. L. Mawdsley, S. Edwards, P. J. Hobbs, J. S. Rodwell, and W. J. Davies. 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Functional Ecology 13:650–660.

Bestelmeyer, B. T. 2006. Threshold concepts and their use in rangeland management and restoration: the good, the bad, and the insidious. Restoration Ecology 14:325–329.

Bezemer, T. M., C. S. Lawson, K. Hedlund, A. R. Edwards, A. J. Brook, J. M. Igual, S. R. Mortimer, and W. H. van der Putten. 2006. Plant species and functional group effects on abiotic and microbial soil properties and plant–soil feedback responses in two grasslands. Journal of Ecology 94:893–904.

Bobbink, R., M. Hornung, and J. G. M. Roelofs. 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. Journal of Ecology 86:717–738.

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., and H. Steltzer. 1998. Positive feedbacks to anthropogenic nitrogen deposition in Rocky Mountain Alpine tundra. Ambio 27:514–517.

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, and M. C. Fisk. 1995. Physiological and production responses of plant-growth forms to increases in limiting resources in alpine tundra: implications for differential community response to environmental change. Oecologia 101:217–227.

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

Bradley, K., R. A. Drijber, and J. Knops. 2006. Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. Soil Biology & Biochemistry 38:1583–1595.

Brant, J. B., D. D. Myrold, and E. W. Sulzman. 2006. Root controls on soil microbial community structure in forest soils. Oecologia 148:650–659.

Bret-Harte, M. S., E. A. Garcia, V. M. Sacre, J. R. Whorley, J. L. Wagner, S. C. Lippert, and F. S. Chapin. 2004. Plant and soil responses to neighbour removal and fertilization in Alaskan tussock tundra. Journal of Ecology 92:635–647.

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

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology & Biochemistry 17:837–842.

Casper, B. B., and J. P. Castelli. 2007. Evaluating plant–soil feedback together with competition in a serpentine grassland. Ecology Letters 10:394–400.

Chapin, F. S., III. 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11:233–260.

Chapin, F. S., III. 2003. Effects of plant traits on ecosystem and regional processes: a conceptual framework for predicting the consequences of global change. Annals of Botany 91:455–463.

Chapin, F. S., III, T. V. Callaghan, Y. Bergeron, M. Fukuda, J. F. Johnstone, G. Juday, and S. A. Zimov. 2004. Global change and the boreal forest: thresholds, shifting states or gradual change? Ambio 33:361–365.

Chapin, F. S., III, M. D. Robards, H. P. Huntington, J. E. Johnstone, S. E. Trainor, G. P. Kofinas, R. W. Ruess, N. Fresco, D. C. Natcher, and R. L. Naylor. 2006. Directional changes in ecological communities and social-ecological systems: a framework for prediction based on Alaskan examples. American Naturalist 168:S36–S49.

Chapin, F. S., III, E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. Nature 405:234–242.

Craine, J. M., D. Tilman, D. Wedin, P. Reich, M. Tjoelker, and J. Knops. 2002. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. Functional Ecology 16:563–574.

Dakora, F. D., and D. A. Phillips. 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant and Soil 245:35–47.

Diaz, S., et al. 2004. The plant traits that drive ecosystems: Evidence from three continents. Journal of Vegetation Science 15:295–304.

Ehrenfeld, J. G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6:503–523.

Ehrenfeld, J. G., B. Ravit, and K. Elgersma. 2005. Feedback in the plant–soil system. Annual Review of Environment and Resources 30:75–115.

Elmqvist, T., C. Folke, M. Nystrom, G. Peterson, J. Bengtsson, B. Walker, and J. Norberg. 2003. Response diversity, ecosystem change, and resilience. Frontiers in Ecology and the Environment 1:488–494.

Eno, C. F. 1960. Nitrate production in the field by incubating the soil in polyethylene bags. SSSA Proceedings 24:277–299.

Evans, R. D., R. Rimer, L. Sperry, and J. Belnap. 2001. Exotic plant invasion alters nitrogen dynamics in an arid grassland. Ecological Applications 11:1301–1310.

Eviner, V. T., and F. S. Chapin, III. 2002. The influence of plant species, fertilization and elevated CO_2 on soil aggregate stability. Plant and Soil 246:211–219.

Fenn, M. E., J. S. Baron, E. B. Allen, H. M. Rueth, K. R. Nydick, L. Geiser, W. D. Bowman, J. O. Sickman, T. Meixner, D. W. Johnson, and P. Neitlich. 2003. Ecological effects of nitrogen deposition in the western United States. BioScience 53:404–420.

Gough, L., C. W. Osenberg, K. L. Gross, and S. L. Collins. 2000. Fertilization effects on species density and primary productivity in herbaceous plant communities. Oikos 89:428–439.

Gough, L., P. A. Wookey, and G. R. Shaver. 2002. Dry heath arctic tundra responses to long-term nutrient and light manipulation. Arctic, Antarctic, and Alpine Research 34:211–218.

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

Groffman, P., et al. 2006. Ecological thresholds: The key to successful environmental management or an important concept with no practical application? Ecosystems 9:1–13.

Gunderson, L. H. 2000. Ecological resilience—in theory and application. Annual Review of Ecology and Systematics 31:425–439.

Hobbie, S. E. 1992. Effects of plant species on nutrient cycling. Trends in Ecology and Evolution 7:336–339.

Hobbie, S. E., A. Shevtsova, and F. S. Chapin, III. 1999. Plant responses to species removal and experimental warming in Alaskan tussock tundra. Oikos 84:417–434.

Kardol, P., N. J. Cornips, M. M. L. van Kempen, J. M. T. Bakx-Schotman, and W. H. van der Putten. 2007. Microbe-mediated plant–soil feedback causes historical contingency effects in plant community assembly. Ecological Monographs 77:147–162.

Klanderud, K. 2005. Climate change effects on species interactions in an alpine plant community. Journal of Ecology 93:127–137.

Kourtev, P. S., J. G. Ehrenfeld, and M. Haggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83:3152–3166.

Litaor, M. L., T. R. Seastedt, M. D. Walker, M. Carbone, and A. Townsend. 2005. The biogeochemistry of phosphorus across an alpine topographic/snow gradient. Geoderma 124:49–61.

Mack, M. C., E. A. G. Schuur, M. S. Bret-Harte, G. R. Shaver, and F. S. Chapin, III. 2004. Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. Nature 431:440–443.

Manning, P., et al. 2006. Decoupling the direct and indirect effects of nitrogen deposition on ecosystem function. Ecology Letters 9:1015–1024.

Matson, P., K. A. Lohse, and S. J. Hall. 2002. The globalization of nitrogen deposition: consequences for terrestrial ecosystems. Ambio 31:113–119.

May, D. E., and P. J. Webber. 1982. Spatial and temporal variation of the vegetation and its productivity, Niwot Ridge, Colorado. Pages 73–81 *in* J. Halfpenny, editor. Ecological studies in the Colorado Alpine: a festschrift for John W. Marr. Institute of Arctic and Alpine Research, University of Colorado, Boulder, Colorado, USA.

May, R. M. 1977. Thresholds and breakpoints in ecosystems with a multiplicity of stable states. Nature 269:471–477.

McArdle, B. H., and M. J. Anderson. 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82:290–297.

McClanahan, T., N. Polunin, and T. Done. 2002. Ecological states and the resilience of coral reefs. Conservation Ecology 6(2):18. ⟨http://www.ecologyandsociety.org/vol16/iss2/art1⟩

Meier, C. L., K. N. Suding, and W. D. Bowman. 2008. Carbon flux from plants to soil: roots are a below-ground source of phenolic secondary compounds in an alpine ecosystem. Journal of Ecology 3:421–430.

Miller, A. E., and W. D. Bowman. 2002. Variation in nitrogen-15 natural abundance and nitrogen uptake traits among co-occurring alpine species: Do species partition by nitrogen form? Oecologia 130:609–616.

Neff, J. C., A. R. Townsend, G. Gleixner, S. J. Lehman, J. Turnbull, and W. D. Bowman. 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419:915–917.

Nilsson, M. C., D. A. Wardle, O. Zackrisson, and A. Jaderlund. 2002. Effects of alleviation of ecological stresses on an alpine tundra community over an eight-year period. Oikos 97:3–17.

Nystrom, M. 2006. Redundancy and response diversity of functional groups: implications for the resilience of coral reefs. Ambio 35:30–35.

O'Connor, N. E., and T. P. Crowe. 2005. Biodiversity loss and ecosystem functioning: distinguishing between number and identity of species. Ecology 86:1783–1796.

Pauli, D., M. Peintinger, and B. Schmid. 2002. Nutrient enrichment in calcareous fens: effects on plant species and community structure. Basic and Applied Ecology 3:255–266.

Pinheiro, J. C., and D. M. Bates. 2000. Mixed-effects models in S and S-PLUS. Springer-Verlag, New York, New York, USA.

Power, M. E., D. Tilman, J. A. Estes, B. A. Menge, W. J. Bond, L. S. Mills, G. Daily, J. C. Castilla, J. Lubchenco, and R. T. Paine. 1996. Challenges in the quest for keystones. BioScience 46:609–620.

Rajaniemi, T. K. 2003. Explaining productivity–diversity relationships in plants. Oikos 101:449–457.

Reynolds, H. L., A. Packer, J. D. Bever, and K. Clay. 2003. Grassroots ecology: Plant–microbe–soil interactions as drivers of plant community structure and dynamics. Ecology 84: 2281–2291.

Rinnan, R., A. Michelsen, E. Baath, and S. Jonasson. 2007. Fifteen years of climate change manipulations alter soil microbial communities in a subarctic heath ecosystem. Global Change Biology 13:28–39.

Robertson, G. P., D. C. Coleman, C. S. Bledsoe, and P. Sollins, editors. 1999. Standard soil methods for long-term ecological research. Oxford University Press, New York, New York, USA.

Ruesink, J. L., and D. S. Srivastava. 2001. Numerical and per capita responses to species loss: mechanisms maintaining ecosystem function in a community of stream insect detritivores. Oikos 93:221–234.

Scheffer, M., and S. R. Carpenter. 2003. Catastrophic regime shifts in ecosystems: linking theory to observation. Trends in Ecology and Evolution 18:648–656.

Scherer-Lorenzen, M., C. Palmborg, A. Prinz, and E. D. Schulze. 2003. The role of plant diversity and composition for nitrate leaching in grasslands. Ecology 84:1539–1552.

Schmidt, S. K., D. A. Lipson, R. E. Ley, M. C. Fisk, and A. E. West. 2004. Impacts of chronic nitrogen additions vary seasonally and by microbial functional group in tundra soils. Biogeochemistry 69:1–17.

Seastedt, T. R., and L. Vaccaro. 2001. Plant species richness, productivity, and nitrogen and phosphorus limitations across a snowpack gradient in alpine tundra, Colorado, USA. Arctic, Antarctic, and Alpine Research 33:100–106.

Shaver, G. R., S. M. Bret-Harte, M. H. Jones, J. Johnstone, L. Gough, J. Laundre, and F. S. Chapin. 2001. Species composition interacts with fertilizer to control long-term change in tundra productivity. Ecology 82:3163–3181.

Sievering, H. 2001. Atmospheric chemistry and deposition. Pages 32–44 *in* W. D. Bowman and T. Seastedt, editors. Structure and function of an alpine ecosystem. Oxford University Press, Oxford, UK.

Sinsabaugh, R. L. 1994. Enzymatic analysis of microbial pattern and process. Biology and Fertility of Soils 17:69–74.

Sinsabaugh, R. L., M. E. Gallo, C. Lauber, M. P. Waldrop, and D. R. Zak. 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75: 201–215.

Sokal, R. R., and F. J. Rohlf. 1994. Biometry. Third edition. W. H. Freeman, San Francisco, California, USA.

Soudzilovskaia, N. A., V. G. Onipchenko, J. H. C. Cornelissen, and R. Aerts. 2005. Biomass production, N:P ratio and nutrient limitation in a Caucasian alpine tundra plant community. Journal of Vegetation Science 16:399–406.

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.

Stevens, C. J., N. B. Dise, J. O. Mountford, and D. J. Gowing. 2004. Impact of nitrogen deposition on the species richness of grasslands. Science 303:1876–1879.

Stursova, M., C. L. Crenshaw, and R. L. Sinsabaugh. 2006. Microbial responses to long-term N deposition in a semiarid grassland. Microbial Ecology 51:90–98.

Suding, K. N., S. L. Collins, L. Gough, C. Clark, E. E. Cleland, K. L. Gross, D. G. Milchunas, and S. Pennings. 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. Proceedings of the National Academy of Sciences (USA) 102:4387–4392.

Suding, K. N., K. L. Gross, and G. Houseman. 2004. Alternative states and positive feedbacks in restoration ecology. Trends in Ecology and Evolution 193:46–53.

Theodose, T. A., and W. D. Bowman. 1997. Nutrient availability, plant abundance, and species diversity in two alpine tundra communities. Ecology 78:1861–1872.

Tilman, D., J. Fargione, B. Wolff, C. D'Antonio, A. Dobson, R. Howarth, D. Schindler, W. H. Schlesinger, D. Simberloff, and D. Swackhamer. 2001. Forecasting agriculturally driven global environmental change. Science 292:281–284.

Treseder, K. K. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytologist 164:347–355.

Underwood, A. J. 1994. On beyond BACI: sampling designs that might reliably detect environmental disturbances. Ecological Applications 4:3–15.

USDA-NRCS [U.S. Department of Agriculture Natural Resource Conservation Service]. 2006. The PLANTS database. National Plant Data Center, Baton Rouge, Louisiana, USA. ⟨http://plants.usda.gov⟩

van Nes, E. H., and M. Scheffer. 2004. Large species shifts triggered by small forces. American Naturalist 164:255–266.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications 7:737–750.

Vitousek, P. M., S. Hattenschwiler, L. Olander, and S. Allison. 2002. Nitrogen and nature. Ambio 31:97–101.

Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry 13:87–115.

Waldrop, M. P., D. R. Zak, and R. L. Sinsabaugh. 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology and Biochemistry 36:1443–1451.

Walker, B., A. Kinzig, and J. Langridge. 1999. Plant attribute diversity, resilience, and ecosystem function: the nature and significance of dominant and minor species. Ecosystems 2: 95–113.

APPENDIX

Statistical results for the effects of nutrient and species-removal manipulations on soil and community characteristics in 2006 (*Ecological Archives* M078-011-A1).